BioMed Research International

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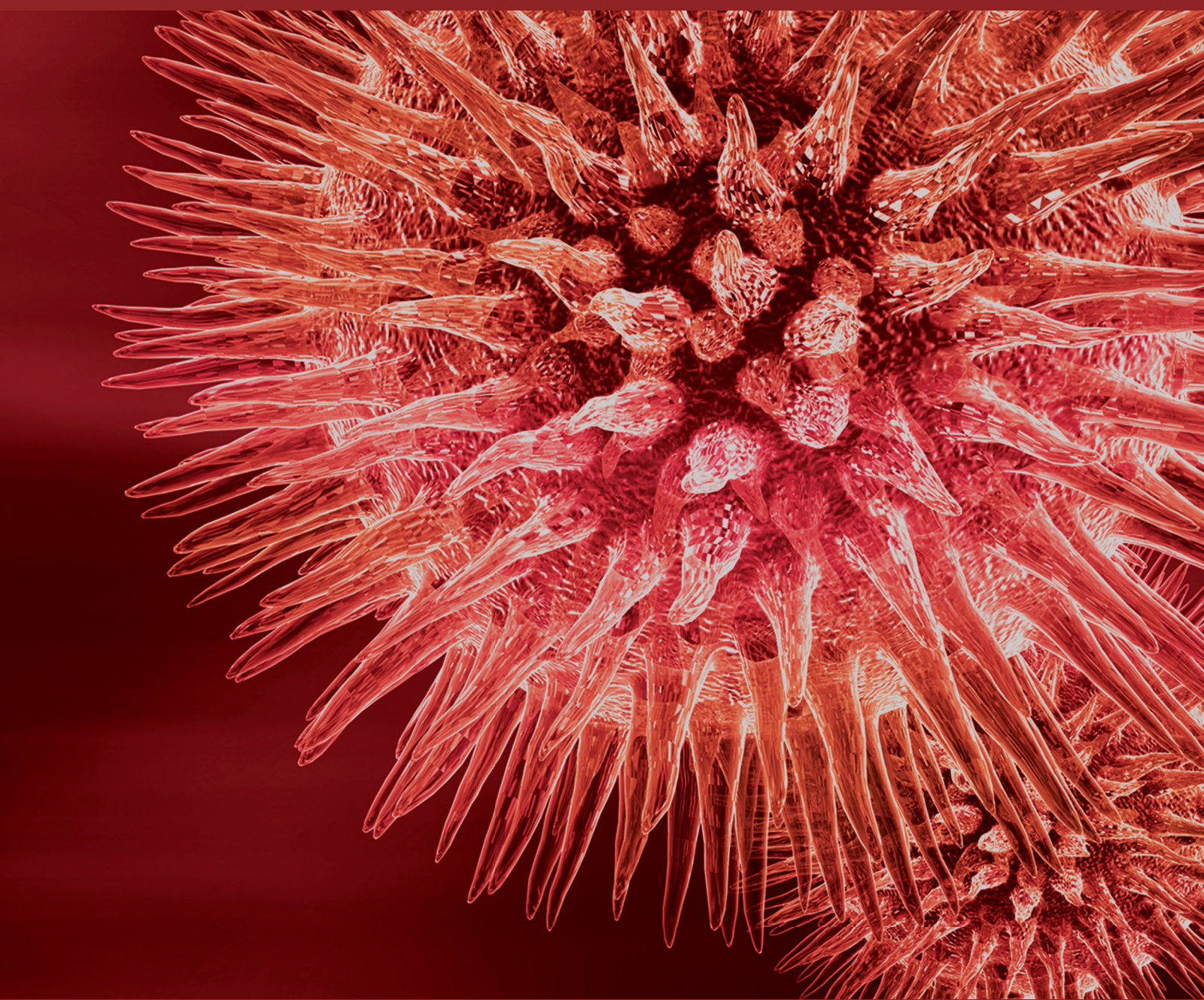

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Contents

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Editorial

Growth Abnormalities of Fetuses and Infants

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The objective of this special issue is to address recent research trends and developments about the advancements of image processing and vision in healthcare. A substantial number of papers were submitted, and after a thorough peer review process, some of these were selected to be included in this special issue. Growth abnormalities (either growth restriction or large for gestational age) during perinatal and postnatal life are a hot topic issue, since they are often linked to alteration of uterine environment caused by placental insufficiency, maternal metabolic syndrome, and in general under- or overnutrition of the fetus. These fetal abnormalities account for the leading causes of perinatal morbidity and mortality. Moreover, under the hypothesis of developmental origin of adult diseases, they bear consequences in later life, programming the infant physiology for a higher risk of noncommunicable diseases, cardiovascular adult diseases, and neurodevelopment delay. Low birth weight, caused either by preterm birth and/or by intrauterine growth restriction, is recently known to be associated with increased rates of cardiovascular disease and noninsulin dependent diabetes in adult life. The "developmental origins of adult disease" hypothesis, often called "the Barker hypothesis," proposes that these diseases originate through adaptations of the fetus when it is undernourished. These adaptations may be cardiovascular, metabolic, or endocrine and they may permanently change the structure and function of the body, increasing coronary heart disease risk factors, such as hypertension, type 2 diabetes mellitus, insulin resistance, and hyperlipidaemia. This hypothesis originally involved from observation by Barker and colleagues that the regions in England with the highest rates of infant mortality in the early 20th century also had the highest rates of mortality from coronary heart disease decades later. As the most commonly registered cause of infant death at the start of 20th century was low birth weight, these observations led to the hypothesis that low birth weight babies who survived infancy and childhood might be at increased risk of coronary heart disease in later life. There is an increased evidence of the link between intrauterine and perinatal alterations and adult diseases. Although the main focus so far has been the timing of delivery and follow-up, the study of the pathophysiology and of possible recovery is of paramount importance and needs the contributions of physicians from several fields, biologists, bioinformaticians, and engineers.

The paper by C. Caissutti and V. Berghella presents a comprehensive review about the most important and employed guidelines about screening and management of gestational diabetes (GDM) that affects up to 7% of pregnant women and is associated with several maternal and perinatal morbidities. It is well known that GDM represents over time a risk factor of type 2 diabetes, for both the mother and the fetus. Nowadays, there are many unsolved questions concerning the indications of screening, the timing and type of screening, the criteria for diagnosis, and the population to screen. The correct identification of universally approved and shared recommendations should improve the GDM pregnancy outcomes (gestational hypertension, prematurity, cesarean deliveries, number of large for gestational age and small for gestational age fetuses, and 1-minute Apgar scores

< 7), improving the health care and maintaining a cost-effective benefit.

The paper by B. Chiofalo et al. takes into account the role of microRNAs (miRNAs) of the placenta in the intrauterine growth restriction (IUGR) disease. IUGR could be considered as a placentation disorder, derived from a dysregulation in trophoblast invasion with characteristic tissue morphology that leads to uteroplacental insufficiency. More than 1880 miRNAs have been reported in humans and most of them are expressed in placenta. They seem to modulate cell development, differentiation, and proliferation, cell type-specific function, and epigenetic processes. In several cases, miRNA is significantly different between physiology and pathological conditions. An abnormal upregulated placental expression regulating some angiogenic regulatory pathways seems to increase vascular resistances, also in growth-restricted human pregnancies. Despite the fact that several authors have demonstrated a relatively easy and feasible detection of some miRNAs in maternal whole peripheral blood, costs of these tests should be reduced in order to increase cohorts and have stronger evidence. A large cohort and an adequate statistical power could identify a panel of biomarkers on maternal peripheral blood for early diagnosis of IUGR.

The paper of W. M. Curtin et al. analyzes the correlation between Doppler abnormalities in fetuses with suspected IUGR delivered at 37 weeks' gestation and placental histopathological lesions, in order to remove the confounding factor that gestational age has on interpretation of placental disease. The correct definition of IUGR nowadays is object of discussion. Up to 70% of fetuses with suspected IUGR may be constitutionally small normal infants and the remainder will be classified as IUGR presumably secondary to a pathologic placental process. In IUGR at later gestational ages, abnormal umbilical artery Doppler patterns are less frequent; the placental pathology is subtler and the lesions can overlap with normal pregnancies. This aspect could distinguish the "true" or "pathologic" IUGR fetuses. In their study the authors show an association between abnormal Doppler patterns and the presence of placental pathology in singleton pregnancies delivered at 37 weeks' gestation for suspected IUGR. In particular, an abnormal MCA Doppler had the strongest association and underscores the limitation of umbilical artery Doppler alone in IUGR at later gestational ages. Further investigation and tools for separating the constitutionally small normal fetus from the IUGR fetus are needed.

The paper of M. G. Clemente et al. describes an observational retrospective study about the postnatal growth in a cohort of IUGR infants. The fetal programming theory postulates that the condition of IUGR is associated with an increased risk of cardiovascular events in adult life, as stroke, type II diabetes, metabolic syndrome, and neurocognitive impairment. At birth, significant differences were found between IUGR and controls neonates with regard to all the auxological parameters (weight, head circumference, and length). During the 1st year, 8 of 12 (70%) IUGR infants exhibited a significant catch-up growth in the 3 anthropometric parameters and a regular growth until the 3rd year of follow-up. The majority but not all infants born with IUGR in our series showed significant postnatal catch-up growth essentially during the first 12 months of life. The modern research describes a new personalized medicine approach through the Newborn Individualized Developmental Care and Assessment Program (NIDCAP), conducted on preterm infants born with severe IUGR by a multidisciplinary research working group. It seems to be effective in ameliorating the neurobehavior, electrophysiology, and brain structure outcomes. An improved knowledge of the causes of IUGR will help to develop measures for its prevention and individualized treatment.

The paper of Q. Guo et al. takes into account the congenital heart defect (CHD), one of the most common birth defects in the world. Around the world, periconceptional folic acid intake in females is thought to reduce the risk of CHD in the newborn. The methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) genes are two of the most important candidate genes for fetal CHD. However, the correlations between the two genes and fetal CHD were inconsistent in various reports. This study is aimed to evaluate the parental effects of the two genes on fetal CHD via three genetic polymorphisms, MTHFR 677C>T (rs1801133), MTHFR 1298 A>C (rs1801131), and MTRR 66A>G (rs1801394). Parents with pregnancy history of fetal CHD were divided into two subgroups in base on ventricular septal defect (VSD) and non-VSD groups. In either maternal or paternal group, the MTHFR 677C>T polymorphism was independently related to fetal non-VSD, while the MTRR 66A>G polymorphism was independently related to fetal VSD. The findings of this study could help to explain why the relationship between the two polymorphisms (MTHFR 677C>T and MTRR 66A>G) and CHD varied among different studies due to different proportion of VSD and non-VSD subjects included in those different population studies.

The paper of D. Darmochwal-Kolarz et al. investigates the role of Interleukin-17 (IL-17), Interleukin-23 (IL-23), and transforming growth factor β (TGF β) in pregnancy complicated by placental insufficiency, fetal growth restriction, and preeclampsia. In recent years, in order to clarify the immunological mechanisms responsible for the proper implantation process, the interest has moved on the Th1/Th2/Th17 paradigm. Interleukin-17 (IL-17) is a cytokine with potent proinflammatory properties and has a proven role in the development of inflammatory processes. Moreover, Interleukin-23, which is produced, among others, by macrophages and dendritic cells, is an important component of the inflammatory response. Finally, transforming growth factor β (TGF β) released, among others, by macrophages, neutrophils, platelets, and lymphocytes, acts primarily to reduce the release of proinflammatory cytokines. In addition, TGF β is involved in the processes of angiogenesis, wound healing, and repair processes, as well as regulation of the entry of cells onto the apoptotic pathway. In the group of patients with placental insufficiency, the maternal sera levels of IL-17 positively correlated with maternal systolic blood pressure and the concentrations of TGF β were significantly lower, while IL-23 was comparable with respect to control group. It seems possible that the increased concentrations of IL-17 and

the deficiency of TGF β in pregnancy complicated by fetal growth restriction and preeclampsia can be responsible for the activation of inflammatory response.

The paper of L. Baken et al. discusses the introduction of three-dimensional (3D) ultrasound in the evaluation of the crown-rump length (CRL), in particular whether the embryonic volume (EV), measured by a Virtual Reality (VR) system, is a better parameter to determine growth restriction in fetuses with structural congenital abnormalities, diagnosed in the first trimester of pregnancy. It is know that the relative increment of the EV is much larger than the increment of the CRL during the same period. Moreover, if a too small CRL is a clinical predictor for miscarriage, chromosomal abnormalities (especially trisomy 18), and fetal growth restriction in the second and third trimester of pregnancy, several authors underlined that EV was not only smaller in trisomy 18 pregnancies but also in trisomy 21 and trisomy 13 pregnancies. In this study, measured CRL and EV were converted to z-scores and to percentages of the expected mean, using published reference curves of euploid fetuses. The EV was smaller than expected for gestational age in fetuses with structural congenital abnormalities, whereas CRL was not. By measuring EV, first-trimester growth restriction becomes more evident and might enable an earlier detection of cases at risk for a congenital abnormality.

The paper of F. Bardanzellu et al. presents a comprehensive review of paracetamol efficacy in Ductus Arteriosus (DA) closure in preterm infants, in which the failure or delay of its spontaneous closure results in the condition of Patent Ductus Arteriosus (PDA). A prolonged situation of PDA can be associated with several short- and long-term complications. Despite years of researches and clinical experience on PDA management, unresolved questions about the treatment and heterogeneity of clinical practices still remain, in particular regarding timing and modality of intervention. Nowadays, the most reasonable strategy seems to be reserving the treatment only to hemodynamically significant PDA. The first-line therapy is medical, and ibuprofen, related to several side effects especially in terms of nephrotoxicity, is the drug of choice. Administration of oral or intravenous paracetamol (acetaminophen) recently gained attention, appearing effective as traditional nonsteroidal anti-inflammatory drugs (NSAIDs) in PDA closure, with lower toxicity. The results of the studies analyzed in this review mostly support paracetamol efficacy in ductal closure, with inconstant low and transient elevation of liver enzymes as reported side effect. More studies are needed to confirm if this therapy shows a real safety profile and to evaluate its long-term outcomes.

The paper of R. Pintus et al. discusses the most recent literature about the metabolomic technology and its application in the cardiologic field, in order to understand the metabolic shifts that occur even before the manifestation of heart diseases and to find possible early predictive biomarkers. Cardiac pathologies are a critical health issue affecting millions of people worldwide, with a constant mortality rate in particular in the elderly, a difficult prognosis, and a worsening in quality of life of affected people. The pathophysiology of heart pathologies is complex. Recent findings pointed out a possible pivotal role of mitochondrial dysfunction and the subsequent altered energy metabolism in cardiac diseases, in particular in case of heart failure. Moreover, every adverse event that may occur during pregnancy "shapes" the health status of the fetus and its development and could affect its life course, also with cardiovascular problems. Congenital malformations, gut colonization by microbiota, individual genetic arrangement, and its interplay with both behavioral and risk factors, such as drugs assumption, can influence the occurrence of heart diseases. During the last decade, animal and human studies have applied metabolomics to cardiovascular research, using both targeted and untargeted approaches; as such, metabolic fingerprints have been identified for several cardiovascular risk factors and diseases. These techniques could be applied to heart tissue and biofluids, such as blood, saliva, and urine, with a minimum compliance needed from the patient, since their collection is not invasive. Metabolomics, for its peculiarities, seems to be so promising that several industries are trying to set up kits to immediately assess the metabolites variations in order to provide a faster diagnosis and the best treatment specific for that patient, offering a further step toward the path of the development of a tailored medicine.

The paper of N. Abdalla et al. describes complete up-to-date findings from the literature regarding the impact of chemotherapy on fetal growth. Cancer and pregnancy rarely coincide. Gynecological cancers are among the most common malignancies to occur during pregnancy, and chemotherapy with or without surgery is the primary treatment option. The main concern of administering chemotherapy during pregnancy is congenital malformation, although it can be avoided by delaying treatment until after organogenesis. The dose, frequency, choice of chemotherapeutic agents, time of treatment commencement, and method of administration can be adjusted to obtain the best maternal treatment outcomes while simultaneously minimizing fetal toxicity. Use of chemotherapy after the first trimester, while seemingly safe, can cause fetal growth restriction. However, the exact effect of chemotherapy on such fetal growth restriction has not been fully established; information is scarce owing to the rarity of malignancy occurring during pregnancy, the lack of uniform treatment protocols, different terminologies for defining certain fetal growth abnormalities, the influence of mothers' preferred options, and ethical issues. Fetal growth abnormalities are recognized sequelae of chemotherapy, and the possibility of fetal growth abnormalities as well as the other side effects of different chemotherapeutic agents should be discussed with the patients.

The type of malignancy and its stage, the use of surgery and/or radiotherapy, the stage of pregnancy, the probability of side effects during treatment, and the patient's own wishes influence the final decision regarding treatment. Moreover, ethical concerns cannot be ignored. Each patient should ultimately be managed individually with the guidance of a multidisciplinary team.

The paper of S. A. Ernst et al. investigates what care-related and maternal risk factors could influence the antenatal nondetection of IUGR during pregnancy and examine if there are specific groups with a higher chance of nondetected suboptimal fetal growth. Approximately 3% to

8% of all infants born in developed countries have been identified as growth restricted. IUGR is a prenatal condition associated with a higher risk for perinatal morbidity and mortality, increasing the stillbirth rate fourfold compared to pregnancies with normally grown fetuses; antenatal non-detection further increases the rate by a factor of 2. An early antenatal detection, choosing the optimal time and method of delivery, and treatment where appropriate could minimize the risks significantly. However, low antenatal detection rates of suboptimal fetal growth through routine fetal ultrasonography have been reported. In fact IUGR has been reported to be antenatally detected only in one-third (25% to 32%) of pregnancies with suboptimal fetal growth. Moreover, the authors identified three factors that influenced IUGR detection: a higher severity of the growth restriction, maternal complications/diseases during pregnancy, and a Doppler examination. The authors did not find statistically significant differences regarding parental socioeconomic status and maternal migration background. Future in-depth studies with larger study populations should further examine factors that could increase antenatal detection rates for IUGR.

The paper of S. Norda et al. discusses the role of neonatal ApoE e4 haplotype on IUGR and its contribution on impaired fetal growth and the possible link of IUGR with cardiovascular and metabolic diseases later in life. Apolipoprotein E (ApoE) is an important circulating serum protein involved in transporting lipids and cholesterol and regulating lipid levels. Its regulatory functions have been attributed to many biophysiological processes including neuronal growth and modulation of oxidant and inflammatory processes. Cord blood of IUGR neonates displays lipid changes towards atherosclerotic profiles. IUGR born babies were found to have lower concentrations of high-density lipoprotein cholesterol (HDL-C), known for having an anti-inflammatory effect and protective properties against the development of atherosclerosis, while triglycerides and oxidized low-density lipoprotein (oxLDL) levels were elevated in samples of umbilical blood compared to adequate weight newborns. This disrupted cholesterol and triglyceride handling plays a role in causing suboptimal fetal development and exposes the newborn to an atherosclerotic environment early, consequentially giving raise to irreversible damage to vessels. The association of ApoE e4 with the development of dyslipidemia and cardiovascular disease is known. In newborns, carrying the ApoE e2 allele has been associated with lower fetal cord blood LDL-C levels and higher levels of HDL-C suggesting a beneficial effect of this genotype on blood lipid configuration, modulating the fetal growth and the severity of IUGR. In this cohort, 4885 preterm infants were analyzed. Neonates were categorized into subgroups of <3rd, 3rd–10th, and >10th birth weight percentile. The identification of the ApoE genotype was carried out. No association was found between genotype and birth weight percentiles in each of the subgroups. ApoE genotype and low birth weight depict two distinct risk factors for cardiovascular disease without being directly associated.

The paper of M. Gatta et al. describes that prematurity has a critical influence on interactive, communicative, and expressive child behaviour, particularly during the first years of life. Few studies have stressed the assessment of mother-father-child interaction in families with preterm children, generating contradictory results. The authors recruited 78 families, 39 families with preterm children, and 39 families with full-term children. Results show that families with preterm children display a low quality of mother-father-child interactions. After six months, family interactions result is generally stable, except for some Lausanne Trilogue Play-scales, reflecting a hard adjustment of parenting style to the evolution of the child. The Lausanne Trilogue Play is a semi-standardized observation situation designed to assess the quality of family interactions. The administration involved the mother-father-child triad invited to cooperate and work together in order to conduct an activity. In families with preterm children, the parenting stress seemed to be correlated with the quality of mother-father-child interactions.

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Review Article

DNA Damage as a Driver for Growth Delay: Chromosome Instability Syndromes with Intrauterine Growth Retardation

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DNA is constantly exposed to endogenous and exogenous mutagenic stimuli that are capable of producing diverse lesions. In order to protect the integrity of the genetic material, a wide array of DNA repair systems that can target each specific lesion has evolved. Despite the availability of several repair pathways, a common general program known as the DNA damage response (DDR) is stimulated to promote lesion detection, signaling, and repair in order to maintain genetic integrity. The genes that participate in these pathways are subject to mutation; a loss in their function would result in impaired DNA repair and genomic instability. When the DDR is constitutionally altered, every cell of the organism, starting from development, will show DNA damage and subsequent genomic instability. The cellular response to this is either uncontrolled proliferation and cell cycle deregulation that ensues overgrowth, or apoptosis and senescence that result in tissue hypoplasia. These diverging growth abnormalities can clinically translate as cancer or growth retardation; both features can be found in chromosome instability syndromes (CIS). The analysis of the clinical, cellular, and molecular phenotypes of CIS with intrauterine growth retardation allows inferring that replication alteration is their unifying feature.

1. DNA and Genomic Integrity

DNA is our genetic heritage; the genetic instructions that cells use to construct their components and function are encoded in their sequence. DNA is also the molecule responsible for transmitting information from generation to generation on a cell and organism scale. This information is provided to each human being in the nucleus of the fertilized egg in a set of 46 DNA molecules forming chromosomes. Subsequent divisions generate millions of cells to form a fetus: each one of these cells has its own set of 46 chromosomes. Amazingly, in spite of having been copied millions of times, the DNA sequence of these chromosomes is remarkably similar to the original molecule. This is surprising since DNA is constantly exposed to endogenous and exogenous mutagenic stimuli. On the one hand, each replication round can result in thousands of lesions while, on the other hand, environmental genotoxic agents are a constant and an inevitable source of DNA damage [1, 2]. Every day, the DNA of a fetus can then accumulate tens of thousands of lesions that could result in mutations [2]. However, the DNA molecule is such an important asset to the cell that a significant share of its genetic information and cellular energy are destined to the detection and repair of DNA damage to preserve the organism's genetic integrity.

2. DNA Damage and the DNA Damage Response

The sources of induced lesions in DNA, can be endogenous or exogenous. The former originate from normal metabolic processes inside the cell, such as DNA replication, which may

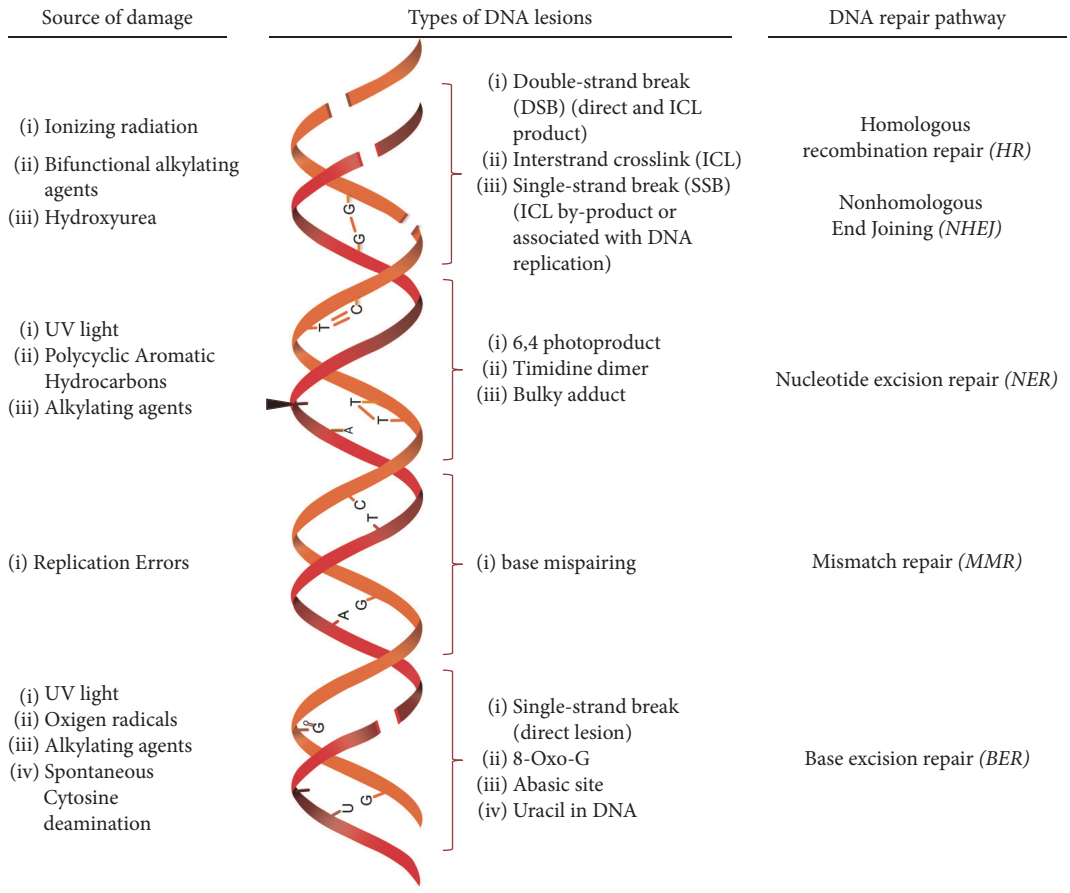


Figure 1: DNA lesions are induced by endogenous and exogenous sources. Every agent causes a particular lesion that will activate specific DNA repair pathways.

incorporate noncomplementary Watson–Crick bases during DNA synthesis. It can also come from lesions caused by oxidative damage that occurs during normal metabolism in the mitochondria and other cellular sites, giving rise to the oxidation of several cellular components, including DNA, resulting in modified bases or breaking of the union between them. Besides, spontaneous decay of the DNA molecule may generate hydrolysis that creates abasic sites and deamination, causing a change in the original bases. Exogenous sources that continually damage our DNA may be of four main origins: (1) biological, such as some virus, (2) physical, like solar radiation or radiation therapy, (3) chemical, like pesticides and medical treatments (chemotherapy), and (4) personal habits, such as smoking (Figure 1). All together, these mechanisms may generate more than ten thousand lesions per cell per day [3].

Two main factors, the type of DNA lesions and the phase of the cell cycle where they are sensed, affect the choice of the DNA repair pathway to be used and the subsequent outcome (Figure 1). Mispaired bases are repaired by mismatch repair (MMR); oxidative damage, abasic sites, and uracil in DNA are corrected by removing the altered base through base excision repair (BER); UV radiation damage and bulky adducts that disrupt the structure of the double helix are repaired by nucleotide excision repair (NER), in which an oligonucleotide of 30 bp containing the lesion is removed [4]. Most DNA lesions interfere with DNA replication and transcription, processes that are indispensable for appropriate cell function. However, double-strand breaks (DSBs), in which both DNA strands lose continuity, are the most dangerous type of DNA damage and have primarily cytotoxic or cytostatic consequences [2, 3, 5].

Interstrand crosslinks (ICLs) are detected and removed through the Fanconi anemia pathway [6, 7], also known as the FA/BRCA pathway. The processing of ICLs occurs during the S phase, resulting in the following intermediary lesions: an adduct and a DSB, which, are further taken care of by known repair pathways. The adduct is repaired by the NER pathway, while the DSB is processed by one of four independent pathways: nonhomologous end joining (NHEJ), homologous recombination (HR), alternative-NHEJ (alt-NHEJ), and single-strand annealing (SSA). The selection of the repair pathway that will take care of the DSB depends on the cell cycle phase and if the $3'$ ends of the DSBs are processed by an exonuclease or not [8]. NHEJ operates in any phase of the cell cycle and does not require any processing since this repair pathway marks both blunt ends of the DSB with a Ku70/Ku80 heterodimer and joins them. Meanwhile,

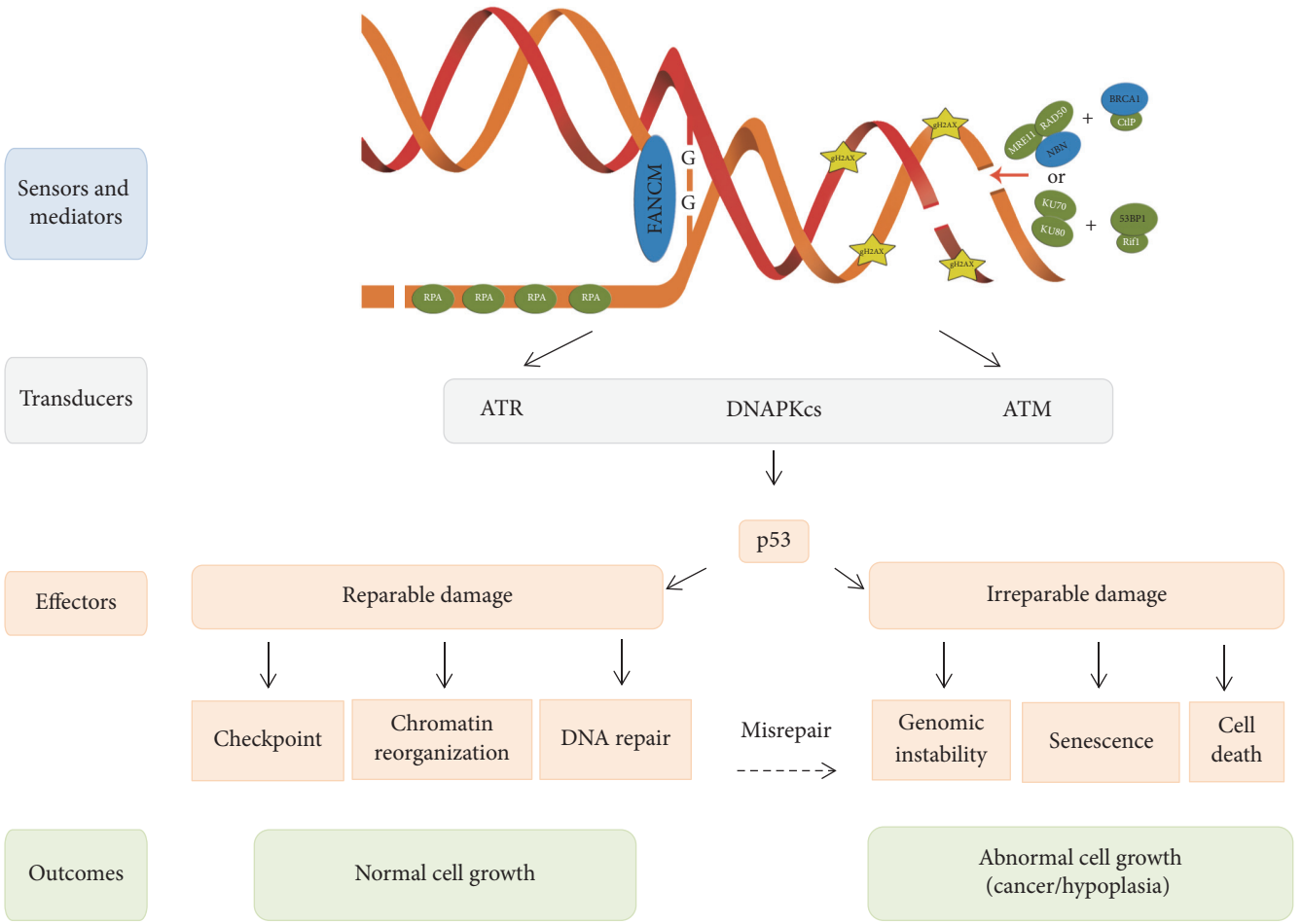


Figure 2: DNA damage response can result in different outcomes following single strand breaks, interstrand crosslinks and double strand breaks. Each DNA lesion is recognized by specific sensor proteins according to the cell cycle phase in which the cell is; during S phase, the protein FANCM identifies a replication fork arrested by an ICL, MRN + BRCA1 sense DSBs; meanwhile, Ku70/Ku80 + 53BP1 can recognize DSBs across the entire interphase; RPA detects and covers ssDNA primarily during S phase. The sensing process is then transduced and amplified on chromatin through a series of posttranslational histone modifications such as phosphorylation of H2AX (γH2AX) on both sides of the DSB. These changes allow the recruitment of the specialized transducer kinase ATR that mainly responds to RPA-covered ssDNA originated by replication stress or DNA lesions such as ICLs; concurrently, ATM responds to DSBs. Kinases activate several effector proteins including the transcription factor p53, which acts downstream regulating diverse outcomes according to the type and quantity of reminding lesions in the cell.

in order to repair a DSB using HR, alt-NHEJ, or SSA, the 3′ blunt ends must be processed to allow the loading of the MRN complex (a heterotrimer integrated by three proteins called MRE11, RAD50, and NBN) to continue the repair process. This occurs primarily during the S and G2 phases of the cell cycle, when sister chromatids are available and can be used for HR error-free repair [7, 9].

3. DNA Damage Response

Even when each specific DNA lesion stimulates a particular DNA repair mechanism, cells trigger a common general program known as the DNA damage response (DDR) which is charged with lesion detection, signaling, and repair promotion as well as cell cycle progression control. It is no surprise that the DDR is an extremely controlled process; the exquisite balance between cell survival and cell death and senescence relies on it [1, 9, 10]. Tissue homeostasis or growth abnormalities such as cancer [11] or tissue hypoplasia depend, among other things, on the amount and type of genomic damage sensed and processed by the DDR.

The DDR is composed of a network of regulatory noncoding RNAs and proteins that act as sensors, transducers, and effectors [1, 12]. Sensor proteins recognize specific lesions (Figure 2). For example, the FANCM protein detects the stalled replication fork caused by interstrand crosslinks (ICLs); the MRN complex is the typical sensor of DSBs during S/G2 phase; the Ku70/Ku80 heterodimer is the primary DSBs sensor during G1, while replication protein A (RPA) overcoats single-strand DNA (ssDNA) found either at processed DSB overhangs to be repaired by HR or at stalled replication forks [13].

Following recognition of the lesions by sensors and mediators, the signal is amplified by transducer proteins.

Table 1: Chromosomal instability syndromes with intrauterine growth deficiency.

Syndrome	Genes	Function in DDR	Repair pathway	Cytogenetic alteration
Fanconi anemia	21 *FANC* genes	ICL detection and processing, generation of adducts and DSBs (sensors, mediators and effectors)	Homologous recombination	Chromatid and chromosomal breaks Radial figures
Seckel Syndrome 1	*ATR*	SSBs detection and signal transduction (transducer)	Homologous recombination Nonhomologous recombination	Chromatid and chromosomal breaks and Rearrangements
Nijmegen Breakage Syndrome	*NBN*	DSBs detection and signaling (sensor and mediator)	Homologous recombination Nonhomologous recombination	Chromatid and chromosomal breaks Aneuploidies Rearrangements affecting chromosomes 7 and 14
Bloom Syndrome	*BLM*	Helicase, process SSBs, and Holliday Junctions (effector)	Homologous recombination	Quadriradials Increase in sister chromatid exchange

The PIKK kinases (Phosphatidyl Inositol 3-Kinase-related Kinases) family is the most important of this group; it is integrated by ataxia telangiectasia and Rad3-related (ATR), Ataxia telangiectasia-mutated (ATM), and DNA dependent protein kinase (DNAPKcs). ATR is activated during the S phase of the cell cycle, in the presence of DNA damage such as base adducts, crosslinks, single-strand breaks (SSBs), replication stress, and DSBs that originate during the S phase, while ATM and DNAPKcs are activated by DSBs at any point of the cell cycle [13, 14]. The DDR kinases activate signaling cascades through posttranslational modifications of various proteins, including phosphorylation, ubiquitylation, and PARylation, which play a central role in regulation of the DDR [15]. Specifically, ATR and ATM can autoactivate or transactivate each other through phosphorylation and then phosphorylate the histone variant H2AX (γH2AX) that acts as a platform to recruit DDR factors and prepare the cell to restore DNA integrity. ATR and ATM's main downstream phosphorylation targets are proteins Chk1 and Chk2 and the important effector p53; their activation allows regulating (a) the chromatin structure surrounding the lesion, (b) checkpoints that stop the cell cycle, (c) DNA repair proteins, (d) proteins that induce senescence, and (e) proteins that lead to cell death (Figure 2) [10, 16].

Finally, if DNA lesions are repaired, the checkpoint that is responsible for stopping the cell cycle is turned off and the cell cycle is allowed to restart through a process called checkpoint recovery. This process is only turned on when the signaling for the DNA lesions is silenced, and the surviving cell recovers its normal homeostasis and growth. When DNA damage cannot be properly repaired, the cell's destiny should be either senescence or death. Otherwise, cells might divide with unrepaired DNA damage through an erroneous activation of the checkpoint recovery process that allows cell survival with genomic instability to promote cell dysfunction and cancer [17].

4. DNA Damage Response and Disease

Unrepaired or misrepaired lesions in DNA may immediately impair replication and transcription, affecting the whole cell function. In an irreparable damage situation, the amount and type of lesions, as well as the cell cycle phase in which the cell is, will influence the DDR response in order to favor immediate cell death or the conversion of DNA lesions into durable mutations or stable chromosomal abnormalities [3].

Cell survival, despite genomic damage, can directly affect cell growth; this can be evidenced by two opposite outcomes: On the one hand, there can be an overgrowth effect, since mutations in critical genes such as oncogenes or tumor suppressor genes can alter cell function and increase the likelihood of cancer development [3, 18, 19]. On the other hand, there can be cell hypoplasia or cell loss; this happens when irreparable damage leads to either cell death or cell senescence, a cancer-protecting condition in which cells are alive but unable to proliferate, limiting the growth of the tissues and conducting to aging (Figure 2). This is evidenced by the phenotype of patients affected by diseases that alter the DDR and DNA repair in which growth alteration and increased cancer susceptibility are important features.

5. Chromosome Instability Syndromes

Mutations in at least 114 genes involved in the DDR lead to disease; some of the mutations are somatic, resulting in various types of spontaneous cancer, and others are genetic diseases with constitutional mutations that lead to syndromes that may or may not be related to the development of cancer [10]. Among the genetic diseases with mutations in DDR genes, the chromosomal instability syndromes (CIS) are a group of rare Mendelian diseases, characterized by increased chromosome breakage resulting from unrepaired or misrepaired DNA strands breaks. Other than chromosomal instability, they also have clinical overlapping features like cancer proneness, premature aging, and growth abnormalities, even though each CIS has a particular phenotype that distinguishes it from the others. Only a subgroup of these patients has prenatal growth alterations; this review focuses on those syndromes (Table 1).

6. DDR Alteration and Growth Failure

Historically, disruption of the DDR has been associated with cell growth abnormalities in a unilateral way: always pointing towards the increased cell number in the form of deregulated cell proliferation in cancer [19]. But there is little information on how DDR alterations can also lead to deregulation of growth in the opposite direction, resulting in lack of growth due to cell death and low cell reproduction. One of the first observations to partner growth deficiency with an altered DDR was the finding that some Seckel syndrome patients (SS), a rare phenotype consisting of primordial dwarfism and microcephaly, had mutations in the *ATR* gene, which encodes the ATR kinase, a DDR transducer. Further analysis of SS patients revealed locus heterogeneity for this phenotype; mutations in other genes related to DDR or DNA repair also led to this clinical picture. This observation has reinforced the association between DDR failure and severe intrauterine growth retardation. Moreover, genes that participate in centrosomal biology are also related to the uncommon phenotype of microcephalic primordial dwarfism, suggesting that centrosomes have a central role for the adequate differentiation of early neuroprogenitors. It has then been proposed that failure in cell proliferation, secondary to abnormal mitosis, is the cause of primordial dwarfism, since the multipolar spindles result in the activation of checkpoints, reducing proliferation and activating apoptosis [20].

For the phenotype of growth restriction to be evident prenatally, the mechanisms regulating growth must be compromised from the beginning of the development in embryonic stem cells (ESCs) which are pluripotent cells derived from the three primary germ layers: ectoderm, endoderm, and mesoderm, and that have the capacity to differentiate into more than 200 cell types. These cells have been proven to have an extremely efficient DDR and proficient DNA repair mechanisms that allow for a rigorous maintenance of their genome integrity; otherwise, unrepaired DNA damage in ESCs would be amplified, affecting normal human development.

ESCs have an extremely active proliferation rate; their cell cycle has a characteristically reduced G1 phase, and repair mechanisms are heightened. When comparing ESCs to fibroblasts, it stands out that MMR is enhanced, BER and NER repair pathways are highly competent, and the repair of DSBs is preferentially made through the reliable HR pathway [21]. Moreover, when HR is not available and DSBs accumulate, apoptosis is the preferred route to remove highly damaged cells. It is interesting that DSB repair in ESCs requires signaling through ATR instead of ATM. ESCs have a special mechanism to deal with DNA damaged cells when inefficient DNA repair or DDR affects the induction of apoptosis or autophagy [22]. This mechanism leads to ESC differentiation that is driven by damage-induced expression of the p53 transcription factor; damaged differentiated cells are efficient in cell cycle arrest and apoptosis. This strategy results in the maintenance of genetic stability in a decreased number of ESCs and a portion of differentiated cells that become senescent, both resulting in growth deficiency [21].

Growth deficiency is at the center of many diseases, including extremely low body size. In mammals, overall size is determined by the number of cells; body mass is then the sum of all the cells that have proliferated minus those that have died. A fetus that is growing harmonically has accurate balance between these two processes; failure of either one of these results in dramatic effects when they occur in the ESC population; a decrease in cell proliferation rate or an increase in apoptosis leads to intrauterine growth retardation (IUGR) [23].

7. Chromosome Instability Syndromes with Intrauterine Growth Retardation

7.1. Seckel Syndrome. Seckel syndrome (SS) is an autosomal recessive disorder, clinically and genetically heterogeneous. So far, at least six genes have been associated with the Seckel phenotype; most of these genes participate in ATR-mediated DDR and in centrosomal function. Two clinical subgroups can be identified: on the one hand, patients with mutations in *ATR*, *NIN,* and *ATRIP* genes only present the Seckel phenotype, while, on the other hand, those with mutations in *CENPJ*, *CEP152, or RBBP8* show allelic heterogeneity and, besides the SS phenotype, can also manifest as a spectrum of disorders known as Primary Autosomal Recessive Microcephalies [24–28]. SS patients who bear mutations in *ATR* show a classical Seckel phenotype, the SS data presented here will be limited to such patients.

ATR is localized in 3q23; it is one of the serine threonine kinases that belong to the PIKK family. They have a fundamental role in the DDR since they participate as transducers in the signaling of DNA lesions, especially when the DDR responds to stalled replication forks and bulky lesions in DNA [24, 25]. ATR responds preferentially to ssDNA during the S phase. It also plays a crucial role in preventing DNA breaks caused by fork pausing when DNA replication machinery finds DNA lesions or complex DNA structure and sequences [24]. ATR stability depends on binding of ssDNA to the ATR cofactor, ATRIP (ATR, interacting protein), and the single-stranded binding protein (RPA). ATRIP is required for ATR localization to the ssDNA regions and hence for ATR activation [25, 27]. Major functions of ATR are activation of cell cycle check point arrest, stabilization of stalled replication forks, and promotion of replication fork restart, which is achieved through its ability to phosphorylate a wide range of substrates that include p53 and H2AX [27, 29].

Cellular phenotype of ATR deficient cells is characterized by decreased phosphorylation of ATR-dependent substrates as well as an impaired G2/M checkpoint arrest. These cells are characterized by markers that signal unrepaired DSBs, like the presence of γH2AX, chromosomal breakage, [26, 29, 30] and micronuclei formation. This cellular phenotype may be originated by a failure to recover from replication stalling, which generates DSBs that are normally repaired by HR when they occur during S/G2. In SS cells, the absence of ATR is critical during embryonic development; this deficiency leads to genomic instability, cell senescence, and cell death that leads to fetal growth and accelerated aging [31].

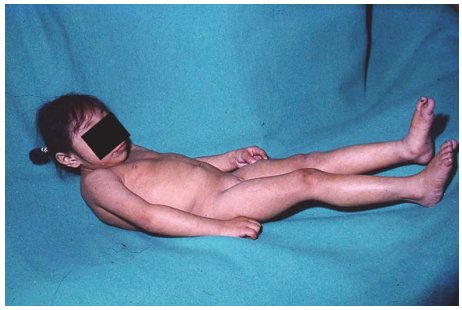


Figure 3: Seckel syndrome patient. Note the severe microcephaly and the "bird-like" appearance facies with micrognathia and receding forehead. The patient is severely disabled with no independent march.

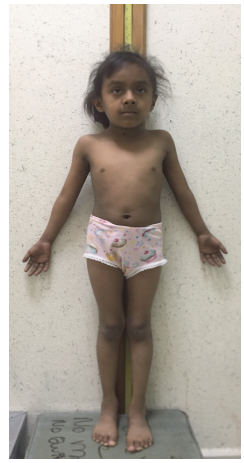


Figure 4: Fanconi anemia patient. This six-year-old girl is 105 cm tall; her height is just below the 5th percentile, and her at-term birth weight is reported to be of 1900 gr. Characteristic features of FA are evident: skin hyperpigmentation, bilateral radial defects consisting of bilateral thenar hypoplasia, and absent fold of the right thumb which cannot be bent.

Moreover, it appears that *ATR* and the other SS genes not only participate in the DDR, but also take part in the control of centrosome maturation. This engagement in centrosome biology is also true for other DDR proteins like BRCA2; biallelic mutations in its gene are responsible for a subgroup of Fanconi anemia patients [32]. The unifying feature between all Seckel patients, irrespective of the molecular defect, is that their cells have an altered cell cycle progression that is particularly evident during high rate cell division, when there is hypersensitivity to replicative stress and centrosomal dysfunction. This is especially important in cells with high division rates, like the ones found in developmental stages where rapid replication is key [24, 27].

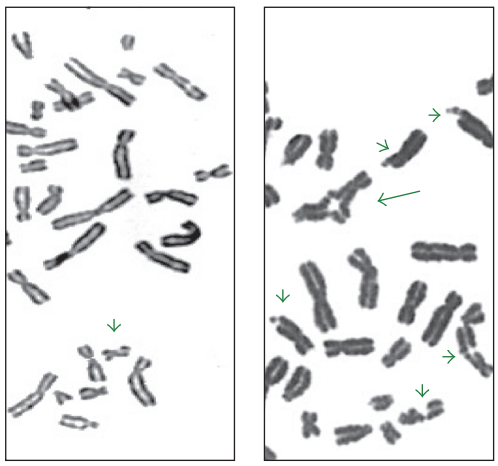
SS is characterized by IUGR, dwarfism, microcephaly (below −4 SD), mental retardation and some other anomalies like luxation of the head of the radius, scoliosis, bone age delay, and seizures (Figure 3). The distinctive facial features include a prominent nose with micrognathia and a receding forehead resulting in a "bird-like appearance" [26, 31, 33]. Since this syndrome has important clinical heterogeneity, other anomalies have been reported, including mandibular hypoplasia, sternal abnormalities, clinodactyly, and low set ears with hypoplastic lobules. Although rare, other reported features are moyamoya syndrome, osteosarcoma, and polyarteritis nodosa [26]. Cerebral malformations, like neuronal migration abnormalities, have been described but are not always present [33]. No glucose metabolism abnormalities have been reported.

Dwarfism can be related to a reduction in the total number of cells generated during development, leading to hypoplastic tissue and reduced organism size [23]. On the other hand, microcephaly may be related to an impaired DNA damage response signal that could alter the cellular threshold for cell death resulting from DNA damage, increasing the levels of apoptosis during development; developing neurons are rapidly proliferating and potentially generate high levels of oxidative damage, which may lead to a higher level of lesions being faced at replication forks [23, 25]. Also an altered mitosis, secondary to impaired spindle formation, could delay mitotic progression and increase the proportion of nonviable cell divisions. Finally, the stem cells may be affected by abnormal centrosomal function. Stems cells have asymmetric divisions in order to preserve the characteristics of a stem cell, and the centrosomes play an important role in such divisions [23].

There is limited clinical information regarding age-related diseases in SS; there is a void of prospective follow-up data from these patients that can only be obtained by following a cohort of SS patients, something that is apparently not being done at this time. Nevertheless, animal models bring some clues over the possibility of an aging phenotype in SS patients since ATR deficient adult mice show premature age-related phenotypes, as well as increased deterioration of tissue homeostasis [29, 34].

7.2. Fanconi Anemia. DNA interstrand crosslinks are extremely noxious DNA lesions that affect central cellular processes like transcription and replication. The FA/BRCA pathway is responsible for the appropriate processing of these lesions; protein products from at least 21 *FANC* genes participate in this pathway [35]. The malfunction of any of those FANC proteins leads to the clinical phenotype known as Fanconi anemia (FA), which is characterized by short stature, congenital malformations from the VACTERL-H spectrum, bone marrow failure, and an increased susceptibility to cancer like acute myeloid leukemia, epithelial head, and neck cancer [36] (Figure 4). Most of the FA families bear biallelic mutations in *FANC* genes that have an autosomic recessive inheritance pattern; only families with mutations in *FANCB* show an X-linked recessive pattern, while those in which *FANCR* is the affected gene demonstrate an autosomic dominant one [35].

The FA/BRCA is an S-specific pathway that has 3 basic steps: it starts with the detection of the ICL by the protein FANCM, followed by the recruitment of the FA core complex that is responsible for the monoubiquitination of the FANCD2/I heterodimer which, in turn, favors the recruitment of effector FANC-repair proteins that restore the DNA to its original form [35].



(a) (b)

Figure 5: Chromosomal instability induced by 40 ng/ml mitomycin C. (a) Lymphocytes from a healthy individual; (b) lymphocytes from a FA patient. Short arrows show chromosomal breaks; the long arrow shows a quadriradial figure. Note the exacerbated chromosome instability found in the FA patient.

The cellular phenotype of FA derived cells is extremely constant. An aberrant FA/BRCA pathway can result in unrepaired DSBs that manifest as chromosomal breaks, or abnormal repair by an error-prone repair pathway that results in radial figures. These alterations are cytogenetic evidence of chromosomal instability, which is the hallmark of FA cells and is exploited for clinical diagnostic purposes (Figure 5). The agents capable of inducing this chromosomal instability are from endogenous origin, like aldehydes [37] and reactive species of oxygen (ROS) [38], as well as exogenous sources like bifunctional alkylating agents (diepoxybutane or mitomycin C). In addition, FA cells have accumulation of cells in G2 phase of the cell cycle, resulting from a functional G2/M checkpoint and a proapoptotic phenotype.

In contrast with the cellular FA phenotype, the clinical picture in FA patients is extremely variable; not every patient will have all the manifestations: one-third of patients do not have congenital malformations [39], while almost 90% will develop bone marrow failure [36]. One of the more constant manifestations in FA patients is growth parameters alteration. An analysis from the international Fanconi anemia registry (IFAR) data showed that over 60% of FA patients presented short stature (below the 5th percentile), while birth length and weight below the 5th percentile was respectively reported in almost 30% and nearly half of FA patients [40]. Moreover, a prospective study of data from 54 IFAR participants showed that the mean standard deviation score (SDS) for height in these patients was significantly below normal for age and sex at -2.35 ± 0.28; meanwhile, mean SDS for weight was better, although below normal (-1.26 ± 0.24). In this study, perinatal growth data was not reported [41].

Other than the IFAR data on growth, there are another two studies that analyze growth parameters of FA patients. Anthropometric measurements from 45 patients with Fanconi anemia from the National Cancer Institute's inherited bone marrow failure syndromes (IBMFS) cohort were assessed. This cohort had a mean SDS for height of -2.1 ± 1.89. The height of over half of the participants (54%) was categorized as short (SDS > 2.0 SD); in this subset of patients, the mean SDS for height was -3.8 ± 1.5 [42]. Finally, the evaluation of 120 FA patients from the FA Comprehensive Care Clinic of Cincinnati Children's Hospital Medical Center revealed that median height SDS was shorter than expected in children and adults, irrespective of gender. Furthermore, this study presented birth information from 70 patients (59 children and 11 adults) from the cohort: 51% of the children and 3 adults were born small for gestational age; their median birth weight at term was 2.02 kg (range 1.5–2.6 kg) [43]. The intrauterine growth restriction seen in FA patients has also been documented in several case reports in which at-term birth weight ranges from 1,780 to 3,200 gr [44–49]. Even though growth abnormalities have been identified in FA patients with mutations in almost every *FANC* gene, it has been shown that patients with mutations in certain genes have a more severe growth delay. That is the case for patients with biallelic mutations in *FANCD1* [47, 48], as well as those who bear mutations in *FANCC* for whom an SDS for height of -3.84 has been found [41].

Endocrine abnormalities have been proven to be an inherent part of the FA phenotype; up to 80% of patients have one or more endocrine abnormalities. Besides growth, thyroid function and glucose homeostasis are frequently affected [40–43]. Considering the existing evidence for a relationship between low birth weight and an increased risk for noncommunicable adult diseases like diabetes, hypertension, heart disease, dyslipidemia, and osteoporosis [50], it is relevant to revise these diseases in FA. The FA cohorts in whom growth was assessed were also evaluated for endocrine status. Glucose homeostasis is affected in a large portion of FA patients; impaired glucose tolerance has been found in 27–68% of them, whereas diabetes has a prevalence of 8–10%. Dyslipidemia has been assessed in only a small portion of FA patients, but it has been found in 17–55% of the evaluated subjects [51]. There are conflicting results over bone

mineral density (BMD) status in FA; the first time this was evaluated, 92% of the patients were found to have osteopenia or osteoporosis [42]; nevertheless, a study that evaluated a larger FA cohort found that low BMD is not a frequent finding [52]. Further research on this topic has raised the question of whether BMD deficit is intrinsic to the FA phenotype or a consequence of hematopoietic cell transplantation (HCT), an interrogation that is still awaiting to be answered [53]. Cardiovascular adult diseases are not relevant in the FA phenotype; heart disease in these patients is from the congenital type as 13% of patients have cardiac malformations [40], while hypertension has only been reported in two patients as the result of renal malformations [54].

Findings from the different FA cohorts show that short stature is an inherent feature of the FA phenotype, this feature does not respond to a single explanation; the apoptosis prone FA cellular phenotype is certainly a contributing factor for this. The fact that FA patients have a constitutionally defective pathway since conception could explain the clinical phenotype of RCIU. Short stature may also be exacerbated by accompanying endocrinopathies. The cooccurrence of many endocrine alterations has encouraged unifying explanations; some have proposed that endocrinopathies are secondary to increased cytokine activity found in FA cells [41], while others favor the view that some endocrine secretory cells might be damaged by high levels of reactive oxygen species which are known to be elevated in FA patients [43]. In line with the developmental origin of adult health and disease theory, it could also be possible that the increased prevalence of glucose homeostasis alterations and dyslipidemia reported in FA patients is a consequence of intrauterine growth restriction of these patients. Nevertheless, the attempted genotype-phenotype correlation of the endocrine phenotype in FA patients does not seem to support that. Patients with mutations in *FANCA* show a mild endocrine phenotype in which height is not severely affected and insulin resistance is mild, whereas mutations in *FANCC*, which are related to shorter stature, have the least insulin resistance [41]. Moreover, no direct relationship between birth weight and glucose tolerance was identified when it was intentionally looked for [43], although the number of patients in which these observations were made is limited.

7.3. Nijmegen Breakage Syndrome. Nijmegen breakage syndrome (NBS) is an autosomal recessive disease caused by biallelic mutations in *NBN*, a gene that encodes nibrin, a protein involved in DNA repair and cell cycle checkpoint regulation. It participates in the former by sensing double-strand breaks as part of the trimeric complex MRN, alongside MRE11 and RAD50. Meanwhile, for the latter, it contributes to the appropriate activation of ATM and ATR which are central transductors of the DNA damage response (DDR) [55]. The malfunction of nibrin translates in a cellular phenotype marked by chromosomal instability, radiosensitivity, reduced phosphorylation of ATM substrates, and S and G2/M cell cycle defects [56]. Chromosome instability is evidenced by cytogenetic methods in 10–60% of cells in the form of breaks and numeric and structural aberrations: translocations and inversions affecting chromosomes 7 and 14 are found in the majority of NBS patients and are considered a cytogenetic characteristic of this syndrome (Figure 6) [57].

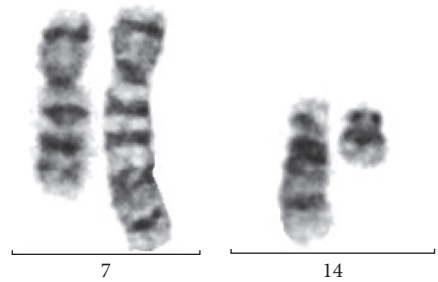
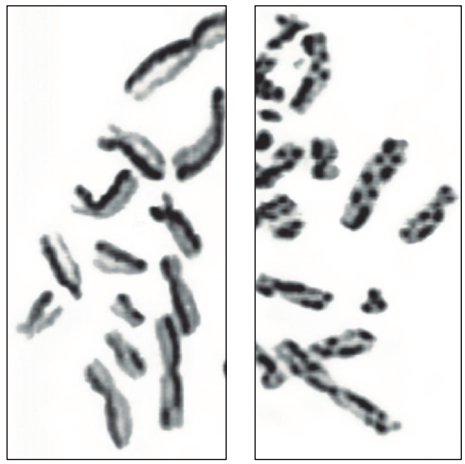


Figure 6: Translocation between chromosomes 7 and 14 is typical in NBS patients' cells.

The clinical impact of these alterations is a phenotype characterized by microcephaly, a distinctive facial appearance consisting of receding forehead and mandible and a prominent mid face with a long nose and philtrum, as well as immunodeficiency that leads to recurrent infections and an increased risk for the development of neoplasia, particularly leukemia and lymphoma. According to the international Nijmegen breakage syndrome study group, growth retardation is also a hallmark of this disease [58], although there is not many details about this feature in the literature.

Most of the information on the natural history of NBS available today comes from patients participating in registries, in which fairly large cohorts of NBS patients are included. There is a large representation of Slavic patients in these cohorts which correlates with the high carrier frequency of a founder mutation of *NBN* in this population. From these studies, it is evident that the most severely affected anthropometric measure in NBS patients is head circumference since all participants display microcephaly, even though only 75% display this feature at birth. When it comes to growth parameters, the same study states that all patients have growth retardation, which is described as proportionate and early occurring [58], although the specific temporality is not defined. A posterior report points out that the growth retardation seen in most NBS patients locates them in the 10th percentile in growth charts and that birth height and weight are usually within normal parameters [59]. Nevertheless, there are other authors who report prenatal and postnatal growth retardation [55, 60], and a tendency for short stature that is more evident during the first year of life [61]. Also, several case reports of NBS patients, in which birth weight is informed, demonstrate prenatal growth restriction [62–67], although this could represent a report bias. The more comprehensive data analysis on growth that includes perinatal information of NBS patients is the one that analyzes data from 67 patients followed for 15 years. Eighty percent of these patients were born at term, the girls were found to have a mean birth weight of 2.7 kg and mean birth height of 51.4 cm, while boys were reported to have a mean birth weight of 2.8 kg and mean birth height of 52.3 cm. Moreover, all patients, irrespective of gender, were found to have a mean height reduced by over 2 SDS at one year old [57]. These data



(a) (b)

Figure 7: Sister chromatid exchange in cells from a healthy individual (a) and from a BLM patient (b).

support the fact that growth retardation in NBS is a prenatal phenomenon.

Nibrin has been found to have essential functions in the regulation of the cell cycle, which translates into an absolute need for this protein for cell proliferation [68]. It would seem logical that mutations of the *NBN* gene that compromise the appropriate function of nibrin would lead to abnormal growth when thought from an organism perspective. Mechanistically speaking, poor cellular growth caused by mutations in a particular gene can be thought of as the main explanation for the growth restricted phenotype, but it has been shown that mutations in other DDR genes that share the cellular phenotype of poor growth, like ATM, do not replicate the compromised growth phenotype at the organism level: growth retardation is absent in ataxia telangiectasia (AT). This data suggests that there are certainly other contributing factors to the abnormal growth phenotype seen in NBS patients [58].

7.4. Bloom Syndrome. Bloom syndrome (BS), is an autosomal recessive disorder caused by homozygous or compound heterozygous mutations in the *BLM* gene (15q26.1) that encodes the RecQL3 helicase. *BLM* has a critical role in the maintenance of genome stability acting at the interface between DNA replication, recombination, and repair [69, 70]. To date, over sixty different mutations, including nonsense and missense, have been identified; all of them result in the inactivation of *BLM* and the consequent loss of its helicase activity. The *BLM* gene encodes a 1,417 amino acid nuclear protein; its expression peaks during the S and G_2/M phases of the cell cycle, which is consistent with its role in DNA replication and recombination. *BLM* specifically unwinds structures like forked DNA duplexes, RNA-DNA heteroduplexes, and R-Loops, which explains its importance during replication fork progression and transcription, besides it is central for Holliday Junctions (HJs) dissolution for HR. The dissolution of HJs by the topoisomerase IIIα-BLM complex cannot be replaced by any other RecQ helicase in the family [69, 70]. It has also been found to have a role in the annealing activity of the ssDNA, the proper sister chromatid segregation during mitosis, and telomere maintenance [70].

BLM can have both pro- and antirecombinogenic functions that are regulated by post/translational modifications, including phosphorylation, sumoylation, and ubiquitination [70].

BLM also enables sister chromatid segregation by processing unresolved replication intermediates that manifest in mitosis as ultrafine DNA bridges (UFBs) and, together with topoisomerase IIIα, RMI1, and RMI2, localizes to UFBs in anaphase. BML deficient cells exhibit and increase frequency of UFBs, suggesting a role of BLM in the resolution of these structures. Failure to resolve these UFBs leads to DNA breakage as mitosis proceeds [70].

Therefore, BS cells are characterized by an increase in chromosomal aberrations, including chromatid gaps and breaks, telomere associations, and quadriradial chromosomes resulting from unsolved recombination between homologous chromosomes. BS cells exhibit increased mutation rates, and the genomic instability includes elevated mitotic HR and unequal sister chromatid exchange (SCEs) (Figure 7). Perhaps the most characteristic feature is the over tenfold increase in SCEs, which results from crossover events during HR repair of damage replication forks [50, 69, 70].

Clinically, the more striking BS feature is IUGR; average birth weight at term is 1,850 g, and birth length is 44 cm. For both boys and girls, weight and length in BS are more than two standard deviations below normal, indicating that growth retardation has a prenatal origin. At postnatal ages, height remains below the normal range and is accompanied by a paucity of subcutaneous fat. Average adult height in males has been found to be 148 cm (130–162 cm) while in females it has been reported to be 139 cm (122–151 cm) [50, 70, 71].

BS patients have overall proportionated short stature but the head is reported to be small and narrow relatively to the body size; it is described as mild microcephaly and is accompanied by malar hypoplasia. Patients have

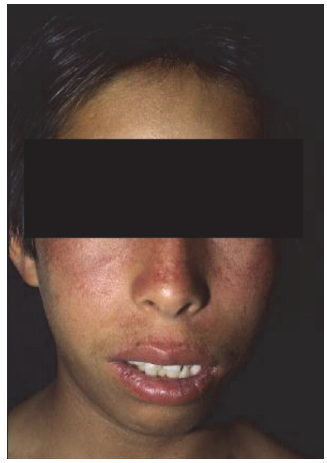


Figure 8: Bloom syndrome patient in whom the characteristic malar erythema is present.

been described to have a high pitched voice [50, 70]. The skin appears normal at birth, but in the first or second year of life, in response to sun exposure, children develop an erythematous rash with a butterfly distribution in the malar area and the back of hands and feet. The rash can include telangiectasia. The severity varies between patients; some can even lose their eyelashes and develop blistering around the mouth. The presence of café-au-lait spots and hypopigmented areas of skin associated with contiguous hyperpigmented areas is common (Figure 8). Intellectual abilities may be limited in some patients and normal in others [50, 70].

Other clinical conditions reported in BS patients are gastroesophageal reflux and diarrhea in infants; mild immunological deficiency with frequent episodes of otitis media; azoospermia or severe oligospermia in males and premature cessation of menstruation in females; minor anatomic defects; and increased risk for neoplasia, which is the main cause of dead in BS [70]. Another common medical condition in BS is diabetes mellitus associated with impaired glucose tolerance and insulin resistance; it has been reported in up to 16% of patients [70]. According to data from the Bloom's Syndrome Registry, diabetes in these patients tends to begin early with a mean age at diagnosis of 26.6 years. It is not associated with ketosis, and it does not have the classical hereditary pattern seen in type 2 diabetes [50, 72].

Short stature in BS is not due to hormonal causes; since the size of cells in persons with BS is normal, it has been hypothesized that there are fewer of them, due to either a decrease in the cell division rate, an increase in apoptosis, or even a combination of both. BS cells have trouble meeting the demands of fast cell divisions encountered in tissues during embryonic development. The leading hypothesis suggests that problems arising during DNA synthesis require a longer S phase to overcome the challenge, which translates into slowing of the cell division rate and an increase in apoptosis [70].

8. Concluding Remarks

This review has focused on discussing phenotypes of CIS in which there is a prenatal alteration of growth. The four syndromes discussed here are rare diseases whose prevalence is difficult to calculate since appropriate identification and diagnosis of affected individuals among the general population are not straightforward. It is important to note that the available information on the natural history of FA, NBS, and BS comes from the analysis of several patients that participate in registries. This strategy results in detailed information that permits a better analysis of the clinical phenotype of these rare diseases. Meanwhile, available data of SS is more anecdotic which results in a nonsystematic gathering of information, which is thus frequently incomplete.

From the available clinical data of these CIS, it is evident that growth is not affected in the same extent for all of these syndromes; the phenotypes that exhibit a more severely stagnated perinatal growth are BS and SS. The severe growth delay reported in these patients has been described as a pathognomonic sign of these diagnoses. In patients with FA, perinatal growth can also be severely affected, but not every FA patient shows this manifestation. Finally, NBS has been described to have growth retardation of prenatal origin of mild severity, almost within the lower normal range (Figure 9). The cephalic circumference can be affected in all of these syndromes; severe microcephaly is pathognomonic in SS and NBS, it is described as mild for BS patients and it is found in less than half of FA patients (Table 2).

When trying to understand why a subset of CIS shows prenatal growth retardation, it could be alleged that the intrinsic chromosomal instability is responsible for it. But this would not explain why prenatal growth retardation is not a universal CIS feature; for example, ataxia telangiectasia patients who have biallelic mutations in the ATM kinase gene do not show prenatal growth delay. It strikes that a molecular common feature shared by the prenatal growth retardation SIC phenotypes is that they are caused by mutations in genes that are necessary for an appropriate response to DSBs during the S phase of the cell cycle. The molecular defect in SS affects the functioning of ATR, the preferred DDR transducing kinase used by highly replicating ESCs. The FA/BRCA pathway, which is affected in patients with FA, detects ICLs upon replication fork arrest. Nibrin, the protein affected in patients with NBS, is induced during S phase for the detection of DSBs. And the BS helicase forms a FANCM-BLM complex needed to sense ICLs during S phase [73]. It could then be argued that altered DNA replication is an important factor for the development of intrauterine growth restriction in these CIS. Nevertheless, it must be kept in mind that the inherent chromosome instability found in these syndromes may also be affecting normal growth in the patients [27, 43, 57, 60, 71].

Being small for gestational age has been associated with a higher risk of noncommunicable adult diseases like glucose homeostasis disorders, dyslipidemia, hypertension, and others. It has been proposed that fetal reprogramming to enhance fetal survival, despite the risk for adult onset diseases, is an outcome that does not take into account

Table 2: Growth features of chromosomal instability syndromes.

Syndrome "Loci"	At-term birth weight/height	Microcephaly	Adult height	Adult onset diseases
Seckel Syndrome "*ATR* 3q23"	Birth weight below −3 SD (2,055 g)	Severe −4 SD	Postnatal growth retardation (below −5 SD)	Not reported
Fanconi anemia "21 *FANC* genes"	Median weight: 2.02 kg (1.5–2.6 kg)	In 20–50%	50–60% of patients with median height < −1.8 SDS Women: −3.4 SDS Men: −4.4 SDS	Cancer Diabetes mellitus Dyslipidemia Altered bone mineral density
Nijmegen Breakage Syndrome "*NBN* 8q21"	Mean weight/height girls 2.7 kg/51.4 cm Mean weight/height boys 2.8 kg/52.3 cm	100%	Mean height for women −1.8 SDS Mean height for men −2.3	Cancer
Bloom Syndrome "*BLM* 15q26.1"	Mean weight 1.89 kg ± 0.35 kg for boys Mean weight 1.87 kg ± 0.35 kg for girls Mean height 43.4 cm ± 4.4 cm for boys Mean height 43.8 cm ± 2.8 cm for girls	Mild microcephaly	Mean height 145.5 cm ± 7.6 cm males Mean height 141.5 cm ± 6.1 cm for females	Cancer Diabetes mellitus

See [27, 33, 40, 43, 50, 57, 69].

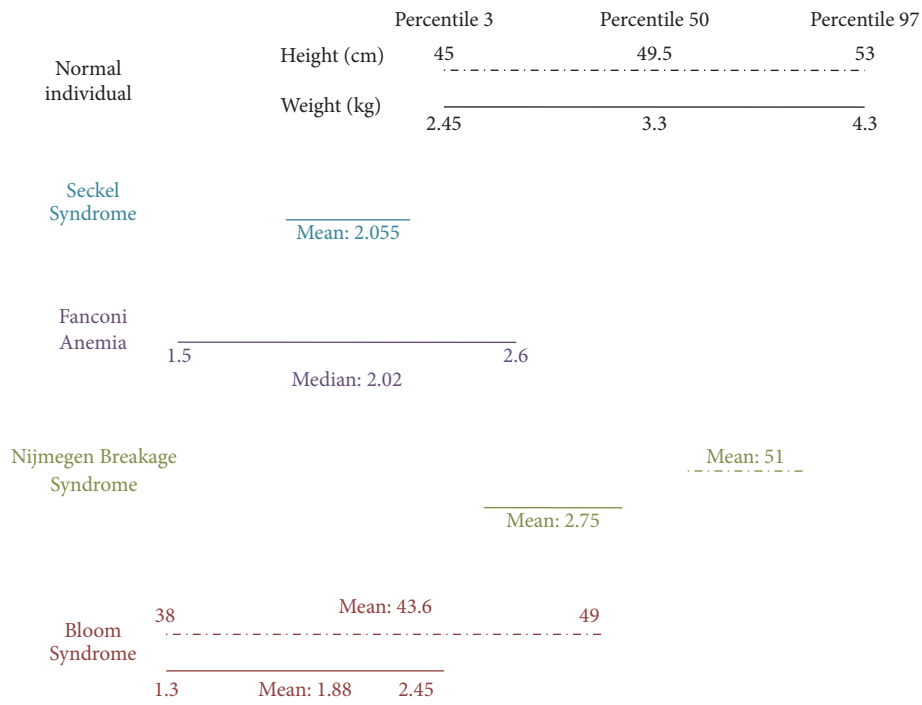


Figure 9: Anthropometric features at birth of chromosome instability syndromes with perinatal growth retardation. Comparison of the available growth data from SS, FA, NBS, and BS syndrome against birth data from the WHO's growth standards for infants. Although mean height and weight values of NBS patients are found to be within normal percentiles according to WHO standards; there are several authors who refer to prenatal growth restriction [55, 63, 65, 79].

the reason for the prenatal growth restraint [50, 72, 74]. It must be kept in mind that noncommunicable diseases are multifactorial entities in which genetic and environmental factors converge, and their cause cannot be attributed to a single explanation. However, when growth restriction is a key feature in syndromes with chromosome instability, the possibility that cell hypoplasia due to DNA repair malfunction is contributing to the growth restriction phenotype must be contemplated. Moreover, since none of these adult onset diseases is reported as an inherent element of the SS and NBS phenotypes, the direct connection between low birth weight and adult noncommunicable diseases is less of a straight

shot. Furthermore, there are other Mendelian syndromes with mutations in DNA repair genes that characteristically show adult noncommunicable diseases but do not have IUGR; such is the case for Werner syndrome, a progeroid disease caused by biallelic mutations in another RecQ helicase (RecQ4) that clinically presents with postnatal short stature and a prevalence of type 2 diabetes mellitus of 70% [50, 70, 74].

Natural history of FA, NBS, and BS documented through the existing patient registries has allowed the identification of noncommunicable diseases to be a part of the clinical spectrum of these syndromes (Table 2). Meanwhile, from the information available through SS case reports, no adult onset disease has been found to be part of this phenotype. To the best of our knowledge, the direct investigation of nonevident endocrinologic alterations, like glucose homeostasis or dyslipidemia, has not been directly made in SS patients. For a rare disease like SS, such a study can only be done if a fairly large group of patients, like the one found in registries, is available. If such a study was to be performed, hidden features in SS patients could be unraveled.

Oxidative stress sensibility is a cellular feature shared by all the SIC syndromes with IUGR that could contribute to the explanation of the premature development of noncommunicable adult diseases in these patients. Reports have shown that BS and NBS cells have endogenous reactive oxygen species (ROS) overproduction and an impaired mitochondrial homeostasis [75]; it is also well known that FA cells show increased levels of oxidative damage [38, 76], and there is evidence that oxidative stress can activate ATR-mediated checkpoint signaling [77]. Since this kind of damage has soundly been related to age-associated diseases that result from tissue degeneration, exacerbated oxidative damage found in these syndromes could result in cellular aging that manifests as overall premature aging with particular symptoms like glucose homeostasis alterations [76, 77].

Fetal growth restriction is a multifactorial condition where wide arrays of factors converge. Fetal aspects, in particular those affecting the genetic constitution of the fetus, have been recognized to play an important role. It has been demonstrated that chromosomal abnormalities and monogenic syndromes caused by mutations in genes that participate in growth or metabolic pathways directly affect fetal growth [78]. Even though they are rare entities, CIS appear as another group of monogenic pathologies that broaden the list of genetic fetal factors of fetal growth restriction.

Consent

The necessary consent for the publication of the photographs was obtained.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

The three authors contributed equally and wrote the first draft of the paper. The final version was approved by everyone.

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Research Article

A Psychological Perspective on Preterm Children: The Influence of Contextual Factors on Quality of Family Interactions

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Preterm birth has a critical influence on interactive, communicative, and expressive child behaviour, particularly during the first years of life. Few studies have stressed the assessment of mother-father-child interaction in families with preterm children, generating contradictory results. The present study wished to develop these fields: (i) comparing the quality of family interactions between families with preterm children and families with children born at full term; (ii) observing the development of family interactions after six months in the families with children born preterm; (iii) assessing family and contextual factors, as parental stress and social support, in parents of preterm children in order to observe their influence on the quality of family interactions. 78 families are recruited: 39 families with preterm children (M = 19,8 months, SD = 11,05) and 39 families with full-term children (M = 19,66 months; SD = 13,10). Results show that families with preterm children display a low quality of mother-father-child interactions. After six months, family interactions result is generally stable, except for some LTP-scales reflecting a hard adjustment of parenting style to the evolution of the child. In families with preterm children, the parenting stress seemed to be correlated with the quality of mother-father-child interactions.

1. Introduction

Every year, in the world, an estimated 15 million babies are born preterm and prematurity is considered the leading cause of neonatal mortality and the second cause of death before 5 years of age [1]. Among them, about 5% of these children are born before 28 weeks, 15% between 28 and 31 weeks, and about 20% between 32 and 33; finally, between 60 and 70% of them are born between 34 and 36 weeks' gestation [2]. Across all Europe the survival of preterm children, born before 37 weeks of gestational age (GA), has recently increased, reaching an incidence of 7–10% [3]. In Italy, the prevalence rate of premature birth is about 6,5% [4].

Regarding the children's behavioural and emotional development, preterm infants show weak relational, emotional, and social skills and difficulties in self-regulation already in the early stages of their development [5]. Specifically, most studies show the presence of higher mean scores on socioemotional scales in preterm children, than their peers born at term, even not reaching clinical cut-off [6].

Several studies that have explored the development of preterm child have shown that poor social/interactive skills, poor behavioural and emotional self-regulation, emotional difficulties, and reduced attention are the most common behaviour problems in preterm infants and children [5].

Currently, literature is also focusing on parental distress. Parents undergo great suffering and concern regarding child's health, describing a stressful and emotional experience related to parenting. Specifically, some researchers were focused on parenting stress, assessed with the Parenting Stress Index-Short Form [7]. In Gray and colleagues [8] study with mothers of children aged 12 months (corrected age)

those whose offspring were born preterm experienced twice as much stress as the mothers of those born at term. The main difference emerged on the parent-infant dysfunctional interaction scale assessed with the Parent-Child Early Relational Assessment [9, 10]. This result suggests that, in the first year of child's life, mothers of preterm babies had more difficulty connecting with their child, with respect to the mothers of at-term children [8]. Considering the parental stress characteristics of parents of preterm children, some studies have noticed the level of perceived social support, as it could be a protective factor for both parents' well-being and mental health since it could be able to reduce parents' stress [11–13]. Actually, the means of perceived social support of "preterm parents" are 5 points higher than the ones perceived from "at-term parents," underscoring its importance in the case of high level of stress [12]. Singer and colleagues [13] also found that the extremely stressful conditions relating to preterm birth do not reduce the adults' perception of their parenting competencies.

Regarding the contribution of the child to the interactions, preterm children are seen as being more passive [14–16], less attentive, and less concentrated and responsive [16–20]. Preterm children appeared to be less inclined to make eye contact with their primary caregiver [21–23] and may be less vocal [20, 24] or more vocal [25] but with less contingency [18]. With respect to children born at term, the preterm ones have less well-developed autoregulatory competencies [26] and little smiles [27] and are mostly described by the expression of more negative emotions [16, 21, 28, 29]. Finally, the preterm children also find it more difficult to give clear clues to caregivers [30, 31].

Concerning maternal interactive style, studies find that the mothers of preterm children are more directive, active, and controlling at 3 months [14, 17, 24, 25] and tend to be less sensitive [14, 24], using a directive scaffolding [32, 33] with a contradictory style, which alternates between passive and overstimulating moments [34]. In summary, many researchers have identified a specific interactive style in the preterm mother-child dyad, in which the child seems to be more passive in his/her interaction with the mother. Some authors attribute this characteristic to a maternal intrusiveness, while others hold that it is related to the maternal reactivity to compensate the child's developmental inadequacy [23, 35].

Regarding affection, while some studies that involved a group of heterogeneous preterm children found no differences [26, 28] others found that mothers of children born extremely preterm mainly express neutral emotions [36]. Some authors found that some characteristics of interaction with preterm children become progressively richer after the first six months of child's life when the environment becomes more complex and demanding [19, 28, 32, 37]. In this regard, a research of Feldman and Eidelman [30] has highlighted that mother's postpartum interactive style predicts both maternal and paternal interactive synchrony with their child during his development.

One of the critical factors of these researchers is that these studies have generated ambiguities due to the way to assess the adult-child interactions and to the heterogeneity of the samples considered [35, 38, 39]. In this lack of knowledge, only a few studies have addressed the assessment of triadic interactions in families with preterm children [36, 40, 41]. In a recent study on the individual, dyadic, and triadic influences on the development of the family system, Feldman [42] found that the infant-risk families (composed of families with preterm children and families with children affected by intrauterine growth restriction) displayed the lowest cohesion and highest rigidity, compared to four group of families: controls and three mother-risk groups (depressed, anxious, and comorbid). Only a recent study used the Lausanne Trilogue Play [43], an observational method also used in the research described in this paper. Only a few variables (regarding affect sharing, timing/synchronization, and child behaviour) were used to observe the quality of family interactions in 83 families with 6-month-old healthy children born between the 28th and the 34th week of gestation, and no differences emerged from the comparison with the control group. This study was the first to use the LTP approach to assess this construct.

In this line, our previous study [44] has attempted to compare the quality of family interactions in a group of families with preterm children and a group of families with children born at full term, exploring differences and similarities. Results show differences in the quality of family interactions emerged between the preterm and at-term children groups. The preterm group showed a significantly lower quality of family interactions than the at-term group. Another aim of this prior research was to consider the associations between the quality of family interactions and contextual factors, as parental empowerment, child's temperament, parental stress, and perceived social support. The parental stress of both parents related to their parental empowerment and maternal stress was related, also, to the partner's parental empowerment. Social support had a positive influence on parental stress, with maternal stress also related to the perceived social support from the partner, which underscores the protective role of the father on the dyad [44].

This paper aims to develop our previous study that contributed to literature regarding family interaction in families with preterm children. The first aim of the present study is to increase the previous sample with a higher size of families with preterm children, comparing the results with those obtained in a group of families with at-term children. We expected to confirm the results of our previous study, which is to find a significant difference between the two observed groups.

Secondly, we aim to observe the evolution of family interactions after six months, retesting a small group of families who have accepted to return for a follow-up.

Finally, we aim to observe if contextual and family variables, as perceived parental stress and social support are influencing factors on the quality of the family interactions. In detail, we expect to observe a significant influence of these aspects on the couple's degree of supportive cooperation during the interactions with the child and on their parental competence to interact with him, eliciting his involvement in the joined activity.

Table 1: Preterm group description.

	Preterm full group		Preterm		Very preterm		Extremely preterm	
	μ	σ	μ	σ	μ	σ	μ	σ
Chronological age (months)	19.8	11.5	19.2	10.6	24.9	12.8	22.6	11.8
Corrected age (months)	19.5	11.3	18.2	10.5	22.5	12.7	19.5	11.3
Birth weight (g)	1294	602.1	1896	599	1121.7	227.65	818	246.19
Gestational age (weeks)	29.66	3.31	33.15	1.14	29.93	1.49	25.58	1.16
	N (%)		N (%)		N (%)		N (%)	
Disability	9 (23%)		3 (23%)		2 (14%)		4 (33%)	
Twins	9 (23%)		2 (15%)		4 (28,5%)		3 (12%)	
Male	24 (61,5%)		7 (53,8%)		8 (57,1%)		9 (75%)	
Female	15 (38,5%)		6 (46,2%)		6 (42,9%)		3 (25%)	
Preterm (P)	13 (33,3%)							
Very preterm (VP)	14 (35,9%)							
Extremely preterm (EP)	12 (30,8%)							

Table 2: Preterm group description at follow-up.

	Preterm full group		P		VP		EP	
	μ	σ	μ	σ	μ	σ	μ	σ
Chronological age (months)	23.7	12.1	24.5	13.09	28	16.49	18.25	2.98
Corrected age (months)	21.5	12.1	22.83	12.71	25.75	16.7	15.12	2.7
Birth weight (g)	1463.43	777.01	2135	707.7	1079	252.69	840	317
Gestational age (weeks)	30.57	3.08	33.1	1.47	30.75	1.35	26.5	0.57
	N (%)		N (%)		N (%)		N (%)	
Disability	5 (35,7%)		1 (16%)		1 (25%)		3 (75%)	
Twins	3 (21,4%)		0		3 (75%)		0	
Male	8 (57,1%)		4 (66,6%)		1 (25%)		3 (75%)	
Female	6 (42,9%)		2 (33,4%)		3 (75%)		1 (25%)	
Preterm (P)	4 (28,55%)							
Very preterm (VP)	4 (28,55%)							
Extremely preterm (EP)	6 (42,9%)							

2. Methods

2.1. Participants and Procedures. 78 children and their families are recruited for this research project. The preterm group included 39 families and their children (M = 19,8 months, SD = 11,05). Families are recruited from two different Italian organizations that offer support and intervention for preterm children and their families: a private Onlus Association "Il Pulcino" (with a recruitment of 31 children) and the Neurorehabilitation Service part of the Children, Adolescents and Families Unit of the Public Health Service ULSS6 in Padua (with a recruitment of 8 children).

Families attending the Neurorehabilitation Service are recruited by a child neuropsychiatrist, who explained the purpose of the research. Families who are part of the "Il Pulcino" Onlus Association are recruited by the professionals of the association, who explained the purpose of the study and, depending on the families' consent, placed them in contact with the responsible research project. All the parents taking part in the project gave and signed their informed consent to the study, approved by the Ethical Committee (CEP 204 SC). Table 1 shows the characteristics of the preterm children and families group.

Mothers have a mean of 38,13 years of age (SD = 4,16) and fathers of 41,4 years (SD = 5,37). The 51,3% of mothers and the 46,2% of fathers have achieved a secondary school degree.

14 families, among those, have been invited to participate in a follow-up section, after six months. In this subgroup children have a mean of 21,5 months of correct age (SD = 12,1); 8 of them are male and 6 are female. Table 2 shows the characteristics of this subgroup.

The control group employed in this study is part of a longitudinal study regarding the development of family interactions [45]. This project involved a hundred of couples who spontaneously conceived their first child, who were followed up from the 7th month of pregnancy until their child was 48 months old. A group of 39 children (M = 19,66 months; SD = 13,10) and their families was drawn from this sample, to match the preterm group in terms of the child's age and gender and the parents' ages. The parents have a mean of 36,74 years of age (SD = 3,85).

The Lausanne Trilogue Play Procedure [43] is administered to both groups of families to assess the quality of their family interactions. The following questionnaires are also administered to the group with preterm children: the Parenting Stress Index-Short Form [7] and the Multidimensional Scale of Perceived Social Support [46]. The observational procedure and the questionnaires are administered at the Childhood, Adolescence and Family Unit (ULSS6 of Padua) to all the families of the preterm group.

3. Materials

Lausanne Trilogue Play [43] is a semistandardized observation situation designed to assess the quality of family interactions. The administration involved the mother-father-child triad–invited to cooperate and work together in order to conduct an activity. The proposed activity is a play session or the planning of a birthday party or a family trip, in connection with child's age. Detailed instructions invited the family to organize the activity, as they usually do at home, just following four rules which reflect these four relational configurations: (I) at first, just one parent interacted with his child, while the other one stayed simply present; (II) then, parents reverse the roles, so that the one who was simply present became the active partner, and vice versa; (III) parents and child play all together; (IV) parents interacted while the child stay simply present. The session was videotaped and later scored using the Family Alliance Assessment Scale (FAAS) 6.3 [47] composed of 15 observational variables (*the variables are grouped into macrocategories: participation (postures and gazes, inclusion of partners), organization (role implication, structure), focalization (parental scaffolding, coconstruction), affect sharing (family warmth, validation, and authenticity), timing/synchronization (interactive mistakes during activities, interactive mistakes during transitions), coparenting (support, conflicts), and infant (involvement, self-regulation)*[48, pp. 24]).

Parenting Stress Index-Short Form (PSI-SF [7]) is a self-report questionnaire that aims to identify stressful parent-child relational systems at risk of leading to dysfunctional behaviour on the part of the parent or the child. The short form (the only one validated in Italy) comprises 36 items scored on a Likert scale from 1 (strongly agree) to 5 (strongly disagree). The items are divided into three scales: (1) parental stress that assesses parent's feelings of being trapped in the parenting role; (2) parent-child dysfunctional interaction that measures the nature of the interaction between parent and child; (3) difficult child that assesses parents' perceptions of their children. Scores above the 85th percentile on the total stress scale are considered borderline clinically significant [49].

Multidimensional Scale of Perceived Social Support (MSPSS [46], Italian version [50]) is a brief self-report scale composed of 12 items that measure three areas: perceived social support from family, from friends, and from significant others. Answers can be scored on a Likert scale from 1 (strongly agree) to 7 (strongly disagree). The instrument has no cut-off score. The score range is from 84 (maximum) to 12 (minimum).

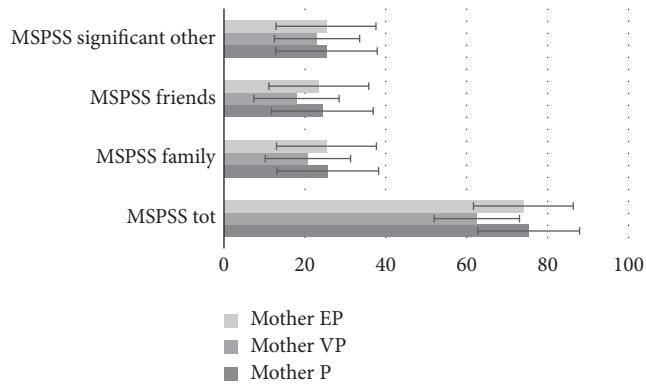

Figure 1: Means of MSPSS scores of each preterm subgroups. * *Note*. MSPSS: Multidimensional Scale of Perceived Social Support; EP: extremely preterm; VP: very preterm; P: preterm.

4. Results

4.1. Preliminary Analysis. As shown in Table 1, the group of preterm children is composed of children with different gestational age, and it includes children with disabilities. A one-way ANOVA has been carried out in order to detect differences, due to the degree of prematurity (gestational age) and to the presence of child disability, in the different variables investigated by the applied tools (parental stress, social support, and quality of family interactions). The ANOVA, confirmed by Bonferroni's post hoc test, does not underline differences between groups about parental stress (PSI) for both degrees of prematurity (mothers total stress: $F(2,36) = 1.598$; $p = 0.216$; fathers total stress: $F(2,36) = 2.392$; $p = 0.106$) and the presence of child disability (mother total stress: $F(1,37) = 2.280$; $p = 0.140$; father total stress: $F(1,37) = 2.077$; $p = 0.158$).

Some significant differences linked to the degree of prematurity emerge for mothers, on the variables of the Multidimensional Scale of Perceived Social Support (Figure 1). There are statistically significant differences in the perceived support total score, $F(2,30) = 8.151$, $p = 0.001$, family support, $F(2,30) = 3.99$, $p = 0.029$, and friends support, $F(2,30) = 9.54$, $p = 0.01$. Bonferroni's post hoc test shows that mothers of very preterm children perceived low social support compared to mothers of low preterm children; these differences have been detected in the total score ($p = 0.002$) and in the family support scale ($p = 0.045$) and in the friends support scale ($p = 0.01$). No difference was observed, instead, for child disability, $F(1,31) = .808$ and $p = 0.376$.

Once more, no differences emerge for fathers in the perceived support total score for both children's groups based on the degrees of prematurity, $F(1,30) = 1.679$ and $p = 0.204$, and on the presence of child disability: $F(1,31) = 2.307$; $p = 0.139$.

Regarding the quality of family interactions, no differences linked to the degree of prematurity emerged from the ANOVA in the LTP total score $F(2,36) = 3.023$; $p = 0.061$; and furthermore none linked to the presence of child disability $F(1,37) = 0.009$; $p = 0.926$. As a result of these preliminary analyses, the preterm group was judged to be homogenous.

Table 3: T-test between preterm group and control group.

	Preterm ($N = 39$)		At term ($N = 39$)		t	p
	μ	σ	μ	σ		
Part I	21.66	4.187	25.37	2.432	−4.724	0.000
Part II	22.42	3.492	25.58	2.585	−4.480	0.000
Part III	22.74	4.105	25.08	2.235	−3.089	0.003
Part IV	19.92	4.109	23.61	3.476	−4.220	0.000
LTP total score	86.71	10.905	99.63	7.684	−5.971	0.000

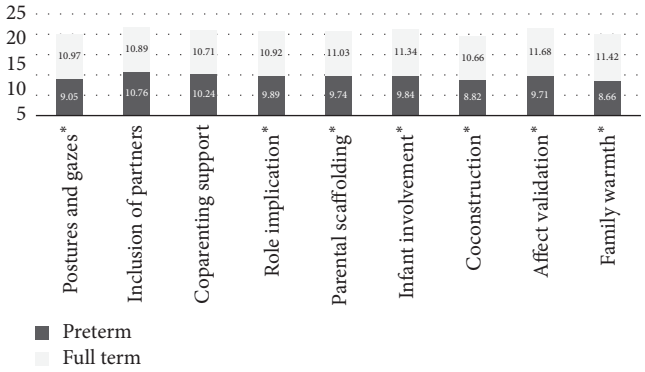

Figure 2: Means of LTP variable scores of each studied group. $^{*}p < 0.05$.

4.2. Family Interactions. Our first aim was to compare the quality of family interactions in two groups of families: families with preterm children and families with children born at full term.

Having confirmed the homogeneity of the preterm group (degree of prematurity and child disabilities), a t-test was run to compare the quality of the family interactions between the preterm group and the full-term group, whose results are given in Table 3. Figure 2 shows the means of the compared LTP variables (LTP of the control group has been coded with the FAAS 4.0 that has 10 variables; 9 of them match with 9 variables of the FAAS 6.3 coding system; the comparison has been done on these nine variables and on the sum of the LTP parts); between them, only two variables do not have different results: the variable inclusion of the partner, $t(74) = -.400$ and $p = 0.690$, and coparenting support, $t(74) = -1.393$ and $p = 0.168$.

4.3. Development of Family Interactions. The second aim of the present study was to observe the development of family interactions in the group of families with preterm children. Six months after the first observation, 14 families have participated in a follow-up session. Wilcoxon test shows a significant change in four of the LTP variables; looking at the means, three of them show a significant decrease: scaffolding ($Z = -2.326$; $p = 0.020$), interactive mistakes during the transitions ($Z = -2.473$; $p = 0.013$), and authenticity ($Z = -2.38$; $p = 0.017$). A significant increase is observed in the means of the scoring of the family warmth variable ($Z = -2.335$; $p = 0.020$).

4.4. Factors of Influence. The third aim of the present study was to observe if contextual and family variables, as perceived parental stress and social support, are influencing factors on the quality of the family interactions. Before verifying the association, we wondered if mothers and fathers PSI scores correlated between them and if they reported different levels of parental stress.

Table 4 shows the correlations between mother and father PSI scores.

A Paired-Sample T-test between mother's and father's parental stress total score confirms that no significant difference has been found among them: $t(38) = -.620$, $p = 0.539$. Also regarding the perception of social support, mother's and father's scores show a significant correlation ($r = .511$; $p = 0.002$). Once again, a Paired-Sample T-test between mother's and father's perceived support total score affirms no difference between them: $t(32) = -.598$, $p = 0.554$.

Following these results, we have performed Pearson's correlations between PSI, MSPSS, and LTP. Table 5 shows the significant results.

No significant results emerge from the perceived social support scales and family interactions.

5. Discussion

Triadic interactive dynamics in families with preterm children have been very little investigated. A small body of literature has not shown significant differences in the comparison with families with children born at full term [51]. Our study fits in this direction, trying to understand the quality of the family interactions and their evolution in the time. From the comparison it has emerged that the group of families with at-term children shows a great quality of triadic interactions, as is deduced by more elevated scores in almost all the LTP variables. The only variables where differences are not revealed are "inclusion" and "coparenting support"; these two variables underline a good mutual support in the parental couple. According to a recent study of Adama and colleagues [52], couples that have faced a premature delivery perceive the partner's support as more significant. In our study, apart from the positive correlations observed, also between mothers and fathers significant agreement emerges regarding the perception of social support and parental stress and from the absence of differences among their PSI scores. The specific differences encountered in the Lausanne Trilogue Play scales, between preterm group and control group, show specific difficulties in the triadic interactions in the preterm sample. Due

Table 4: Pearson's correlations between mother and father's PSI (Parental Stress Index) scores.

		PSI father PS	PSI father P-CDI	PSI father DC	PSI father stress tot
PSI mother PS	Pearson's correlation	.491**	.272	.407*	.455**
	Sig. (2-tailed)	.001	.094	.010	.004
	N	39	39	39	39
PSI mother P-CDI	Pearson's correlation	.485**	.381*	.320*	.452**
	Sig. (2-tailed)	.002	.017	.047	.004
	N	39	39	39	39
PSI mother DC	Pearson's correlation	.481**	.363*	.576**	.540**
	Sig. (2-tailed)	.002	.023	.000	.000
	N	39	39	39	39
PSI mother stress tot	Pearson's correlation	.546**	.374*	.488**	.540**
	Sig. (2-tailed)	.000	.019	.002	.000
	N	39	39	39	39

* *Note*. PS: parental stress, CDI: parent-child dysfunctional interaction; DC: difficult child. * $p < 0.05$; ** $p < 0.01$.

Table 5: Pearson's correlation between PSI and LTP scores.

		PSI mother P-CDI	PSI mother DC	PSI mother stress tot	PSI father P-CDI	PSI father DC	PSI father stress tot
LTP inclusion of partners	Pearson's correlation	−.358*	−.089	−.271	−.159	−.109	−.132
	Sig. (2-tailed)	.025	.590	.095	.333	.511	.423
	N	39	39	39	39	39	39
LTP role implication	Pearson's correlation	.061	.160	.100	.327*	.253	.326*
	Sig. (2-tailed)	.713	.329	.545	.042	.120	.043
	N	39	39	39	39	39	39
LTP coconstruction	Pearson's correlation	−.382*	−.097	−.238	.085	.148	.043
	Sig. (2-tailed)	.016	.557	.145	.605	.369	.793
	N	39	39	39	39	39	39
LTP support	Pearson's correlation	−.328*	−.430*	−.386*	.012	−.168	−.117
	Sig. (2-tailed)	.041	.006	.015	.943	.308	.478
	N	39	39	39	39	39	39
LTP self-regulation	Pearson's correlation	−.072	−.042	−.053	−.373*	−.249	−.299
	Sig. (2-tailed)	.664	.798	.750	.019	.126	.064
	N	39	39	39	39	39	39
LTP interactive mistakes during activities	Pearson's correlation	−.252	−.273	−.281	.143	.102	.046
	Sig. (2-tailed)	.121	.093	.083	.386	.535	.782
	N	39	39	39	39	39	39
LTP interactive mistakes during transitions	Pearson's correlation	−.015	−.053	.084	−.239	−.324*	−.207
	Sig. (2-tailed)	.928	.747	.610	.142	.044	.207
	N	39	39	39	39	39	39

* $p < 0.05$.

to the mentioned risk factors involving parents and preterm children, several studies have focused on parent-child interactions with the aim of investigating the general quality of the early adult-child relationship and development. Globally, data show that prematurity has a negative influence on interactive, communicative, and expressive levels of mother-child interaction during the first years of life [35, 38]. Our results confirm these pieces of evidence also at the triadic level (mother-father-child interactions).

Regarding the development of the family interactions, they generally have stable results after six months which are considered, even if we notice some significant variation: the triadic family interactions seem in fact to worsen after six months in the dimensions of scaffolding, authenticity, and

interactive errors, while they are improving in the dimension of the family warmth.

Preterm children seem to demonstrate, in the first years of life, a smaller level of vigilance, attention, activity, and responsivity compared to children born at full term [27]; this difference seems to be reduced with their growth [16].

Similarly, even if parent behaviour shows an elevated stability, according to the literature it seems to become mainly intrusive [16]. The mean age of our sample at the time of the first observation was of 21,5 months and of 28,2 months in the follow-up.

During the second observation, children of preterm sample have participated more actively in the game, as observed by the scores obtained in the variable child's involvement. This might contribute to mainly activating some interactive errors and make the adaptation harder for the parents to the real abilities of their child through scaffolding. It seems therefore that parents fail to evolve, in this range of time, their ability to involve their child in an interactive exchange, probably anchored to a vision of a child as always small, immature, and not very active, as observed in the literature [53].

The atypical growth that characterizes the development of premature children could explain the difficulty of parents to adapt positively to the child's acquisitions. In fact, after birth, premature babies undergo to rapid "catch-up" [54], rapid and sudden acquisitions of skills that may not allow the parent to adapt in a functional way. At the same time, however, the greater involvement of the child in the gameplay is also associated with greater family warmth, as if it would be easier for the parents to catch the affective states of the child and respond appropriately. Some studies found that parents of preterm infants are sensitive and responsive in the interaction [22, 28, 55] but they tend to express responsiveness verbally more than in their facial expressions [25]. They use social monitoring and eye contact [39] and positive affection expressed verbally and nonverbally [26], although birth weight influences the intrusiveness of mothers [39].

The lower authenticity could be influenced by the repetition of the second observational procedure.

Another aim of our study was to observe the influence of parental stress and perception of social support experienced by these parents, on the quality of family interaction. In our preterm group the mean of the parental stress total score does not exceed the clinical cut-off; but, in agreement with the literature [56], it seemed to be higher. Moreover, literature also means that mothers reported greater stress than fathers did, and these differences remained remarkably stable over time [57]. This gender difference was not confirmed in our study and it brings hypothesis that these children are exposed, consistently, to the distressed parental cares. This relationship could affect child symptomatology [58].

Perceived stress is associated with different variables of the Lausanne Trilogue Play and we can see that the subscales of maternal stress are mainly associated with components of the triadic interactions concerning parental and family interaction (support and cooperation, partner inclusion, and coconstruction), while the paternal stress is more associated with aspects of interactions related to the child (self-regulation). In this line, Olafsen and colleagues [59] reached an association between parenting stress and negative reactive temperament in the child, at one year of child's life.

These are negative type correlations, thus when perceived stress increases, the LTP-scale score decreases. Paternal stress correlates positively with some of the LTP-scales, especially with the ability to solve interactive errors and to respect roles. This result could be explained by the tendency of fathers in paying more attention, during the interaction, to the game structure rather than to the relationship. Perception of less support may be associated with the condition of bigger vulnerability that children born at a lower gestational age can have, which often leads parents to limit contact with the outside world, and this could lead, in the first period, to an experience of social isolation [60].

6. Conclusions

The research presented expands, confirms, and is in continuity with the findings of the previous studies, highlighting the presence of some limits in triadic family interactions in families with preterm children. The families involved, in fact, obtain lower scores than the normative sample in seven of the nine compared LTP-scales. Therefore, such families show a good level of parenting support and cooperation and parental alliance, a strength of this group. They also show a level equal to the regulatory one in creating a good interactive environment through bodily signals.

After six months, this group of families seems to show a certain stability in the triadic interactions excluding the dimensions of scaffolding, authenticity, and solution of interactive errors in which they worsen, reflecting a hard adjustment of parenting style to the evolution of the child and therefore, generally, a difficult triadic adaptation.

This finding draws attention to family development in cases of prematurity of the child, to support the parental couple and the child along with its growth. It can occur that parents might cling to an idea of a child being immature and fragile, without being ready to change some interactive patterns in relation to growth.

Although compared to the two previous studies the sample has increased considerably, its sample size is not yet sufficient to generalize the results. It is, therefore, necessary to continue to involve new families in the different observations and above all to carry on a follow-up, in order to generalize the data and get more accurate information on the specificity of these families.

In order to offer them a support adjusted to their needs, current resources and the difficulties are listed.

This study opens several future prospects by observing its results; among these, there is the need to deepen the differences in triadic interactions and constructs analysed according to the degree of prematurity, particularly in the group of families with very preterm children and disability. It would also be interesting to continue medium and long-term observations, to investigate how the triadic interactions

evolve over time, taking in count the second childhood as well. This kind of longitudinal design could underline the importance of adopting an intervention-research approach [61].

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Review Article

Echocardiographic Techniques of Deformation Imaging in the Evaluation of Maternal Cardiovascular System in Patients with Complicated Pregnancies

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Cardiovascular diseases (CVD) represent the leading cause of maternal mortality and morbidity. Knowledge of CVD in women is constantly evolving and data are emerging that female-specific risk factors as complications of pregnancy are conditions associated with an increased risk for the long-term development of CVD. Echocardiography is a safe and effective imaging technique indicated in symptomatic or asymptomatic pregnant women with congenital heart diseases who require close monitoring of cardiac function. Deformation imaging is an echocardiographic technique used to assess myocardial function by measuring the actual deformation of the myocardium through the cardiac cycle. Speckle-tracking echocardiography (STE) is a two-dimensional (2D) technique which has been found to be more accurate than tissue Doppler to assess both left ventricular (LV) and right ventricular (RV) myocardial function. The use of 2D STE however might present some technical issues due to the tomographic nature of the technique and the motion in the three-dimensional space of the myocardial speckles. This has promoted the use of 3D STE to track the motion of the speckles in the 3D space. This review will focus on the clinical value of the new echocardiographic techniques of deformation imaging used to assess the maternal cardiovascular system in complicated pregnancies.

1. Epidemiology

Cardiovascular disease (CVD) remains the leading cause of maternal mortality, and maternal cardiac diseases are a major cause of nonobstetric morbidity, complicating 0.2 to 4% of pregnancies in industrialized countries. Older age at the first pregnancy and the increasing prevalence of hypertension, type II diabetes, and obesity represent the main risk factors for cardiovascular complications in pregnancy and postpartum [1]. Moreover, pregnant women with known heart diseases require multidisciplinary obstetric and medical management to assess maternal and fetal risks; modern ultrasonographic technologies are pivotal to allow these patients to reach the childbearing age. In industrialized countries, congenital heart diseases account for 75–82% of CVD in pregnancy, with shunt lesions predominating (20–65%), whereas in nonindustrialized countries the major cause is rheumatic valvular disease (56–89% of CVD in pregnancy). Finally, cardiomyopathies are rare, but they may potentially cause severe complications, and peripartum cardiomyopathy is the most common of them [2].

2. Physiologic Cardiovascular Modification during Pregnancy

During pregnancy, there are many physiological changes due to the increased metabolic demand of the mother-fetus couple, which requires an adequate uteroplacental circulation. Impairment of these mechanisms of adaptation can

cause a fetal or maternal disease, such as growth retardation and preeclampsia, or unmask an underlying cardiac disease. Pregnancy is associated with an increase in heart rate, which starts in the first trimester, peaks in the third trimester (15–25% increase over the baseline heart rate), and returns to preconceptional values by 10 days postpartum. There is also a hormonally mediated increase in blood volume, red cells, and stroke volume (about 20–30%), although there are many difficulties in calculating it [3]. These changes lead to the increase in cardiac output by 30% in the first and second trimester, reaching sometimes 45% in a singleton pregnancy at 24 weeks. Whether or not cardiac output changes in the third trimester is currently debated. However, we know that it decreases rapidly in the first 2 weeks after the delivery, until 24 weeks postpartum. The increase in cardiac output seems to be due to increased stroke volume during early gestation, to increased heart rate later on [2–4]. Immediately after delivery, cardiac output further increases because of the decompression of the inferior vena cava and autotransfusion from the uterus.

Blood pressure typically decreases during normal gestation and it is usually 10 mmHg below baseline values in the second trimester. There is no agreement about systolic blood pressure change during pregnancy [2–8], but it appears likely that both diastolic and mean arterial blood pressure decrease from the early first trimester until 26–28 weeks and then they rise again towards the end of the pregnancy [3, 7–9]. Blood pressure changes are induced by a reduction in systemic vascular resistance [10]. The uteroplacental circulation and systemic vasodilatation contribute to the decline in vascular resistance, which is the steady component of ventricular afterload. Vascular resistance decreases in the early first trimester, presents its nadir in the middle of the second trimester, and returns close to prepregnancy levels within two weeks after delivery [11]. Associated with the decrease of vascular resistance, there is an increase in global arterial compliance (approximately 30%), which is the pulsatile component of ventricular afterload [2, 3]. The abovementioned hemodynamic changes can be considered the pathophysiological bases of cardiac remodeling during pregnancy [2, 12]. In fact, a 15% increase of left atrial diameter has been related to the increase in preload. It starts in the first 5 weeks and plateaus at 28–34 weeks' gestation. Similarly, the left ventricle (LV) increases its end-diastolic dimension by 7–12% beginning at 12 weeks and shows a plateau at 24–32 weeks [4]. Moreover, LV develops eccentric hypertrophy by increasing left ventricular wall thickness (15–25%) and mass (by 50%, mainly in the third trimester). These parameters of left ventricular remodeling may remain above normal limits until 6 months postpartum [10].

3. The Evaluation of Myocardial Function: Strain Imaging

Echocardiography is the most frequently used imaging technique to assess cardiac function and hemodynamics in CVD. Echocardiography allows a rapid assessment of systolic and diastolic function of cardiac chambers, regional wall motion, and valve anatomy and function [13]. Due to the safety of ultrasounds, the wide availability of the technique, and its portability and repeatability, echocardiography is very useful to assess the cardiovascular system of pregnant women with suspected or confirmed heart disease [2].

Deformation imaging is an echocardiographic technique used to assess myocardial function by measuring the actual change in length of the myocardium through the cardiac cycle. Echocardiography can evaluate myocardial deformation through two methods: the first, tissue velocity imaging (TDI), is a Doppler based method, whereas the second, speckle-tracking echocardiography (STE), is based on the analysis of conventional two-dimensional grayscale images.

Strain and strain rate, indices of myocardial deformation, can be obtained with both TDI and STE. Using color TDI, the SR is calculated as the velocity between 2 points along the myocardial wall (velocity gradient) normalized for the distance between the 2 points. TDI SR data can be integrated over time to obtain strain that is the most frequently used measure of myocardial deformation and measures the relative lengthening or shortening of myocardial fibers compared to baseline values. Since TDI is a Doppler based method and velocity can only be measured along the direction of the ultrasound beam, only a limited number of strain components can be measured by TDI. Usually, apical views are used to calculate the longitudinal strain and the parasternal short-axis views are used to calculate radial strain [14].

Conversely, STE is based on the detection on 2D images of the motion of acoustic markers (called "speckles") generated by the interaction of ultrasounds with the myocardium. The position of the speckles can be tracked during the cardiac cycle by using specific software packages. The movement of speckles can be used to measure strain and calculate SR. To analyze the different components of myocardial deformation (strain) with STE is necessary to acquire several views: 4-chamber, 2-chamber, and apical long-axis views to compute global longitudinal strain (GLS) and short-axis views for circumferential and radial strains [15].

Strain and SR are used to estimate global and regional myocardial function. The main advantages of these parameters are (i) the relative independency by loading conditions as opposed to conventional chamber function parameters like ejection fraction (EF) or stroke volume and (ii) the ability to differentiate the active contraction of the myocardium from passive motion resulting from the global heart translation or from tethering by the surrounding myocardium [16].

Moreover, since it measures directly myocardial function, deformation imaging can detect subclinical myocardial dysfunction, when EF or other chamber function parameters are still in the normal range because the heart has activated its compensatory mechanisms. Since, during pregnancy, there is a continuous variation of the loading conditions of the heart, the use of STE can be particularly useful to study the changes occurring in the myocardial function during either normal or pathological pregnancy.

3.1. Strain: Basic Concepts. Strain measures the relative shortening/thickening of a myocardial segment during systole

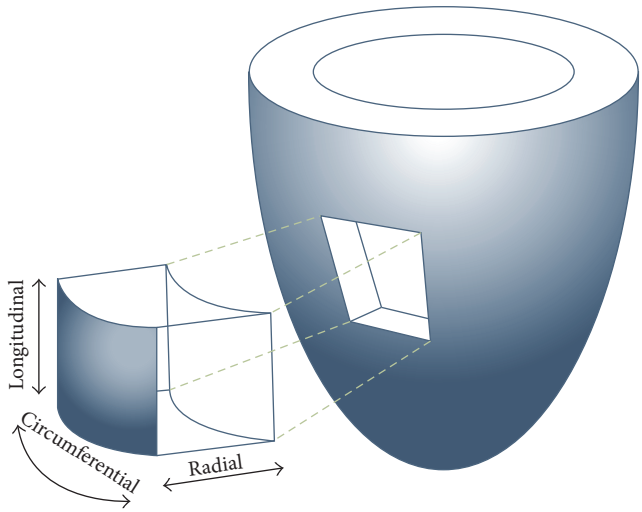

Figure 1: The image shows the three main components of myocardial deformation: longitudinal, radial, and circumferential.

compared to its initial length/thickness in diastole and it is a parameter used in echocardiography to describe the myocardial deformation.

The linear strain (amount of deformation) can be defined by the formula

$$\varepsilon = \frac{\Delta L}{L0}, \tag{1}$$

where ε is strain, $L0$ is baseline length, and ΔL is change in length.

Strain is unitless and it is expressed as a percentage. Strain can assume negative or positive values, which reflect shortening/thickening or lengthening/thinning, respectively [17].

There are several components of myocardial deformation: longitudinal (measuring basal-apical shortening), radial (measuring myocardial thickening), and circumferential (measuring circular perimeter shortening) (Figures 1 and 2).

Since, during systole, the length of the LV shortens and its circular parameter decreases, the systolic values will be lower than diastolic ones. Accordingly, values for normal longitudinal and circumferential strain will be negative. Conversely, during systole, the thickness of myocardial segments increases. Therefore, the systolic values of radial strain will be higher than diastolic ones and values for normal radial strain will be positive [14]. SR describes the rate at which the myocardial deformation occurs and it is expressed as seconds^{-1} [18, 19]. Experimental studies have shown that the SR is less dependent on LV load variations and better reflects actual myocardial contractility than strain [20]. Nevertheless, the SR signal is noisier and less reproducible than strain; therefore, strain was the most frequently reported parameter in clinical studies assessing myocardial function. The utility of strain and SR has been documented in several clinical and experimental studies which showed that they can provide more information on pathophysiological mechanisms of cardiac dysfunction compared to conventional parameters describing cardiac chamber function (e.g., EF, stroke volume) [21, 22].

3.2. Strain Obtained from Tissue Doppler Imaging. The first echocardiographic method used to evaluate strain was TDI. By this method, SR was derived from the velocity data using the equation

$$\mathrm{SR} = \frac{(V_1 - V_2)}{L}, \tag{2}$$

where V_1 is the velocity at point 1, V_2 is the velocity at point 2, and L is the distance between points 1 and 2, usually set at 10 mm. However, since it is a Doppler method, to avoid underestimation of velocities, it is essential that the ultrasound beam is aligned parallel to the direction of the myocardial wall motion being studied [19].

The myocardial velocities can be measured using either *spectral pulsed tissue Doppler (TVI PW)* or *2-dimensional color-coded TDI image loop*. However, myocardial velocities obtained from the spectral pulsed TDI curves are higher than those from 2D color-coded TDI images, because the former measures peak velocities whereas the latter measures mean velocities [23].

It is important to take into account the fact that measurements performed by TDI may be influenced by both blood flow and movement of the other adjacent structures. Moreover, to minimize the effects of respiratory variations, the patient should be asked to suspend his/her breathing for several heartbeats and the operator should use respiratory maneuvers during acquisition to improve the quality of acquisitions [24].

3.3. Strain Obtained by Speckle-Tracking Echocardiography. Speckle-tracking echocardiography is a relatively new ultrasound imaging technique based on the analysis of the spatial dislocation of spots generated by the interaction between the myocardial fibers and the ultrasound beam. These spots are defined as "speckles" which are merged in functional units (kernels) which have a unique pattern within the myocardium. Therefore, each kernel can be individually tracked during the cardiac cycle and its motion can be analyzed by dedicated software packages (Figure 3). By knowing the position of the kernels at the beginning and at the end of systole and the time between two frames (from frame rate), the software can automatically calculate the deformation of the myocardium (strain), the rate of the displacement, and the rate of the deformation (SR) [25].

This method, different from TDI, is angle-independent, provides accurate measures of myocardial deformation, and guarantees good intraobserver and interobserver reproducibility [26, 27]. GLS is a parameter of global LV function obtained by averaging the peak values of systolic strain obtained from the 17 segments of the LV [28]. Accordingly, to obtain GLS, it is necessary to acquire and analyze three apical views of the LV: 4-chamber, 2-chamber, and apical long axis.

A number of studies have demonstrated that GLS is an accurate parameter to evaluate myocardial function [29, 30].

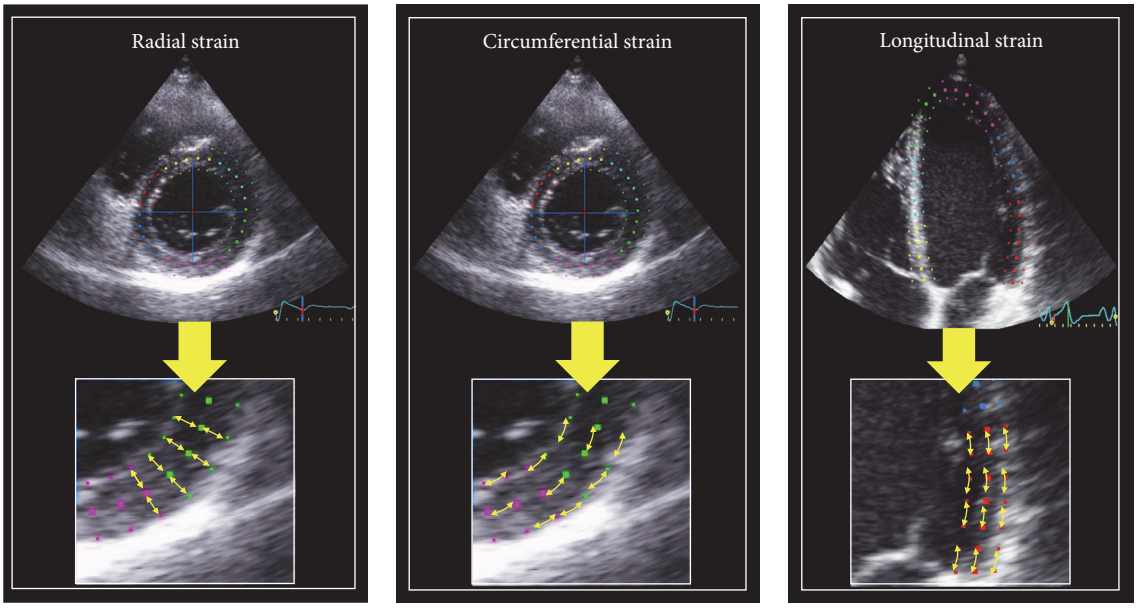

Figure 2: The figure shows speckle-tracking analysis and the main components of myocardial deformation: radial (a), circumferential (b), and longitudinal (c).

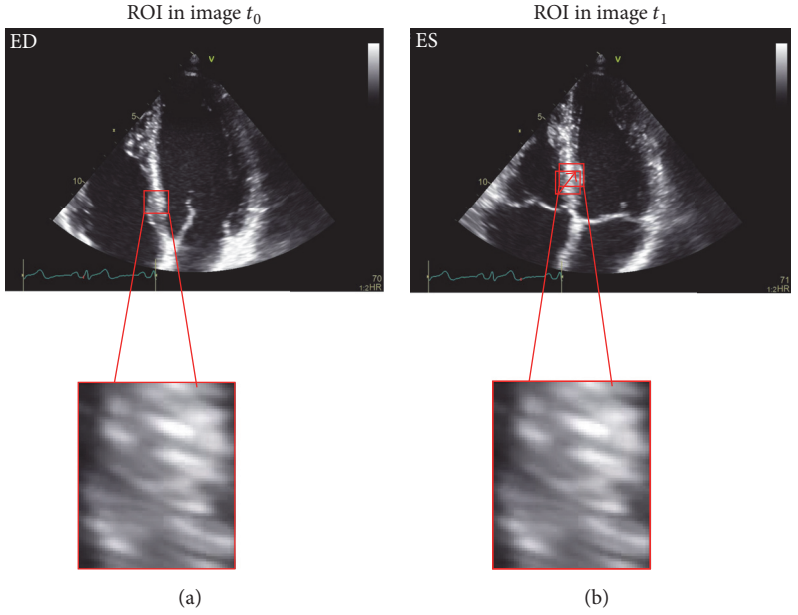

Figure 3: The image shows a region of interest (kernel) at t_0 (a) and the relative change in its position at t_1 (b). Myocardial speckles in the grayscale image are tracked frame-by-frame.

It has been reported to be more sensitive than EF in identifying subclinical left ventricular dysfunction in cardiomyopathies [31] and has shown an independent prognostic value [32, 33]. Use of GLS to assess myocardial function has been recommended in a variety of clinical scenarios [15, 27, 34]. The reference values for STE have been included in the recent recommendations for cardiac chamber quantification by echocardiography in adults [35].

The strain and SR values obtained with TVI and STE are well correlated, even though the STE approach is more rapid and reproducible and allows a more complete evaluation of LV myocardial deformation due to its independence of the ultrasound beam alignment [26].

3.3.1. 2D Speckle-Tracking Echocardiography Image Acquisition. To obtain images to be analyzed with the STE software

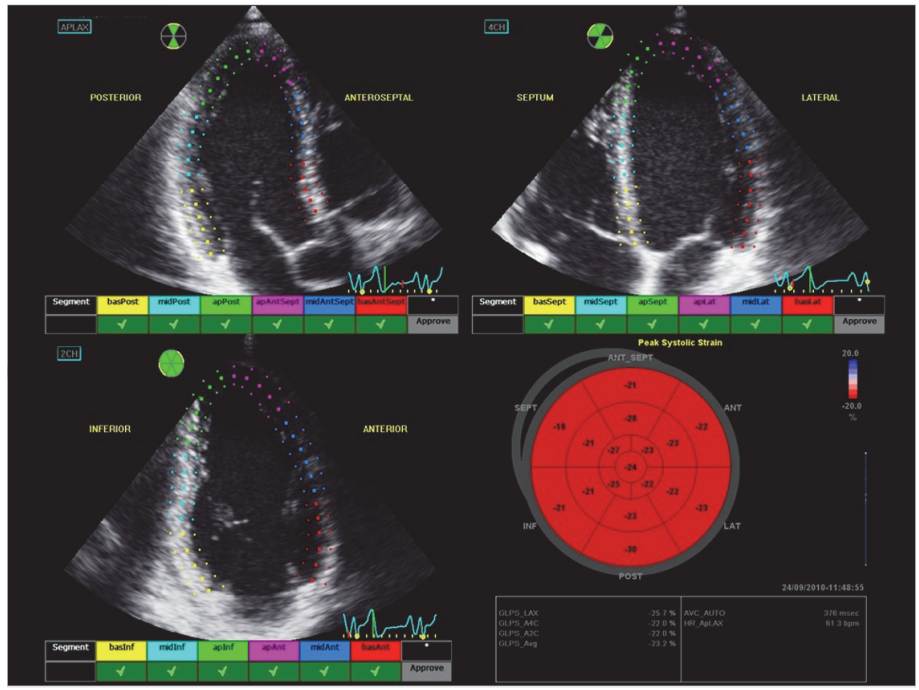

Figure 4: Regions of interest (dotted lines) have been traced on apical long axis, 4- and 2-chamber views. Each region of interest is divided into 6 segments for each view. The operator may accept/reject segments in case of suboptimal tracking of speckles.

package, it is recommended to optimize image quality by using a grayscale second-harmonic 2D imaging technique with careful adjustment of image contrast. The gain settings should be optimized, the depth should be reduced, and the focus should be in the middle of the left ventricle. Finally, images should have adequate temporal resolution (50–90 frames per second) [19]. Lower temporal resolutions will not allow a sufficient number of systolic frames to track the motion of the kernels. Higher temporal resolution will impact the spatial resolution of the images by reducing the number of scan lines [36]. Moreover, it is essential to optimize the LV border visualization. Care must be taken to avoid LV foreshortening and image acquisition should be performed during breath-hold to minimize respiratory interference. It is essential that the electrocardiographic trace is stable to avoid artefacts during the evaluation and at least three cardiac cycles should be acquired for each loop [37]. Artefacts, such as reverberation or shadowing, could affect strain computation and provide wrong strain values, which might erroneously suggest cardiac dysfunction.

3.3.2. 2D Speckle-Tracking Echocardiography Image Analysis. The assessment of strain by 2D STE is a semiautomatic method. The images recorded are processed using dedicated software packages usually available on workstations or included in the echo machine. The apical long-axis view should be analyzed first to identify the end-systolic frame looking at the movement of the aortic valve. A pulsed-wave spectral Doppler envelope of LV outflow may be helpful to set the timing of cardiac events if the images of the aortic cusps are suboptimal [17].

Endocardial border is identified by the software in all apical views resulting in a manually adjustable region of interest, which should exclude the bright pericardium [38].

The software package automatically divides each region of interest into 6 segments for each view and the operator may eventually accept/reject segments having the possibility of modifying the shape and the thickness of the region of interest in case of error of identification or incorrect tracking (Figure 4).

After operator approval of tracking, a deformation (strain) curve will be generated for each segment and the software package reports the peak value of strain occurring before aortic valve closure. GLS will be computed by averaging the segmental values of peak strain.

To make interpretation of regional strain easier, the software generates a "bullseye" map with red and blue color coding, where the segments on the external ring represent the basal LV segments, the middle ring segments represent the midventricular segments, and the most internal ring represents the apical segments [39] (Figure 5).

Several independent studies confirmed that a peak GLS in the range of −20% can be expected in a healthy person [35].

A large study conducted on 247 healthy volunteers showed that longitudinal strain was significantly more negative in women than in men. Therefore, separate lower limits of normality for longitudinal strain should be used in men (−16.9%) and in women (−18.5%) [40].

3.3.3. Three-Dimensional Speckle Tracking (3D STE). 2D STE has become a popular and clinically useful technique, but it is limited by the assumption that kernels move linearly

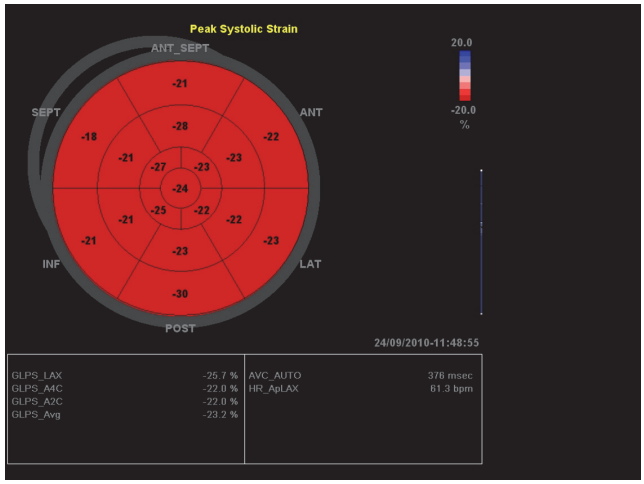

Figure 5: Bullseye display of segmental peak-systolic longitudinal strain. Global longitudinal strain is computed for each view.

along planes that coincide with the 2D echocardiographic view. Conversely, myocardial motion is quite complex and comprises rotation and displacement in the 3D space. Since using 2D STE might cause the loss of some kernels in the through-plane motion during the cardiac cycle, it has been suggested that 3D STE may be a more suitable tool to track the motion of the kernels in the 3D space [41].

To obtain myocardial deformation by 3D STE, we need a pyramidal full-volume data set of the LV that can be acquired using a matrix array transducer. To obtain a large data set which contains the whole LV with adequate temporal resolution (30–36 volumes per second), we need a multibeat acquisition to create a single volumetric data set by stitching together subvolumes obtained from 2 to 6 consecutive cardiac cycles [42]. To avoid the stitching artefacts, acquisitions should be done during suspended respiration and they are feasible only in patients with regular cardiac rhythm [43]. Since with 3D STE it is possible to follow kernels in a volumetric space, the software can calculate simultaneously the longitudinal, the radial, and the circumferential components of myocardial deformation, plus a composite parameter (area strain) which takes into account the simultaneous deformation in both longitudinal and circumferential direction at mid myocardium [44]. Data relating to the normal values in adult nonpregnant patients are known [45].

However, although it is theoretically appealing, at present, 3D STE still has several limitations that have not allowed for its wide use in daily clinical practice (Figure 6).

4. Clinical Applications of Deformation Imaging in Pathological Pregnancies

4.1. Preeclampsia. Preeclampsia is a hypertensive complication that affects 5–7% of pregnancies and is one of the most common causes of maternal morbidity and mortality [46]. In fact, it is considered a complex multiorgan disease potentially involving the kidney, liver, and cardiovascular and hematologic systems, as well as the brain. Autopsy data have shown a tenfold prevalence of myocardial contraction band necrosis in preeclamptic patients if compared with pregnant women that died from other causes [47]. Some studies have demonstrated persistent maternal cardiac impairment and hemodynamic changes years after delivery [48, 49]. Valensise et al. demonstrated that signs of left ventricular diastolic dysfunction and persistent heart remodeling persist in nonpregnant women before a second pregnancy with recurrent preeclampsia. These findings could raise an issue concerning preeclampsia as a cause or effect of heart remodeling [48–51].

The majority of published papers about echocardiographic assessment of maternal heart in preeclamptic women have used conventional parameters of cardiac function and remodeling [50–55]. In preeclamptic patients, the use of TDI and 2D STE demonstrated a reduction of both left and right ventricular diastolic and systolic function, also in preclinical stages of the disease, when cardiac output and EF are still preserved. LV-mass, LV-mass index, and LV wall thickness in preeclamptic women are higher than in healthy controls, reflecting the increase in LV afterload. Myocardial performance index (MPI), an index of reduced cardiac systolic and diastolic function, also increased. An impairment of right ventricular systolic function has been also described, reflecting the increase of pulmonary resistance secondary to LV diastolic dysfunction [56].

Moreover, several authors agree that longitudinal, radial, and circumferential strains are impaired in preeclampsia and may remain impaired also for months after the delivery, even in patients with preserved cardiac output and EF [48, 49, 55, 57, 58]. An interesting consideration is that coexisting LV hypertrophy and regional longitudinal systolic dysfunction could reflect a regional subendocardial impairment, probably due to subendocardial ischemia and/or fibrosis seen by Bauer et al. in preeclamptic women autopsies [47, 55]. Currently, new parameters based on 3D STE are emerging, showing an increased ability to detect subclinical myocardial impairment and early systolic and diastolic cardiac dysfunction. Myocardial dysfunction precedes chamber impairment and 3D STE can provide an assessment of global and regional LV function [59, 60]. Furthermore, some authors, using 3D STE, demonstrated that early onset preeclamptic patients presented worse cardiac remodeling than late onset preeclamptic patients, underlining the clinical relevance of detecting earlier and subtler cardiac dysfunction signs [58]. Other authors distinguish between preeclamptic and nonproteinuric hypertensive women, showing less impairment of longitudinal, circumferential, and radial strain in the latter [59]. These findings could mean that hypertension may not be the only cause of preeclamptic heart impairment, although women with nonproteinuric hypertension presented a worse cardiac function than healthy patients [57, 61].

4.2. Intrauterine Growth Restricted Pregnancies. Knowledge of heart disease in women is constantly evolving and emerging data show that complications of pregnancy such as preeclampsia and intrauterine growth restriction (IUGR) are predictors for the development of heart disease later in life [62]. Up to 10% of all pregnancies are affected by IUGR and

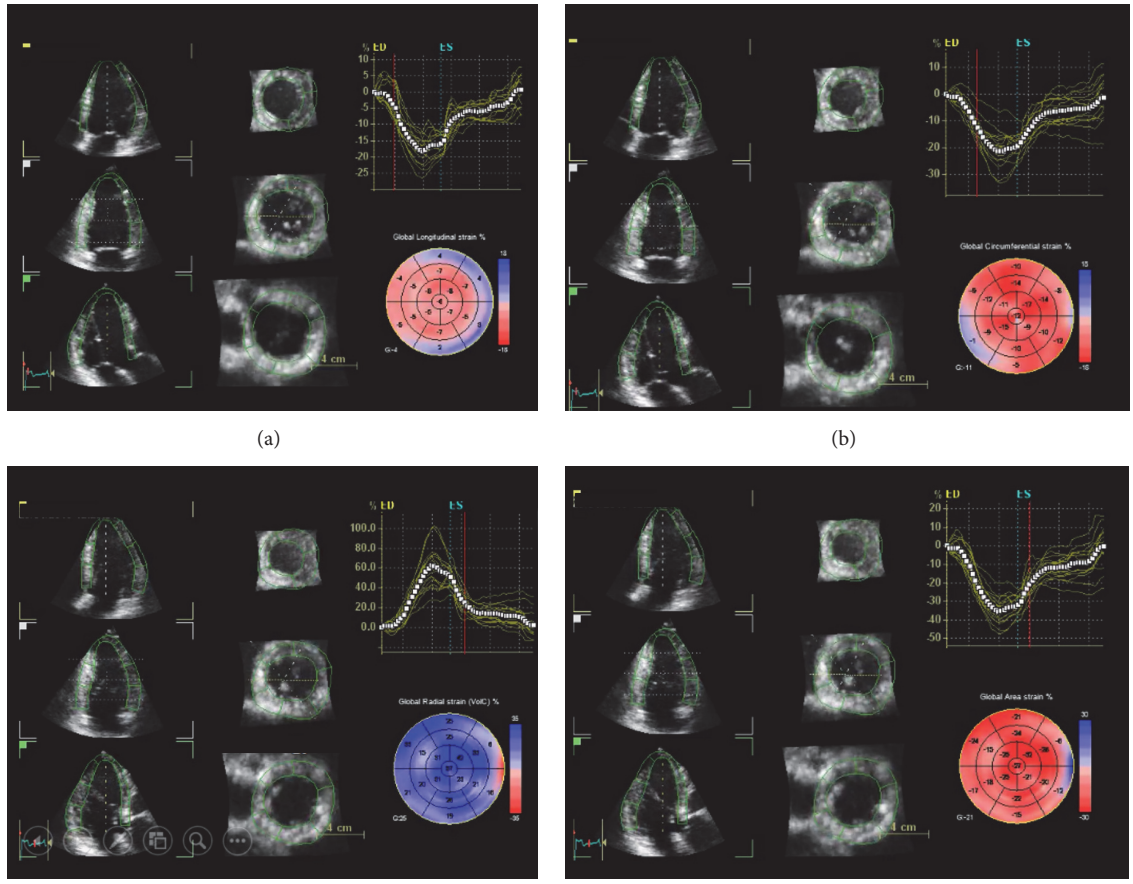

Figure 6: Three-dimensional speckle-tracking echocardiography. The software package calculates simultaneously the longitudinal (a), the circumferential (b), and the radial (c) components of myocardial deformation, plus a composite parameter (area strain) (d).

its definition is controversial. The main reasons of IUGR are placental insufficiency and defective trophoblastic invasion, currently evaluated by the estimated fetal weight and umbilical artery Doppler flow velocity [63]. Fetal Doppler evaluation is a useful method to predict fetal compromise and permits distinguishing between severe IUGR and small for gestational age (SGA) fetuses [64]. However, different classifications are also reported in the literature and they could generate confusion in the definition of both maternal and fetal risk.

While maternal cardiac modifications occurring during normal pregnancy are well known, in normotensive pregnancies with IUGR, there are contradictory lines of evidence about maternal hemodynamics [3]. Some authors reported reduced cardiac output and left ventricular compliance [65], whereas others reported reduced maternal systolic cardiac function and increased total vascular resistance, without alterations of left diastolic function compared to physiological pregnancies [66].

Moreover, IUGR patients, compared with preeclamptic pregnancies, seem to present lower cardiac index, left ventricular diastolic dysfunction, and higher total vascular resistance index. Unlike preeclampsia, cardiac geometry and intrinsic myocardial contractility were reported to be preserved, but a third of IUGR patients present reduced diastolic reserve and an overt diastolic chamber dysfunction, despite a normal EF [67]. This suggests that the cardiovascular response is similar to that seen in preeclamptic patients, though less severe. Lack of physiological adaptation to the pregnancy, assimilating IUGR patients to a nonpregnant hemodynamic condition, could explain the reason of high resistance, low blood volume, and hypotensive condition, which characterized IUGR patient's condition [65]. The use of cardiac indices in isolation lies at the basis of several studies available in the literature, which make use of conventional 2D and Doppler transthoracic echocardiography [68]. A further improvement to the clinical presentation in IUGR patients could be gathered by including in the analysis the correlation between age and diastolic indices. The introduction of TDI and 2D STE techniques for analysis of myocardial deformation might allow an earlier diagnosis and better grading of cardiac dysfunction [69]. While several authors described the feasibility of STE in studying fetal heart function and morphology, in particular, the segmental and global systolic and diastolic velocities, strain, and SR values, few studies described its application for the evaluation

of IUGR patients [70]. Krause et al. investigated maternal longitudinal mechanical dyssynchrony, a useful tool used for the evaluation of LV function, finding that pregnancies complicated by IUGR recorded significantly higher degrees of inter- and intraventricular dyssynchrony than those of normal controls [71].

5. Conclusion

Reduced maternal cardiac function in pregnancies that are complicated by preeclamptic and intrauterine growth restriction is the result of both reduced intrinsic myocardial contractility and reduced diastolic filling. Myocardial dysfunction can be present even in the presence of a normal ejection fraction, with significant decreases in radial, circumferential, and longitudinal strain values.

The use of 2D and 3D STE techniques to evaluate ventricular mechanics may help detect subclinical left ventricular dysfunction in women affected by obstetrical pathologies as preeclampsia and intrauterine growth restriction. Early detection of left ventricular dysfunction with the institution of appropriate treatment may reduce the risk of future CVD.

Abbreviations

CVD: Cardiovascular disease
EF: Ejection fraction
GLS: Global longitudinal strain
LV: Left ventricle
SR: Strain rate
STE: Speckle-tracking echocardiography
3D STE: Three-dimensional speckle tracking.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Review Article

Paracetamol in Patent Ductus Arteriosus Treatment: Efficacious and Safe?

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In preterm infants, failure or delay in spontaneous closure of Ductus Arteriosus (DA), resulting in the condition of Patent Ductus Arteriosus (PDA), represents a significant issue. A prolonged situation of PDA can be associated with several short- and long-term complications. Despite years of researches and clinical experience on PDA management, unresolved questions about the treatment and heterogeneity of clinical practices in different centers still remain, in particular regarding timing and modality of intervention. Nowadays, the most reasonable strategy seems to be reserving the treatment only to hemodynamically significant PDA. The first-line therapy is medical, and ibuprofen, related to several side effects especially in terms of nephrotoxicity, is the drug of choice. Administration of oral or intravenous paracetamol (acetaminophen) recently gained attention, appearing effective as traditional nonsteroidal anti-inflammatory drugs (NSAIDs) in PDA closure, with lower toxicity. The results of the studies analyzed in this review mostly support paracetamol efficacy in ductal closure, with inconstant low and transient elevation of liver enzymes as reported side effect. However, more studies are needed to confirm if this therapy shows a real safety profile and to evaluate its long-term outcomes, before considering paracetamol as first-choice drug in PDA treatment.

1. Introduction

Ductus Arteriosus (DA) is the vascular communication connecting pulmonary artery to aorta and represents one of the fundamental shunts of prenatal life circulation [1, 2].

The expression "Patent DA" describes the situation of a physiological or pathological open DA, which we will consider in this review with the abbreviation "PDA." The conditions of "Patent DA" and "Persistent DA" are often confused and indicated with the same general expression "PDA," even if they differ in terms of morphology, clinical effects, and management [1, 3, 4]. In healthy full-term newborns DA generally undergoes functional closure between 24 and 72 hours of life [5, 6], favored by higher postnatal levels of PaO2, removal of placenta from neonatal circulation, increase in pulmonary flow, and decline of prostaglandin E2 (PGE2) local receptors number [7, 8], reducing PGE2 vasodilating effect on DA [9–11].

The effect of PGE2 on vascular smooth muscle relaxation results in maintenance of ductal patency and occurs through activation of adenylate cyclase, leading to cAMP increase, via interaction with G-protein receptors [12, 13].

The anatomical closure of DA is generally complete in a few weeks [9], through the evolution into the structure called ligamentum arteriosum [14].

For several reasons, including the persistence of high levels of circulating PGE2 in preterm neonates, spontaneous DA closure often fails or is delayed in such patients, and this condition has been associated with various and severe short- and long-term complications [2, 6].

According to Benitz and Committee on Fetus and Newborn [6], on the 4th day of life PDA would persist in about 10% of infants with gestational age (GA) between 30 and 37 weeks, in 80% of those with GA between 25 and 28 weeks and in 90% of preterms born at 24 weeks of GA. From the 7th day of postnatal life the percentage of infants with PDA in these groups would reduce, respectively, to about 2%, 65%, and 87%.

According to the same review, DA would spontaneously close in 73% of infants with more than 28 weeks of GA and

in 94% of infants with birth weight (BW) greater than 1000 grams [6].

There are few studies on PDA spontaneous closure in newborns with lower GA and BW, or in infants with respiratory distress syndrome (RDS), because a PDA therapeutic closure is often performed in these categories of patients. In a randomized trial comparing prophylactic indomethacin to placebo, non-pharmacologically treated PDA has not led to the development of clinical effects in 50% of infants of BW between 500 and 999 grams [6].

The spontaneous closure of PDA during early postnatal life in 35% of ELBW infants and in 70% of infants with GA greater than 28 weeks has been demonstrated in a prospective study by Koch et al. [15]; in another study, 75% of infants with GA less than 27 weeks with PDA at discharge moment has shown spontaneous ductal closure within the first year of life [5, 16].

Prolonged condition of PDA in preterms can be associated with important complications, such as severe RDS, prolonged need for assisted ventilation, pulmonary hemorrhage, bronchopulmonary dysplasia (BDP) [17], necrotizing enterocolitis (NEC), renal function damage, intraventricular hemorrhage (IVH), periventricular leukomalacia (PLV), cerebral palsy, or death [1, 14, 18–22].

These conditions depend on the magnitude of left-right shunt volume through PDA, regulated by the balance between PDA dimension and arterial resistance fall in the pulmonary circle during the early hours of postnatal life and resulting in lung hyperflow and development of pulmonary congestion and edema. If this condition persists, deterioration of respiratory function can occur. The impact of this "ductal steal" on systemic circulation causes a reduction in cardiac output increasing, the mechanism that allows facing the rising in systemic resistances of postnatal period. This condition can lead to vital organs perfusion impairment, such as brain, kidney, and bowel [6, 9].

To prevent such complications, the practice of DA closure is common and it is performed at first pharmacologically, but, in case of drugs failure or contraindication, with surgical ligation [18].

Despite years of researches and clinical experience on PDA management, many unresolved issues about its evaluation and treatment, with consequent heterogeneity of clinical practices in different centers, still remain, particularly regarding timing and modality of intervention. In fact, the available strategies vary from prophylactic treatment to early or delayed therapy [6, 23].

Recent studies, however, do not recommend prophylaxis in case of non-hemodynamically significant PDA, because it exposes the infants to indomethacin or ibuprofen adverse effects, without substantial short-term or long-term benefits [1, 12, 14]. The most reasonable strategy seems to be, nowadays, reserving the treatment only to hemodynamically significant PDA (hsPDA) [5, 24].

For this purpose, the first-line therapy is medical and nonsteroidal anti-inflammatory drugs (NSAIDs) are drugs of choice, preventing the conversion of arachidonic acid into prostaglandins via cyclooxygenase (COX) inhibition, in both the existing isoforms COX-1 (constitutive) and COX-2 (inducible) [18, 23, 25]. Reduction in prostaglandin levels leads to DA muscular wall constriction through the hypoxia of ductal vasa vasorum and consequent local angiogenesis, formation of neointimal tissue, and apoptosis. These mechanisms, in conjunction with platelet recruitment and activation, lead to processes of obstruction and fibrosis and, as a result, anatomical ductal closure [26–30].

2. Biochemical Markers of PDA

Many biochemical markers have been correlated with PDA such as B-type Natriuretic Peptide (BNP), the segment of the amino terminal B-type Natriuretic Peptide (NT-proBNP), and the cardiac Troponin T (cTnT), whose levels increase in case of hsPDA with right to left shunt, and could help in disease staging and management [5, 12]. Rising levels of BNP could represent a compensatory diuretic mechanism facing the increase in cardiac preload induced by the hyperaldosteronism condition subsequent to renal hypoperfusion and activation of renin-angiotensin system.

El Kuffash et al. [31] and Czernik et al. [32] also evaluated urinary proBNP as a simple and noninvasive PDA indicator, becoming higher in ventilated neonates nonresponders to treatment; according to Vettukattil [33] urinary NT-proBNP-to-creatinine ratio may be related to medical treatment response.

However, not all the authors agree with these results. Rostas and McPherson [34] affirm that BNP and NT-proBNP are not effective and really useful biomarkers to orient PDA therapy.

Some studies have also pointed out a role of systemic inflammation, which could improve COX-1 activity and PGE2 production, in ductal patency maintenance [35, 36]; this inflammatory pathway could also play a negative role influencing drug therapy response [37, 38].

In this perspective, Hillman et al. [35] performed the first study (on a sample of 132 newborns), investigating high sensitivity C-Reactive Protein (CRP) levels, as marker of low grade inflammation [39], detecting significantly higher levels of this mediator in patients diagnosed for PDA [35]; successively, Meinarde et al. [40] detected the same result analyzing 88 newborns.

These findings, though detected on small samples of patients, focus on the possible influence of inflammatory conditions on PDA, also in case of occurrence in prenatal life (such as chorioamnionitis) [35, 36].

Moreover, such systemic inflammatory status could also determine the high oxidative stress detected in PDA patients [35, 41]. In fact, according to some studies, reactive oxygen metabolites seem to be involved in ductal closure regulation and it has also been hypothesized that NSAID's activity in PDA closure could also be partly mediated by their ability in reactive species and oxidative stress reduction [28, 42–44].

In the study of Inayat et al. [45], in preterms with hsPDA, a poor antioxidant status within the first 48 hours after birth has been demonstrated through the detection of lower levels of superoxide dismutase (SOD), urinary catalase, and plasma and urinary 8-isoPGF2a, with an impairment in urinary prostaglandin E2, plasma and urinary thromboxane B2, and

plasma SOD after pharmacological PDA treatment [45]. Further studies are needed to establish the correct relation between inflammation, oxidative stress, and PDA and to assess if there could be a possible role of such conditions related mediators in predicting PDA or monitoring therapeutic effects of closure treatment.

Metabolomic analysis also revealed a promising technique in the diagnosis and treatment of PDA; in fact, differently by the other mentioned mediators, it seems to have the singular ability to detect, at birth moment and only through the H-NMR evaluation of the first urine sample, the successive condition of persistent patency of DA at 3-4 days instead of its spontaneous closure and may also predict the exact individual response to the therapy. Moreover, different metabolic profiles of expression have been detected between responders and nonresponders to ibuprofen therapy. These interesting findings, though on small preterm groups, have been evidenced by preliminary results in the studies of Fanos et al. [46] and Castell Miñana et al. [47].

Further studies will help to fully understand the applicability fields of metabolomic holistic marker.

3. PDA Treatment

3.1. Indomethacin. Among nonselective COX inhibitors, intravenous (iv) indomethacin was the first drug used for PDA treatment, presenting a closure rate of about 70–85% without any other short-term benefits [26]. Since indomethacin has been used as a prophylaxis in PDA management, it has been shown to reduce the incidence of intraventricular hemorrhage (IVH ≥ grade 3 by 30%) and severe pulmonary hemorrhage by 35%, symptomatic PDA development, and necessity of surgical ligation [1, 14, 33, 48–52], without effects on mortality or long-term neurodevelopmental outcome [14].

Instead of this previous evidence, the recent prospective double cohort study of Liebowitz and Clyman [53], published on 2017, has pointed out also a protective effect of prophylactic indomethacin on development of BDP and death, instead of delayed PDA treatment (after 7 postnatal days) in extremely premature neonates.

However, for its high vasoconstrictor power, this drug has been associated with several side effects such as impairment in renal function until acute or chronic renal failure, oliguria, proteinuria, hyperkalemia [23], cerebral white matter damage, NEC, intestinal perforation (especially when coadministered with corticosteroids), and platelet dysfunction [2, 54].

Renal side effects are the most frequently reported and oliguria is generally reversible within 48 hours after last drug administration [23].

3.2. Ibuprofen. Recognizing these indomethacin related side effects, ibuprofen was subsequently introduced in the clinical practice, either orally or in iv manner; each course of therapy is composed of the standard dose of 10 mg/Kg/dose/day on the first day of treatment followed by two subsequent doses of 5 mg/Kg/dose/day on 2° and 3° days [33].

Ibuprofen shares with indomethacin the mechanism of action and the efficacy in PDA closure (success rate 70–85%) [26], but its lower vasoconstrictor effect leads to a reduced impact on microcirculation and consequent less impairment of renal function; this difference could be partly determined by a preferential effect of indomethacin on COX-1 instead of COX-2 but also by other mechanisms not exactly known [55, 56].

However, ibuprofen is not free from other significant side effects, such as pulmonary hypertension and hyperbilirubinemia [6, 9, 14].

Now it represents the first-choice drug for hsPDA treatment, but it is not recommended in prophylaxis because of the lack of efficacy in reducing intraventricular hemorrhage incidence, unlike indomethacin [1, 48, 49].

A recent randomized trial of Demir et al. [57], published on January 2017, has evaluated the ibuprofen intrarectal way of administration, which became as effective as the oral way in VLBW neonates with hsPDA. After treatment, in both groups the authors demonstrated higher levels of Cystatin-C, a biomarker of glomerular filtration which can suggest nephrotoxicity, indicating the necessity of a closely clinical observation especially in patients with a damaged renal function.

Higher doses of ibuprofen have been shown to improve closure rate, in particular using a treatment course of 20-10-10 mg/Kg/dose after standard doses failure, but the potential side effects of this drug regimen must be still clarified [14, 48]. Although some studies suggest the safety of high dose treatment [58], El-Mashad et al. [55] recommend the administration of low ibuprofen doses, underlying its inhibitory effect on hepatic glucuronidation of bilirubin and its high albumin binding affinity, which can increase the risk of bilirubin encephalopathy [59, 60].

Other studies show a major increase in Cystatin-C level after high dose regimen instead of standard doses [14, 48].

Drug responses can vary in different individuals, as it is known from pharmacogenomics. It is a current practice to administer the same ibuprofen dose for newborns of different GA and postnatal ages and this could result in a paradox from pharmacokinetic and pharmacodynamic perspective; however, other factors should be considered, such as the existence of two different ibuprofen enantiomers (S- and R-ibuprofen with half-lives of 25 and 10 hours, resp.) and the presence of genetic polymorphisms that can determine different clinical and side effects, dividing treated patients in extensive and poor metabolizers [9].

For example, polymorphisms in cytochromes P450 CYP2C8 and CYP2C9 are widespread in the population and some genetic variants can improve enzyme activity influencing NSAIDs metabolism, which represent their substrates. As reported by Agùndez et al. [61], individuals showing CYP2C8∗3 (rs11572080; rs10509681), CYP2C9∗2 (rs1799853), or CYP2C9∗3 (rs1057910) variations more frequently present gastrointestinal bleeding as an adverse effect of NSAIDs administration.

The possible role of CYP2C8 and 2C9 polymorphisms in influencing ibuprofen response, in preterm neonates treated for hsPDA, has been evaluated by Durrmeyer et al. [62]; in this study, similar drug responses have been reported among the studied individuals carrying different genetic variants, in a multivariate analysis; predictor factors for drug response seemed to be a higher gestational age and the non-Caucasian

ethnicity, suggesting the possibility of this element influence on interindividual variability in ibuprofen response [62].

Moreover, other authors also evaluated P450 polymorphisms in healthy volunteers treated with ibuprofen; among these, Ochoa et al. [63] demonstrated the influence of CYP2C9∗2 and CYP2C9∗3 genetic variants on the pharmacokinetics of the enantiomers S-ibuprofen and R-ibuprofen and showed a possible gender predisposition in drug metabolism influence. In addition, Karaniewicz-Ada et al. [64] detected impaired ibuprofen enantiomers metabolism in individuals carrying CYP2C8∗3, CYP2C9∗2, and CYP2C9∗3 alleles, and also other studies evidenced the effect of CYP2C8∗3 variant on R-ibuprofen (with higher clearance) [65, 66] and fewer side effects in individuals showing CYP2C8∗3 variants [65].

More studies must be performed to fully understand the role of these purposed genetic determinants for ibuprofen responses. The goal of each pharmacotherapy would always be the administration of the exact individualized dose, showing the highest rate of success with the best safety profile and the lowest toxicity. In this perspective of person-based medicine, studies of pharmacokinetic and metabolomic are highly promising.

3.3. Nephrotoxicity of NSAIDs. Acute Kidney Injury (AKI) is an important issue in premature neonates of NICU, significantly contributing to morbidity and mortality of such critical population, since it is well known that these patients become more susceptible to kidney damage [67–71].

In preterms, AKI occurrence is generally a multifactorial event, but exposition to nephrotoxic medication plays an important role, potentially interfering with postnatal nephron generation and representing an avoidable cause of neonatal renal damage. Long-term effects of drug-induced AKI on both kidney function and general healthy outcome remain still understudied. However, recent data suggest that prematurity incomplete nephrogenesis, in addition to nephrotoxic administered toxins, could predispose to chronic kidney damage (CKD) [67, 72–74].

According to the recent study of Hanna et al. [67], published on 2016, the exact number of cases of nephrotoxin-associated AKI and its contribution on the development of CKD in neonatal population are not exactly known, for the lack of studies on a great number of patients but also for the absence of a systematic follow-up after neonatal AKI. Only small single center reports are actually available [75], but further studies will help to define the most appropriate application of nephrotoxic drugs and the correct surveillance in order to reduce the risk of CKD in treated patients [67].

The nephrotoxic effect of NSAIDs is related to prostaglandin important role during kidney and cardiovascular system adaptation after birth [23, 76, 77].

Prostaglandins neonatal circulating levels become higher than in successive life, since these mediators act as afferent arteriolar vasodilators and regulators of renal water clearance, facing the postnatal systemic resistances vasoconstriction. For these reasons, the inhibition of prostaglandin synthesis negatively affects renal blood flow and glomerular filtrate, generally resulting in transient oliguria [67, 78].

Moreover, neonatal kidney, not completely developed, is susceptible to the lack of prostaglandins. In fact, its maturation process closely depends on these mediators. This has been demonstrated both prenatally and in the postnatal period but appears more pronounced in preterms, whose urinary excretion of prostaglandin E2 (PGE2) and prostaglandin I2 (or prostacyclin) becomes higher than in neonates born with normal GA or with a month of postnatal life [23, 76, 77].

During studies conducted to evaluate NSAIDs renal damage, urinary PGE2 revealed a useful and noninvasive biomarker of nephrotoxicity, becoming significantly decreased in the urine of preterm infants after treatment for PDA closure, both with indomethacin and with ibuprofen, and this reduction became stronger in case of more severe side effects [23, 79].

Neonates with higher risk of nephrotoxic damage after ibuprofen administration are those with lower basal levels of PGE2; in the review of Fanos et al. [23] it is pointed out that no treatment should be considered in neonates with PGE2 urinary levels lower than 35 pg/ml; in neonates with rapid decrease of PGE2 urinary levels during treatment, ibuprofen suspension must be taken into account and neonates with PGE2 levels lower than 5 pg/ml after or during ibuprofen treatment will probably develop significant renal adverse effects [23].

Unlike this, the study of urinary isoprostanes, whose excretion increases in case of high oxidative status, revealed a lower level of these metabolites after ibuprofen administration, suggesting a possible protective effect of this NSAID against oxidative stress [23, 80].

Other sensitive and promising urinary biomarkers of kidney injury are represented by Cystatin-C and Neutrophil Gelatinase-Associated Lipocalin (NGAL); NGAL urinary excretion increases early during AKI and its detection becomes significantly helpful in monitoring nephrotoxicity in newborns [81].

3.4. Paracetamol. More recently, oral or iv administration of paracetamol (acetaminophen) gained attention in PDA treatment; the first case report on this topic has been published by Hammerman et al. on 2011 [82, 83]. Successively, this drug has been evaluated through many trials as safe and effective compared to traditional NSAIDs in PDA closure, with fewer side effects [2, 18, 84–86].

Before paracetamol introduction, in case of contraindication for NSAIDs, such as active or recent intracerebral hemorrhage (<48 h), thrombocytopenia (<50,000/mm^3), bleeding diathesis (meaning INR > 1.5 and/or hematuria, blood in the stool, tracheal secretions or at the injection site), sepsis, NEC, intestinal perforation, pulmonary hemorrhage, hepatic damage with severe hyperbilirubinemia, renal dysfunction (oliguria <1 ml/kg/h also after adequate hydration, serum creatinine >110–140 μmol, and BUN > 14 mmol/l), and hypersensitivity to ibuprofen [22, 48, 87], the only available solution was surgical ligation with all the connected risks [22, 55, 59].

However, further studies are needed before this drug can be recommended as first-line therapy; long-term outcomes of

treatment and its possible late side effects at 18 or 24 months of postnatal age must be fully clarified [24, 26].

Yang et al. [88] demonstrated a probably higher renal safety of this drug describing a significantly lower reduction in PGE2 urinary excretion and minor incidence of oliguria comparing two groups of infants treated with paracetamol versus ibuprofen.

These advantages would be related to the different drug mechanism of action, because paracetamol is not a classical NSAID, having only a weak antiplatelet and anti-inflammatory activity. It exerts mainly central effects (analgesic, antipyretic) and reduces the synthesis of prostaglandins through the inhibition of prostaglandin synthetase (PGHS), as it happens with NSAIDs, but acting in a different enzyme site, called peroxidase region (POX) [12, 18, 55].

However, some hepatic side effects have been described after iv paracetamol administration, which may determine a transient increase in liver enzymes concentration [89] or, according to other studies, more serious acute liver toxicity events [48, 90–93].

Hepatotoxicity in neonates is not determined directly by paracetamol itself but can be caused by N-acetyl-p-benzoquinone imine (NAPQI) metabolite production by hepatic cytochrome P450 (CYP)-dependent mixed function oxidase enzyme. The mechanisms of NAPQI formation, sulphate elimination, and glucuronide production rate are still not exactly known in preterms [94, 95].

The hepatic paracetamol metabolism occurs through sulphation, glucuronidation, and oxidation. Administering therapeutic doses of paracetamol, glucuronidation, or sulphation is activated as first mechanism, producing non-toxic metabolites. Also hepatic oxidation of paracetamol by CYP1A2, 3A4, and 2E1 generates the highest reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) which is conjugated by glutathione into a renal metabolite that becomes safe. Instead, after an excessive dose of paracetamol, sulphation and glucuronidation pathways saturate and the resulting excessive dose of NAPQI consumes glutathione reserves becoming toxic [34]. It is well known that, in adults, the toxic paracetamol dose is about ten times higher than therapeutic concentration and paracetamol metabolism changes with the growth [96]; further evaluations could allow us to fully understand the extremely premature neonates metabolism [34].

It is described that neonates show an extremely variable glucuronidation rate and a limited ability for glutathione conjugation [97], with the predominance of sulphation [98], and that CYP is expressed early in postnatal life in full-term neonates while this is not well known in preterms [99].

However, clinical evidence shows a low or absent hepatic toxicity in neonates, suggesting the existence of a large therapeutic serum concentration range for paracetamol [34, 55, 100, 101].

This could depend on some mechanisms that seem to protect neonates in case of overdose such as slow oxidative metabolism and slow hepatic production of toxic metabolites and high rate of glutathione synthesis [48, 93, 102, 103].

N-acetylcysteine can detoxify NAPQI and becomes safe in neonates, so that it is used in case of subtoxic serum paracetamol concentration [94, 104] but there are no studies investigating its administration in PDA treatment [48].

For this lack of clear information about neonatal paracetamol metabolism, Cook et al. [95] performed a population pharmacokinetic model in order to define intravenous paracetamol effects and toxicity determinants and successively evaluated its predictive value with the aim of generalizing this knowledge to the whole neonates population. Their results evidenced that body weight (instead of gestational age, postmenstrual age, and unconjugated bilirubin levels) represents the principal predictor of intravenous paracetamol pharmacokinetics and the only covariate showing the adequate features to be included in the final proposed model, influencing both clearance and volume of drug distribution. According to these findings, the author suggests that the use of a parsimonious intravenous paracetamol dosage based on equivalent per kilogram (in all neonates, from extremely preterms to full-term newborns) could accommodate pharmacokinetics maturational changes, without the necessity to modify dosages and administration times according to gestational or postmenstrual age, as previously proposed by other studies. Cook et al. [95] also conclude with the observation that further studies will confirm if this simplified regimen really becomes unable to induce hepatotoxicity in all subcategories of neonates, considering the limited number of participants to the mentioned study but also the poor available knowledge about the real drug pharmacodynamics in neonates [95].

Serum paracetamol levels were evaluated in three studies of PDA management. In the study of Oncel et al. [105], these became 7.3 mcg/mL, 15.5 mcg/mL, and 14.7 mcg/mL during the three days of therapy. In the study of Yurttutan et al. [106], serum paracetamol levels after 24 h from administration became lower than 18 mcg/mL [48, 105–107].

Härkin et al. [108] analyzed 87 serum samples from 21 paracetamol treated patients and detected concentrations lower than 25.2 mg/L, without relevant accumulation. All these values resulted in therapeutic range for children (10–30 mcg/mL) [48, 107].

To examine the possible side effects of this drug, treated patients should be evaluated for alimentation disturbances, abdominal distension, oliguria, hypertension, and renal and hepatic functionality both during and after the treatment, also considering long-term consequences of clinical and subclinical side effects [94].

According to Tan and Baral [12], acetaminophen protein adducts or long chain acylcarnitines can be considered sensitive biomarkers helpful in monitoring the occurrence of potential hepatotoxic effects.

The effects of prophylactic paracetamol administration on PDA closure have been retrospectively evaluated by Aikio et al. [109] on 102 neonates born with <32 weeks of GA, demonstrating a reduction in PDA incidence from 30,7% to 14,7% after paracetamol introduction before the age of 72 hours of life, without an increase in adverse effects. However, more studies are needed to attest efficacy and safety of early PDA closure with paracetamol [109].

3.5. Surgical Ligation. Surgical closure of PDA, after failure of drug therapy or in case of contraindications to available drugs, is not exempt from adverse effects, such as vocal cord dysfunction, impaired neurological outcome, risk of BDP [14, 26, 110], retinopathy of premature (ROP), chylothorax and diaphragmatic paralysis, bleeding, pneumothorax, and cardiorespiratory failure [5].

For the high rate of complications related to PDA ligation, especially in terms of acute and severe hemodynamic side effects and worsening in neurodevelopmental outcomes, early prophylactic ligation is not recommended [33, 111, 112] and there is a spread trend of conservative ductal management, reserving this surgical approach only to those patients showing medical consequences of a large hsPDA after failure of two or more courses of medical treatments and those who need ventilator and oxygen support [14, 113–115].

Benitz and Committee on Fetus and Newborn [6] also underline that rapid and complete ductal closure occurring with ligation often leads to hemodynamic and respiratory complications, and supportive intensive care can be needed [6, 116].

4. Discussion and Recent Literature Review

Terrin et al. [82] performed on 2016 the first meta-analysis and systematic review on the results of the studies published between 2013 and 2014 evaluating paracetamol administration for PDA treatment (2 RCTs and 14 uncontrolled studies); the author reported a similar PDA closure rate of paracetamol instead of ibuprofen and a comparable safety profile, underlying that the analyzed studies included a relatively small number of neonates to consider these results as definitive [82].

In the same manner, the aim of this review is to discuss the recent published literature (2015-2016) evaluating paracetamol administration for hsPDA treatment in preterm neonates, comparing this drug to other NSAIDs or placebo or no intervention in order to add new evidence to what is already known about paracetamol efficacy and safety.

According to these features, we analyzed 15 studies (6 randomized controlled trials RCT, one of these is still ongoing, and 9 uncontrolled studies) (Table 1) and 16 reviews (Table 2), found between the articles in English language of MEDLINE using paracetamol, acetaminophen, Patent Ductus Arteriosus treatment, PDA, and preterm neonates as key words.

Data about the population characteristics (BW and GA, number of evaluated patients), type of study, kind and dose of administered drug, main outcome (closure rate), secondary outcomes (mortality, morbidity, or ductal reopening if mentioned), and safety profile for each mentioned study can be found in Tables 1 and 2.

On Table 3 we reported the different echocardiographic criteria to define hemodynamically significant PDA according to each author representing the cut-off for treatment; the table also shows the heterogeneous characteristics of the studied populations, which can make the results hardly comparable and must be taken in account during their interpretation. On Table 4, advantages, disadvantages, and side effects of PDA treatment strategies have been reported.

In the studies we discussed in this review, the standard dose of indomethacin (0,2 mg/Kg/dose/12 h for three doses), ibuprofen (10 mg/Kg/dose/day followed by 5 mg/Kg/dose/day on 2° and 3° days of therapy for 1–3 courses), or paracetamol (15 mg/Kg/dose/6 h for 3–7 days) has been used. Otherwise, we have indicated in the text the different dosage.

4.1. Results of Randomized Controlled Studies (2015-2016). At first, we report the results of five randomized controlled trials (RCTs) performed administering paracetamol and attesting its efficacy in PDA closure, comparable to ibuprofen and indomethacin. Paracetamol has also showed a safer profile, with less side effects than NSAIDs [18, 55, 85, 88, 108].

A total of 641 preterms have been included and randomized; among these, 272 received paracetamol (149 oral versus 123 iv), 139 received iv indomethacin, 25 received placebo, 105 received oral ibuprofen, and 100 received iv ibuprofen.

Bagheri et al. [18] demonstrated comparable global closure rates between oral paracetamol and oral ibuprofen, with only minimal complications in paracetamol group. Considering its high safety, the author concluded paracetamol may be used as first-choice treatment but other studies should be performed to confirm it and to evaluate also spontaneous closure of PDA. In any case, we must underline the high GA (mean 31,53 weeks) of the newborns in this study, which could make these results hardly comparable with other trials.

In the study of Dash et al. [85], enteral paracetamol showed a PDA closure rate of 100% and no hepatotoxicity was detected. This surprising high result about paracetamol efficacy deviates from other studies' results, but it must be considered that this RCT evaluated patients showing a mean GA of 31,6 weeks, higher than neonates in other trials.

In contrast to many authors' results, Dash et al. [85] reported a high intestinal bleeding occurrence in paracetamol group (26.3%) and this result, according to El-Mashad et al. [55], could be influenced by the high osmolality of paracetamol used in their trial. The authors confirmed the global safety of enteral paracetamol treatment and concluded attesting its validity in preterms hsPDA management; however they affirm that more data are needed, especially long-term studies, to evaluate neurodevelopmental outcome effects of paracetamol administration [85].

Härkin et al. [108] demonstrated a faster hsPDA closure rate in paracetamol group (95%) than in placebo group. The authors used a different drug dosage, administering 20 mg/kg of paracetamol at 24 hours of life, followed by 7,5 mg/kg every 6 h for 4 days and the ductus closed at a mean of 177 hours of postnatal life in treated patients versus 338 hours in controls. However, GA influenced ductal closure; in fact, in extremely preterm infants (<27 weeks' GA), paracetamol did not show a significant effect; among these, 4 preterms (50%) required PDA ligation. Paracetamol seemed to be more efficacious in males than in females; this result did not show a statistical relevance but, among the studies considered in this review, this is the only trial evaluating and pointing out a difference in gender drug response. No adverse effects or signs of hepatotoxicity have been described and serum paracetamol concentration became as a result included in the safety range,

Table 1: Experimental studies investigating paracetamol administration in PDA treatment (2015-2016).

Authors	Year	Patients	GA (weeks)	BW (gr)	Treatment	Study	Dose	Results	Side effects
Härkin et al. [108]	2016	48	<32	Mean: 1.220 Para group, 1.120 placebo group	iv Para ($n = 23$) versus placebo	Controlled trial, fase I.II, double blind, randomized	20 mg/kg at 24 h followed by 7,5 mg/kg every 6 h for 4 days	Faster closure in Para group (95% CI 0.25–0.97, $P = 0.016$). Mean (95% CI) postnatal closure age 177 h (31.1–324) for Para versus 338 h (118–557) for controls ($P = 0.045$) GA > 27 w: mean postnatal closure age 80 h in Para group versus h placebo 322 h ($P = 0.004$). GA < 27 w: $n = 8$, not Para effect on ductus ($P = 0.63$), 4 (50%) required PDA ligation (Para $n = 3$, placebo $n = 1$). Para closure in males (HR 0.31, 95% CI 0.12–0.85, $P = 0.023$), higher than females (95% CI 0.27–1.96, $P = 0.52$)	No adverse effects or hepatotoxicity
Valerio et al. [87]	2016	48	23–32	Mean: "first-line" group 853,3 ± 286,9; "rescue" group 887,7 ± 297	iv Para ($n = 48$) ($n = 30$ for ibu contraindications. $N = 18$ after ibu failure)	Observational longitudinal prospective study	ibu 10-5-5 mg/Kg versus Para 15 mg/kg every 6 h	No significant differences in closure rate "first-line" versus "rescue" groups after 2 (56.7% versus 61.1%, $P = 0.7624$) or 3 cycles (63.3 versus 77.8% $P = 0.2959$).[(1)] Surgical ligation rate higher in "first-line" (26.7%) versus "rescue" group (16.7%). Reopening rate In "first-line" higher versus "rescue" group (10 versus 0%), and 91% of needing surgical closure had GA ≤27 w	No hepatotoxicity

Table 1: Continued.

Authors	Year	Patients	GA (weeks)	BW (gr)	Treatment	Study	Dose	Results	Side effects
Bagheri et al. [18]	2016	129	<37	Mean: 1.646,26 Para group, 1.642,62 ibu group	Oral Para ($n = 67$) versus oral ibu ($n = 62$)	Randomized trial	ibu 20-10-10 mg/Kg versus Para 15 mg/kg every 6 h	After 1° course of treatment: closure in 82.1% $n = 55$ patients oral Para vs. 75.8% $n = 47$ oral Ibu ($P = 0.38$). 2° course: closure oral Para group versus oral ibu group 50% versus 73.3% ($P = 0.21$)	No significant complications for both drugs
Dani et al. [84]	2016	110	$25–31+^{6}$	—	Para iv ($n = 55$) versus ibu iv ($n = 55$)	Randomized multicenter controlled study	Para 15 mg/kg/dose every 6 h for 3 days. Ibu 10-5-5 mg/kg/day	On course	—
Tofé Valera et al. [117]	2016	3	<32	<1.900	3 iv Para for ibu contraindications	Case series	Para 15 mg/kg every 6 h for 3–6 days	100% closure (3/3 Patients)	Hypertransaminasemia $n = 1$ patient
Yang et al. [88]	2016	87	<37	Mean: Para group 2.219 ± 606, ibu group 2.091 ± 657	Oral ibu ($n = 43$) versus oral Para ($n = 44$)	Randomized controlled trial	10-5-5 mg/kg ibuprofen versus Para 15 mg/kg every 6 h for 3 days	Plasma and urinary PGE2 levels in Para group (45.0 ± 36.9 ng/l) significantly lower than ibu group (73.5 ± 44.8 ng/l, $P = 0.002$). Closure rate similar between Para ($n = 31$, 70.5%) and ibu groups (=33, 76.7%, $P = 0.50$). Lower oliguria in Para group ($n = 1$, 2.3%) than ibu group ($n = 6$, 14.0%)	Low adverse events in Para group

Table 1: Continued.

Authors	Year	Patients	GA (weeks)	BW (gr)	Treatment	Study	Dose	Results	Side effects
Memisoglu et al. [118]	2016	11	23–30+^{3}	415–1.580	iv Para for contraindications to Ibu or Indo	Case series	15 mg/kg every 6 h for 3 days	Closure rate 90.9% (n = 10/11)	No adverse or side effects
Sancak et al. [119]	2016	18	n.k.	<1.500	iv Para (n = 10) versus oral Para (n = 8)	Retrospective study	15 mg/kg every 6 h for 3 days	After 2 courses of treatment, higher closure rate in oral Para group versus iv Para group (88% versus 70%), not statistically significant (P = 0.588)	Liver function tests normal
Weisz et al. [120]	2016	26	<28	n.k.	Oral Para for Indo failure	Retrospective cohort study	15 mg/kg every 6 h for 3–7 days	Echo indices improved in 46% (n = 12) infants (3 closed and 9 reduced to mild shunt), all avoiding ligation. No echo improvement in 54% (n = 14) infants (n = 8/14 underwent ligation)	No differences in 2^{ry} outcomes

Table 1: Continued.

Authors	Year	Patients	GA (weeks)	BW (gr)	Treatment	Study	Dose	Results	Side effects
Mohanty et al. [121]	2016	40	<32	n.k.	Para for ibu contraindication	Prospective study	n.k.	72.5% (n = 29/40) successful closure versus 29.5% (n = 11/40) not response	No major complications
El-Mashad et al. [55]	2016	300	<28	<1.500	iv Para (n = 100) versus iv ibu (n = 100) or iv Indo (n = 100)	Prospective randomized Study	Para 15 mg/kg/6 h iv. Ibu iv 10 mg/kg on 1° day, 5 mg/kg on the 2° and 3° days. 0.2 mg/kg/12 h ind iv for 3 doses	No significant difference in closure rate (P = 0.868). Cumulative closure rate higher after 2° course (88% in Para group, 83% in ibu group, 87% in Indo group). After closure, improvement in ventilatory settings (P < 0.001)	Serum creatinine levels and BUN higher in Indo versus ibu group (P value < 0.001). PLT and UOP lower in Indo than ibu group (P < 0.001). Increase in bilirubin levels in ibu group (P < 0,05). No difference of HB level or liver enzymes (P > 0.05). GIT bleeding rate higher in Indo and Ibu groups (P < 0.05)
Roofthooft et al. [22]	2015	33	<28	<1.500	Para for contraindication to ibu or failure of treatment	Prospective observational single center Study	ibu 1 or 2 courses (10 mg/kg on 1° day, 5 mg/kg on the 2° and 3° days). Para iv (15 mg/kg/6 h) 3–7 days	Group A: 46% Para efficacy Groups B-C: Para treatment ineffective in all patients (2 died, in the others surgical ligation)[(2)]. After previous exposure to ibu, Para ineffective in 100%	No adverse effects

Table 1: Continued.

Authors	Year	Patients	GA (weeks)	BW (gr)	Treatment	Study	Dose	Results	Side effects
Dash et al. [85]	2015	77	Mean: 28,5 Para group, 28,9 ibu group	≤1.500	Enteral Para (n = 38) versus iv Indo (n = 39)	Randomized controlled trial	Para per os (15 mg/kg/dose, 6 hourly for 7 days) or iv Indo (0.2 mg/kg/dose, once daily for 3 days)	Closure rate 100% (n = 36/36) in enteral Para group versus 94.6% (n = 35/37) in iv Indo group (P = 0.13). 2° outcomes similar in the two groups. 26,3% of GI bleeding in Para group	No hepatotoxicity in Para group
Tekgündüz et al. [122]	2015	13	24–31	470–1.390	iv Para for contraindication to oral ibu	Case series	N = 1 patient 15 mg/kg/dose every 6 h N = 12 patients, 10 mg/kg/dose every 6 h for 3 or 4 days	PDA closure in 76.9% (n = 10/13)	Hepatotoxicity N = 1 patient
Peña-Juárez et al. [123]	2015	10	30–36	840–1.600	Oral Para	Case series	15 mg/kg/dose every 6 h for 1-2 days	Closure in n = 7/10 patients (70%); n = 3/10 (30%) patients underwent surgical ligation N = 2 patients died (causes not related to drug administration)	No significant changes in liver function and platelet count

Para = paracetamol. Ibu = ibuprofen. Indo= indomethacin. iv = intravenous. GA = gestational age. BW= birth weight. w = weeks. gr = grams. BUN = serum blood urea nitrogen. UOP = urine output. HB = hemoglobin. PGE2 = Prostaglandin E2. GI = gastrointestinal. n.k. = not known data. (1) "First-line" group: paracetamol as first-choice therapy for ibuprofen contraindications. "Rescue" group: paracetamol after ibuprofen failure or for development of contraindications during its administration. (2) Group A: paracetamol first choice for ibuprofen primary contraindications. Group B: paracetamol after ibuprofen incomplete courses for development of contraindications during its administration. Group C: paracetamol after failure of two complete ibuprofen courses.

Table 2: Reviews investigating paracetamol administration in PDA treatment (2015-2016).

Authors	Year	Patients	GA (weeks)	BW (gr)	Treatment	Study	Dose	Results	Side effects
Benitz and Committee on Fetus and Newborn [6]	2016	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	Absent long term outcomes improvement for early routine PDA closure in preterms	Rapid, complete therapeutic closure often leads to severe hemodynamic and respiratory collapse
Oncel and Erdeve [48]	2016	149	<30	n.e.	Oral or iv Para for Ibu contraindication or failure (after 2 courses)	7 Studies	Para 15 mg/Kg every 6 hours for 3 days	Closure rates similar Para versus Ibu. GA < 28 w: reduced efficacy of Para	Less GI bleeding/jaundice in Para group. Transient increase in liver enzymes after iv Para. No differences in other side effects
Tan and Baral [12]	2016	88	23–37	n.e.	Oral or iv Para	12 Studies	Para 15 mg/Kg every 6 hours for 3 days	Closure rate 76.1% (n = 67/88)	Transitory liver enzymes elevation in 6 patients
Vettukattil [33]	2016	n.e.	n.e.	n.e.	n.e.	n.e.	Para 15 mg/Kg every 6 hours for 3 days. Ibu 10-5-5 mg/Kg on 1° day, 5 mg/kg on the 2° and 3° days	Evidence supporting intervention in PDA closure in extremely preterms within 72 h of birth	n.e.
Rostas and McPherson [34]	2016	n.e.	n.e.	n.e.	n.e.	n.e.	Para 15 mg/kg/dose every 6 hours for 3–6 days	Para reasonable strategy for PDA treatment if COX inhibitors contraindicated or failure. The same efficacy enteral Para versus enteral Ibu	Increase GI bleeding in Ibu group. Appropriate monitoring for toxicity is required during Para administration
van den Anker and Allegaert [24]	2016	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	No indication for prophylactic therapy of PDA	Less adverse effects of Para versus Ibu or Indo

Table 2: Continued.

Authors	Year	Patients	GA (weeks)	BW (gr)	Treatment	Study	Dose	Results	Side effects
Sivanandan and Agarwal [124]	2016	n.e.	n.e.	n.e.	Para indicated in infants with NSAIDs contraindications	n.e.	n.e.	Similar efficacy Para versus NSAIDs (limited data available from randomized trials). Actually, Para not first choice, more trials needed	n.e.
Bancalari and Jain [125]	2016	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	Prophylactic Indo reduce vasopressor-dependent hypotension in preterms. More studies needed to evaluate transient hypotension long-term consequences on respiratory or neurologic outcomes	n.e.
Chandrasekaran [126]	2016	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	Para seems effective and safer than COX inhibitors in closing PDA	n.e.

Table 2: Continued.

Authors	Year	Patients	GA (weeks)	BW (gr)	Treatment	Study	Dose	Results	Side effects
Terrin et al. [82]	2016	371	n.e.	n.e.	Para for Ibu resistance or contraindication	Meta-analysis on 14 uncontrolled studies and 2 RCTs	30–60 mg/kg/die	GA < 28 w: reduced efficacy of Para (67% closure rate). GA ≥ 28 w: 89% closure rate; RCTs showed no significant difference Para versus Ibu groups for mortality, morbidity or ductal reopening	Para: safer profile GI bleeding (95% CI = 0.07–1.03), hyperbilirubinemia (95% CI = 0.34–0.97), oliguria (95% CI = 0.25–1.79.) Para: transient increase in ASTs or γGT in a small n° of patients
Ohlsson and Shah [127]	2015	250	n.e.	n.e.	Oral Para versus oral Ibu	Meta-analysis on 2 large RCTs	n.e.	No significant difference oral Para versus oral Ibu in closure rate after 1° course (95% CI 0.67, 1.22). No significant differences Para versus Ibu groups in 2^{ry} outcomes except duration for hyperbilirubinemia and need of supplemental oxygen	Lower in Para group
Evans [14]	2015	250	<36 (n = 90 < 30 w)	n.e.	Oral Para	2 Studies	Para 15 mg/kg/dose. Ibu 10-5-5 mg/Kg on 1° day, 5 mg/kg on the 2° and 3° days	Similar efficacy Para versus Ibu in 2 RCT (79% versus 81% and 77% versus 72%). Reopening rate 1° RCT (24.1% versus 16.1%; P = 0.43 Para versus Ibu group). Para group (n = 1 patient) Ibu group (n = 2) surgical ligation (GA 26 w). 2° RCT closure rate: Para 56.3% (n = 45), Ibu 47.5% (n = 38 P = 0.268). Reopening rate n = 5 and n = 6 of Para versus Ibu. N = 4 in each group closure after 2° course	Less collateral GI effects (bleeding/jaundice) in Para group

Table 2: Continued.

Authors	Year	Patients	GA (weeks)	BW (gr)	Treatment	Study	Dose	Results	Side effects
Jain and Shah [128]	2015	n.e.	n.e.	n.e.	Oral Para versus oral Ibu	2 RCTs	Para 15 mg/kg per dose every 6 hours for 2 to 7 days	No differences Para versus Ibu closure rate or 2^{ry} outcomes: (1° study, 95% CI, 0.60–1.15 68 and 2° study, 95% CI 0.57–2.62). NSAIDs first choice therapy but Para safe option	No difference in side effects
El-Khuffash et al. [31]	2015	36	24^{+4}–27^{+6}	645–954	Para iv	Retrospective	15 mg/kg/die for 3–6 days	Para closure rate 25% (n = 9/31). No responders 11% (n = 4/31). PDA ligation 64% (n = 23/31). Median days PDA constriction 0.9 days [0.5–1.9] post-treatment. PDA definitely closed at discharge n = 30/31. N = 10 infants received Ibu prior to Para treatment. In this cohort, Ibu not efficacious in any infant. N = 4/9 infants pretreated with Ibu:PDA closure (44%)	No side effects
Le et al. [129]	2015	338	n.e.	n.e.	Ibu resistance or contraindication	12 studies, 2 RCTs	15 mg/kg/dose every 6 h for 3 days	>76% success PDA closure (case reports); PDA closure rates from 72.5 to 81.2% in RCTs	Few incidents of elevated liver enzymes

n.e. = not explained in the review. Para = paracetamol. Ibu = ibuprofen. Indo = indomethacin. iv = intravenous. GA = gestational age. BW = birth weight. w = weeks. gr = grams. GI = gastrointestinal. NSAIDs = nonsteroidal anti-inflammatory drugs. COX = cyclooxygenase.

Table 3: Echocardiographic criteria to define hemodynamically significant PDA (hsPDA), representing the cut-off for treatment according to the authors, and Heterogeneous characteristics of the studied populations.

Authors	Year	PDA size	LA/Ao ratio	(i) Studied population (ii) Postnatal age at diagnosis	(i) Birth weight (ii) Gestational age
Roofthooft et al.[1] [22]	2015	>2 mm	>1,6	(i) 33 VLBW with these echo features (ii) Median 51 days group A, 30 days group B, none in group C[2]	(i) <1500 g (ii) <28 w
Dash et al. [85]	2015	≥1,5 mm	>1,5	(i) 77 preterms with these echo features (ii) <48 hours	(i) ≤1500 (ii) Mean: 28,5 w Para group, 28,9 w Ibu group
Peňa-Juárez et al.[3] [123]	2015	≥1 mm	>1,8	(i) 10 preterms with these echo features (ii) <10 days	(i) 840–1600 g (ii) 30–36 w
Härkin et al. [108]	2016	Diameter > 50% LPA	>1,4	(i) Among 63 screened VLBW patients, 48 had these echo features and underwent randomization (76,2%) (ii) n.e.	(i) Mean: 1220 g Para group, 1120 placebo group (ii) <32 w
Valerio et al.[4] [87]	2016	≥1,4 mm/Kg	≥1,4	(i) Among the 196 studied preterms, 102 had PDA (52%), and, among these patients, 48 (47,1%) had these echo features (ii) Echo performed at 48–72 hours	(i) Mean: 853,3 g "first-line" group, 887,7 g "rescue group"[5] (ii) 23–32 w
Bagheri et al. [18]	2016	>1,5 mm	>1,2	(i) 160 patients enrolled for hsPDA but 31 excluded. Final group: 129 patients (ii) ≤14 days	(i) Mean: 1646 g Para group, 1642 g Ibu group (ii) <37 w
El-Mashad et al. [55]	2015	>1,5 mm	>1,6	(i) 300 preterms with these echo features (ii) ≤ 14 days	<1500 g <28 w
Dani et al. [84]	2016	>1,5 mm	>1,3	(i) On course (ii) 48–72 hours	(i) n.e. (ii) 25–31^{+6} w
Tofé Valera et al. [117]	2016	>2 mm	>1,5	(i) 3 preterms (ii) 3, 5 and 14 days	(i) <1900 g (ii) <32 w
Yang et al. [88]	2016	>1,4 mm	>1,4	(i) Among 96 neonates with these echo features, 87 underwent randomization (ii) 15 hours–10 days	(i) Mean: 2091 g Ibu group, 2219 g Para group (ii) <37 w
Memisoglu et al. [118]	2016	>1.4 mm/kg	>1.4	(i) 11 preterms (ii) n.k.	(i) 415–1580 g (ii) 23–30^{+3} w
Benitz and Committee on Fetus and Newborn [6]	2016	≥1,5 mm	≥1,5	(i) n.e. (ii) n.e.	(i) n.e. (ii) n.e.

Table 3: Continued.

Authors	Year	PDA size	LA/Ao ratio	(i) Studied population (ii) Postnatal age at diagnosis	(i) Birth weight (ii) Gestational age
Tan and Baral [12]	2016	≥1,4 mm	≥1,5	(i) n.e. (ii) n.e.	(i) n.e. (ii) 25–37 w
Vettukattil [33]	2016	>1.4 mm	>1.4	(i) n.e. (ii) n.e.	(i) n.e. (ii) n.e.

PDAsize: mm or mm/Kg; PA = Pulmonary Artery; La/Ao ratio = left atrium/aorta diameter; LVO/FVC = Left ventricular output and systemic flow through superior vena cava; LPA = Left Pulmonary Artery; Qp/Qs = Pulmonary/Systemic Flow Ratio; Para = paracetamol; Ibu = ibuprofen; echo = echocardiographic; w = weeks; g = grams; n.e. = not explained in the text; n.k. = not known data; [1]PDA/LPA > 0,8; [2]Group A: paracetamol first choice for ibuprofen primary contraindication. Group B: paracetamol after ibuprofen incomplete courses for development of contraindication. Group C: paracetamol after failure of two complete ibuprofen courses; [3]Qp/Qs ratio > 1,8; [4]LVO/FVC ratio ≥ 4. [5]"First-line" group: paracetamol as first-choice therapy for ibuprofen contraindications. "Rescue" group: paracetamol after ibuprofen failure or for development of contraindications during its administration.

showing that early iv paracetamol accelerates hsPDA closure without relevant side effects.

Yang et al. [88] demonstrated a similar PDA closure rate between oral paracetamol and ibuprofen, but less adverse events were detected in newborns receiving paracetamol, with lower incidence of oliguria. In conclusion this study evidenced lower toxicity, also corresponding to lower plasma and urinary PGE2 levels, in paracetamol group.

El-Mashad et al. [55] performed the first large prospective randomized study comparing the efficacy and side effects of paracetamol, ibuprofen, and indomethacin simultaneously. For this purpose, $n = 300$ neonates have been enrolled and treated with iv paracetamol ($n = 100$), iv ibuprofen ($n = 100$), or iv indometacin ($n = 100$). Global PDA closure rate did not show significant differences among the three groups and an improvement in ventilatory setting was also demonstrated after successful PDA closure. In NSAIDs groups the authors detected a significant increase in creatinine and serum blood urea nitrogen levels associated with a significant platelet count and urine output (UOP) reduction. Only among the ibuprofen treated patients was there also a significant increase in bilirubin levels. The effect in platelet reduction is absent after paracetamol treatment and this could be explained, according to the authors, by its lack of action on thromboxane, unlike NSAIDs. In conclusion, in this study, paracetamol has shown the same efficacy of indomethacin and ibuprofen in preterm neonates PDA closure but less side effects, especially for its low impact on renal function, platelet count, and GI bleeding.

4.2. Results of Uncontrolled Studies (2015-2016). Below, we report major results and findings of analyzed uncontrolled studies. A total of $n = 202$ patients have been treated with paracetamol and, among these, $n = 2$ showed hepatotoxicity.

The efficacy of paracetamol after ibuprofen failure has been evaluated in two trials by Roofthooft et al. [22] and Valerio et al. [87], finding different results. According to Roofthooft et al. [22], paracetamol is not effective in hsPDA closure in newborns with a postnatal age > 2 weeks, showing a global paracetamol success of 18% in the studied sample, significantly lower than literature data. However different effectiveness levels have been detected in various categories of treated patients: closure rate (or PDA diameter reduction) was 46% after paracetamol first-choice therapy, while this drug failed when administered after two cycles of ibuprofen. In this second group of patients, paracetamol showed 100% of failure, suggesting that, after ineffective ibuprofen treatment, paracetamol in VLBW is not effective in closing PDA, maybe for its late administration (mean 14 days) or for the patients features in this study (lower GA than other trials or variability in the hemodynamic significance criteria among the studies, showed in Table 3), although it became a safe treatment, without renal or hepatic side effects.

In the study of Valerio et al. [87], comparing the efficacy of paracetamol between a "rescue" group (after failure of ibuprofen) and a "first-line" group (contraindication for ibuprofen), no significant difference in PDA closure efficacy was detected. More patients of the "first-line" group underwent PDA surgical ligation (26.7%) and showed PDA reopening (10 versus 0%); it could also depend on more critical conditions of these patients. GA represented a closure predictor in "first-line" group and, in attempt to obtain a predictor index for PDA closure, Valerio and his collaborators demonstrated the role of CRIB score (a clinical score resulting in combination of GA, BW, sex, patient's temperature, and maximum base excess in the first 12 h of life) [130], as an independent predictor of PDA closure in "rescue" group. The authors conclude confirming the efficacy of iv paracetamol for PDA closure in VLBW and ELBW preterm population and they also suggest the oral route seems to be valid but not recommended for such infants, showing intestinal mucosa immaturity with consequent unpredictable absorption.

Instead of the results of Roofthooft and Valerio and his group [87] attested paracetamol efficacy also when administered after ibuprofen failure, suggesting the necessity of other trials to improve our knowledge.

Paracetamol efficacy becomes greater when started in the first week of life and this may be related to the higher prostaglandin circulating levels during early postnatal life; moreover, efficacy of paracetamol when administered after NSAIDs failure could be also depend on an additive effect [82].

Four small case series have been performed by Tekgündüz et al. [122], Memisoglu et al. [118], Peña-Juárez et al. [123], and Tofé Valera et al. [117] on 13, 11, 10, and 3 preterms, finding a hsPDA closure rate of 76.9%, 90,9%, 70%, and 100%,

Table 4: Comparison among different strategies of PDA treatment.

	No treatment	Indomethacin	Ibuprofen	Paracetamol	Surgical ligation
Advantages	(i) Avoid drug exposition (ii) PDA could close spontaneously	(i) In PDA prophylaxis, it reduces intraventricular hemorrhage (IVH) incidence (30%) and early pulmonary hemorrhage (35%), development of symptomatic PDA, necessity of surgical ligation (ii) Efficacy in PDA closure	(i) Efficacy in PDA closure (ii) Lower nephrotoxicity than indomethacin	(i) Efficacy in PDA closure (ii) Lower side effects instead of NSAIDs	(i) Rapid and complete ductal closure
Disadvantages	Possible lack of closure	(i) Toxicity (ii) Contraindications: active or recent hemorrhage, thrombocytopenia, sepsis, NEC, intestinal perforation, hepatic damage with severe hyperbilirubinemia, renal dysfunction	(i) Toxicity (ii) Contraindications: active or recent hemorrhage, thrombocytopenia, sepsis, NEC, intestinal perforation, hepatic damage with severe hyperbilirubinemia, renal dysfunction	(i) Toxicity still to be fully defined (ii) Long term outcomes still to be fully defined	(i) Risks of an invasive procedure (ii) Long term outcomes still to be fully defined
Standardization of dosages	—	0,2 mg/Kg/dose/12 h	10 mg/Kg/dose/day followed by 5 mg/Kg/dose/day on 2° and 3° days of therapy	15 mg/Kg/dose/6 h	—
Standardization of therapy length	—	3 doses	1–3 courses	3–7 days	—
Route of administration	—	Intravenous	(i) Intravenous (ii) oral	(i) Intravenous (ii) oral	—
Need of monitoring	—	Yes, especially for nephrotoxicity	Yes, especially for nephro- and hepatotoxicity	Yes, especially for hepatotoxicity	Yes, rapid and complete ductal closure can lead to hemodynamic and respiratory complications
Side effects	Respiratory distress syndrome (RDS), prolonged need for ventilation, pulmonary hemorrhage, bronchopulmonary dysplasia (BDP), necrotizing enterocolitis (NEC), renal function damage, intraventricular hemorrhage (IVH), periventricular leukomalacia (PLV), cerebral palsy, death	Nephrotoxicity (until acute or chronic renal failure), cerebral white matter damage, necrotizing enterocolitis (NEC), intestinal perforation, platelet dysfunction	Nephrotoxicity, pulmonary hypertension, hyperbilirubinemia, necrotizing enterocolitis (NEC), intestinal perforation, platelet dysfunction	Transient and inconstant increase in liver enzymes	Hemodynamic side effects, cardiorespiratory failure, risk of BDP, retinopathy of prematurity (ROP), vocal cord dysfunction, chylothorax, diaphragmatic paralysis, bleeding, pneumothorax, impaired neurological outcome

respectively, after paracetamol administration. Globally, hepatotoxicity (hypertransaminasemia) was described in two patients and no other significant side effects were detected.

The retrospective cohort studies of Weisz et al. [120] and Mohanty et al. [121], including, respectively, 26 and 40 patients, also demonstrated a positive response in paracetamol treated preterms for hsPDA in 46% and 72,5% of neonates in absence of complications, demonstrating that paracetamol could be a safe therapy in such infants.

The two different administration routes of paracetamol have been compared by Sancak et al. [119] in 18 VLBW newborns; hsPDA closure rate seemed to be higher in those treated with oral compared to iv paracetamol administration after two courses of therapy, but this result was not statistically significant. Both the treatments did not show hepatic toxicity. In the future, larger trials should be performed in order to define the possible differences between the two administration routes.

4.3. Results of Reviews (2015-2016). Our analysis on 16 reviews investigating the role of paracetamol in hsPDA treatment (Table 2) shows that most authors support the efficacy of this drug in ductal closure, becoming comparable to NSAIDs, with inconstant transient lower side effects (in terms of elevation of liver enzymes) instead of GI bleeding, oliguria, and hyperbilirubinemia showed after ibuprofen therapy [12, 14, 24, 34, 48, 82, 118, 127, 129]. However, an appropriated monitoring in order to early detect paracetamol toxicity is recommended [34].

In the review of Tan and Baral [12], among $n = 88$ treated patients, $n = 6$ showed transitory liver enzymes elevation [12].

A safer profile in terms of gastrointestinal bleeding and hyperbilirubinemia after paracetamol administration instead of ibuprofen has been described by Evans [14] and Terrin et al. [82].

All these authors agree with the possibility of using paracetamol in case of ibuprofen or indomethacin contraindications and/or failure but other studies are needed to confirm the safety profile of this therapy, to establish the lowest effective dose and to evaluate long-term outcomes, in particular the possibility of neurocognitive impairment, before considering it as the drug of first choice [12, 24, 34, 127].

Le et al. [129] agree with the idea that paracetamol seems to be a good alternative in PDA treatment and should be considered, in case of ibuprofen contraindication, before ligation. The author also recommends performing other trials because two studies published on 2013 found low iv paracetamol success rate [89, 131] in small groups of patients ($n = 29$ and $n = 3$); moreover, the high mean postnatal age (22 days) at paracetamol administration in the study of Roofthooft et al. must be also considered [131].

According to the reviews of Oncel et al. [48] and Terrin et al. [82], paracetamol efficacy would be lower in extremely preterm neonates (<28 weeks of GA), probably for structural limitations in these subjects, which present a higher expression of prostaglandin receptors in the wall of the ductus and a thin-walled DA, with a lower represented neointimal mounds. In these patients, administration of PG inhibitors can be followed by functional closure but less frequently by the structural ductal closure [48].

El Kuffash et al. [31] evaluated late treatment with iv paracetamol beyond the 2nd week of life which became effective in hsPDA closure, avoiding PDA ligation. Anyway, more studies are needed to confirm the role of paracetamol late administration.

Evans [14] described a similar reopening rate after PDA treatment both in paracetamol and in ibuprofen groups and also concluded underlying the same efficacy of the two drugs, which should be confirmed through other trials.

4.4. Results about Conservative Treatment. Results in support of conservative PDA treatment have been reported by Slaughter et al. [132] in their cohort study published on January 2017 and by Letshwiti et al. [133]. The first is a large study on $n = 12.018$ newborns (≤28 weeks' GA) affected by PDA, evaluated and treated in 25 different hospitals. The 32% of all infants have been treated with NSAIDs (27% received indomethacin and 7% ibuprofen) and no association was demonstrated between NSAID treatment (performed between 2 and 28 postnatal days) and the odds of mortality or BPD, mortality, or moderate-severe BPD at 36 weeks of postmenstrual age versus similar not treated preterms. The lack of definition of echocardiographic PDA features in treated and not treated population could represent a limit that makes this study about the conservative approach difficult to compare to other studies' evidence.

The study of Letshwiti et al. [133], published on December 2016, compares three different PDA management approaches: early targeted treatment, symptomatic treatment, and no treatment. Infants in symptomatic treatment group were treated in case of hsPDA. Patients in early targeted group received ibuprofen in case large PDA is evaluated by echocardiography in the first 48 h; in conservative treatment group PDA was managed with increased PEEP and fluid restriction. The author demonstrated a lower rate of ibuprofen therapy and ligation in conservative group and a significantly decreased incidence of chronic lung disease compared to symptomatic treatment group (18% versus 51%) and to patients early treated (18% versus 46%).

4.5. Results Evaluating Neurocognitive Impact. Most authors affirm the necessity to assess the long-term neurocognitive impact of PDA treatment, because it could represent a limitation in PDA management: a reduction in this outcome could support a conservative approach to PDA. In this regard, Janz-Robinson et al. [134] reported data of a retrospective population-based cohort study attesting the association between PDA treatment (medical or surgical) and risk of developmental delay, cerebral palsy, sensorineural or conductive deafness, or bilateral blindness instead of nontreated patients, at age 2-3 years, especially for neonates <25 weeks' GA. These results may validate a permissive tolerance of PDA.

On the contrary, the results of the follow-up study of Oncel et al. [135], published on 2017, attest there are no differences in neurodevelopmental outcomes at 18 and 24 months in $n = 61$ ex preterm infants (GA < or = 30 weeks), treated for PDA with oral ibuprofen or paracetamol [135].

These results suggest the necessity to fully understand the exact relation between treatment and neurodevelopmental outcome before recommending the most appropriate strategy for PDA management.

5. Patent Ductus Arteriosus and Cardiovascular Programming

Several recent studies pointed out the association between risk of adult life cardiovascular diseases and insults occurring during fetal growth and in very early postnatal period; the concept of "developmental programming" has been proposed to explain this correlation [136, 137].

According to this theory, each condition of fetal adaptation to an inadequate or deprived environment during embryonal development can constitute an insult that will make the individual more susceptible and vulnerable throughout the whole life, as a permanent adverse effect [136].

This happens because fetal and early postnatal life are "critical periods" in which tissues development is realized through high cells proliferation rate, and each occurring adverse event can conduce to negative consequences in organs growth [136, 138]. All these correlations are best demonstrated on animals models, but there is also much evidence in epidemiological human studies [136].

It is known that adverse events in the first phases of life could influence blood pressure and determinate cardiovascular disease in adult life [136, 139–142]; moreover, low birth weight may be associated with long-term cardiovascular diseases [143–146].

It is in this perspective that we consider the correct management of PDA fundamental, a cardiovascular pathology occurring in the critical prematurity period, whose incorrect treatment could potentially lead to negative "programming" with permanent cardiovascular function impairment and a variable impact on heart health.

6. Conclusions and Future Perspectives

The goal of the studies on PDA management would be to perform an individualized therapy, choosing the sartorial treatment for each of the patient characteristics, which could be the most effective as much as possible, personalized, and with the lowest side effects.

Between the available drugs for PDA treatment, paracetamol seems to be a promising alternative and most authors agree with the necessity of more trials to establish the safer dose in preterms and its efficacy. If these arguments will be confirmed, paracetamol could become the first-line therapy for hsPDA treatment.

However, non-well designed trials about proper paracetamol dosing or large randomized controlled trials (RCT) on short and long-term safety have been published in the last two years; as a consequence, we are still waiting for these important data before thinking to change the actual standard care for PDA into paracetamol.

In the next future we hope that "omics" technologies, namely, metabolomics, could be useful to assess and fully clarify the different metabolic pathways of mediators able to early predict, in an individualized way, closure of PDA allowing the avoidance of drug toxicity or a profile of metabolites able to predict the exact response to treatment or drug safety, as it is known that the high interindividual variation in therapy response is strongly related to the subject's biochemical state.

Additional Points

What Is Known. (i) In preterm infants, spontaneous closure of Ductus Arteriosus (DA) often fails or is delayed, and this condition can be associated with several short- and long-term complications. (ii) Nonsteroidal anti-inflammatory drugs (NSAIDs), traditionally used in Patent Ductus Arteriosus (PDA) treatment, are related to several side effects especially in terms of nephrotoxicity.

What Is New. (i) The results of the studies analyzed in this review mostly support paracetamol efficacy in ductal closure, showing inconstant low and transient elevation in liver enzymes as side effect. (ii) Other studies are needed to confirm the safety profile of this therapy, to establish the lowest effective dose, and to evaluate long-term outcomes before considering it the drug of first choice.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Review Article

Metabolomics and Cardiology: Toward the Path of Perinatal Programming and Personalized Medicine

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Heart diseases are one of the leading causes of death in Western Countries and tend to become chronic, lowering the quality of life of the patients and ending up in a massive cost for the Health Systems and the society. Thus, there is a growing interest in finding new technologies that would allow the physician to effectively treat and prevent cardiac illnesses. Metabolomics is one of the new "omics" sciences enabling creation of a photograph of the metabolic state of an individual exposed to different environmental factors and pathologies. This review analyzed the most recent literature about this technology and its application in cardiology in order to understand the metabolic shifts that occur even before the manifestation of these pathologies to find possible early predictive biomarkers. In this way, it could be possible to find better treatments, ameliorate the patient's quality of life, and lower the death rate. This technology seems to be so promising that several industries are trying to set up kits to immediately assess the metabolites variations in order to provide a faster diagnosis and the best treatment specific for that patient, offering a further step toward the path of the development of a tailored medicine.

1. Introduction

Cardiac pathologies are a critical health issue affecting millions of people worldwide with a constant mortality rate in particular in the elderly, a difficult prognosis, and a worsening in quality life of affected people. In fact, they tend to become chronic and lead to several complications that may affect other vital organs such as brain, lungs, and kidneys. Indeed cardiovascular diseases (CVDs) are globally the number one cause of death: more people die every year from CVDs than from any other cause (17.5 million deaths, an estimation of 31% of all deaths worldwide). People with cardiovascular pathologies or who are at high cardiovascular risk need early detection since the 80% of premature heart diseases are preventable [1].

Among the complications of these pathologies there are pulmonary edema or respiratory tract infections, kidney insufficiency, and stroke. In children, cardiovascular diseases or congenital heart malformations can lead to pulmonary hypertension and neurodevelopmental problems due to the lack of oxygen supply [2].

The pathophysiology of heart pathologies is complex. Indeed, recent findings pointed out a possible pivotal role of mitochondrial dysfunction and the subsequent altered energy metabolism in cardiac diseases, in particular in case of heart failure [3].

In general, patient management could be quite challenging and demanding; thus there is a need for the clinician to have the best tools that can improve and facilitate the diagnosis and the prognosis for these diseases.

2. Metabolomics: The Skeleton Key of Cardiac Diseases?

During the last decade, animal and human studies have applied metabolomics to cardiovascular research, using both

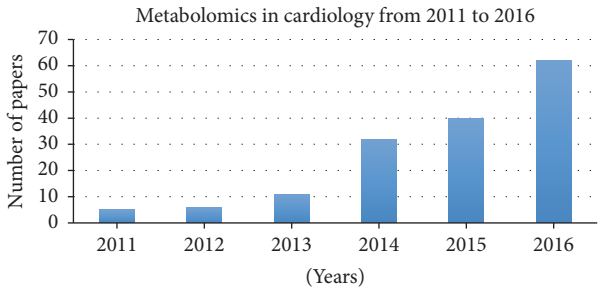

Figure 1: PubMed results concerning the studies of metabolomics and cardiology from 2011 to 2016.

targeted and untargeted approaches; as such, metabolic fingerprints have been identified for several cardiovascular risk factors and diseases [4].

Indeed, by entering the keywords "metabolomics" and "cardiology" on PubMed, this will show 161 papers, 156 written from 2011 to 2016 (Figure 1).

In fact metabolomics is a new technique that allows investigators to study the metabolic network involved in heart diseases so as to better understand their pathophysiological mechanism. Griffin et al. highlighted how the classical metabolomics technique could be applied in cardiology; indeed high resolution Magnetic Resonance Imaging (MRI) and mass spectrometry (MS) are extremely useful for gaining information about cardiac disease processes since they are both highly discriminant for a range of pathological processes starting from cardiac ischemia (angina and myocardial infarction) to heart failure [5]. These techniques could be applied to both heart tissue and biofluids such as blood and saliva with a minimum compliance needed from the patient, since their collection is not invasive. Metabolomics is one of the newest "omics" science and before its broad application, scientists tried to investigate the metabolic variation in both physiological and pathological states using proteomics or transcriptomics, but these techniques have several limitations; for instance, they are not "real-time" meaning that in case of disease occurrence the modification in the proteome or transcriptome modifications are much slower than modifications in the metabolome [6].

In Table 1 the most relevant studies are reported concerning metabolomics in cardiology on PubMed from 2011 to 2016. There are 18 studies, involving 3.874 patients in total: 2351 suffered from acute cardiac pathologies, while 736 suffered from chronic heart illnesses and 786 were controls. Most studies (9) performed ^{1}H-NMR analysis. The prevalent biofluid analyzed was plasma (10 studies) followed by serum (7 studies), urine (3), and breath (1).

In 2011 Kang et al. investigated metabolomics urinary profiles of elderly patients with ischemic heart failure, using ^{1}H-nuclear magnetic resonance (^{1}H-NMR) [23]. The patients compared to controls showed different levels of ketonic bodies as a marker of altered myocardial metabolism meaning that one of the pathological features of this pathology could be a reduction in fatty acids oxidation and an increase of glucose metabolism.

Among others, the study performed by Desmoulin et al. in 2013 underlines the predictive power of metabolomics [19]. It is a prospective study on a cohort of acute heart failure patients admitted in the cardiac intensive care unit and it assessed survival at 30 days. The plasma was collected on admission. They found out that lactate and cholesterol were the discriminating metabolites predicting 30-day mortality; in particular patient with high lactate and low cholesterol on admission showed increased mortality. This lactate/cholesterol rate in plasma could be a useful and simple parameter to apply in clinical practice in order for the physician to make the best decision in heart failure care.

Another interesting study performed by Deidda et al. in 2015 questioned whether there could be any changes in patients metabolome according to the worsening of their conditions [4]. They compared blood samples of patients affected by mild to moderate impairment of left ventricle ejection fraction and of others affected by severe left ventricle ejection fraction impairment and controls. After the statistical analysis, they identified 3 metabolic clusters related to the 3 groups. The responsible metabolites specific for each heart failure stage are 2-hydroxybutyrate, glycine, methylmalonate, and myoinositol and they might reflect both an increase in energy demand and an impaired ability to generate ATP (see also Table 1). The fingerprint identification of a still-free-of-symptoms myocardial impairment, which directly correlates with the more sensitive echocardiographic parameters of myocardial contractility, could enable a better monitoring of at-risk individuals, allowing the anticipation of systolic function worsening and/or the development of an episode of overt failure.

In line with presented data, a latest review published in the Journal of the American College of Cardiology stated that metabolomics is transforming the ability to predict, identify, and better understand several cardiac diseases, by allowing monitoring of the effectiveness of therapeutic interventions, thus leading to advancing the objective of personalizing the practice of medicine [24].

3. Perinatal Programming and Cardiology

The perinatal programming of adult diseases (DOHaD theory) states that every adverse event that may occur during pregnancy "shapes" the health status of the fetus and its development and could affects its life course [25]. Thus this theory emphasizes the importance of this delicate period of life in which everything must be timed properly in order to avoid future complications such as cardiac diseases in adolescence and adult life.

In fact Bassareo et al. investigated the cardiac outcome of young adults born with extremely low birth weight (ELBW) [26]. At 25 years of age they are at risk of major cardiac consequences such as sudden death due to the prolongation in QT interval or they are at higher risk of hypertension due to the reduced brachial-flow mediated vasodilatation compared to those born appropriate for gestational age (see also Table 2) (Bassareo PP, "Long Term Problems in Young Adults Born ELBW" [26–30]).

It is therefore a big challenge, on the opposite side of the life span, to try to predict the cardiac outcome of the neonate during pregnancy. Metabolomics seems to have made it

Table 1: Recent relevant works concerning metabolomics in cardiology are shown chronologically with the main metabolites shifts.

Authors	Patients	Methods	Sample	Metabolites results
Feng et al. 2016 [7]	59 CHD patients and 43 healthy controls	Untargeted metabolomics method	Plasma, urine	↑ GlcNAc-6-P and mannitol in CHD
Ahmad et al. 2016 [8]	41 patients with end-stage heart failure	Tandem flow injection Mass spectrometry	Plasma	↑ long chain acetylcarnitines in chronic heart failure
Oni-Orisan et al. 2016 [9]	123 patients with coronary artery disease (CAD) versus 39 controls	Mass spectrometry	Plasma	↓ cytochrome P450-derived epoxyeicosatrienoic acids metabolites in CAD
Deidda et al. 2015 [4]	24 heart failure patients versus 9 controls	^{1}H-NMR	Plasma	↓ 2-hydroxybutyrate, in HF patients ↑ glycine, methylmalonate, and myoinositol in HF patients ↑ acylcarnitines, carnitine, creatinine, betaine amino acids, ketone bodies in HF patients
Zordoky et al. 2015 [10]	44 HF patients versus 20 controls	LC/MS ^{1}H-NMR	Serum	↓ phosphatidylcholines, lysophosphatidylcholines, sphingomyelin in HF patients
Cheng et al. 2015 [11]	401 HF patients versus 114 controls	Mass spectrometry	Plasma	↓ phosphatidylcholines, arginine ↑ ornithine, spermidine, spermine phenylalanine, tyrosine

Table 1: Continued.

Authors	Patients	Methods	Sample	Metabolites results
Würtz et al. 2015 [12]	1373 cardiovascular events	Quantitative nuclear magnetic resonance	Serum	↑ phenylalanine, monounsaturated fatty acids in cardiovascular events ↓ omega 6 fatty acids, docosahexaenoic acids in cardiovascular events
Zhong et al. 2014 [13]	157 hypertension patients versus 99 controls	^{1}H-NMR	Serum	↑ VLDL, LDL, lactic acid, acetone ↓ valine, alanine, pyroacemic acid, inose, p-hydroxyphenylalanine, methylhistidine in hypertension patients
Vaarhorst et al. 2014 [14]	79 cases of coronary heart disease	^{1}H-NMR	Plasma and serum	↑ ornithine, TMAO ↓ valine, arginine, creatinine
Shi et al. 2014 [15]	45 cases of coronary heart disease versus 15 controls	^{1}H-NMR	Plasma	↑ leucine, N-acetyl glycoprotein, α-glucose, β-glucose, phenylalanine, acetone, HDL, glutamate, glutamine, methylalanine, lysine, tyrosine, ornithine, taurine, proline, lactic acid, tryptophan, valine, acetyl-glutamic acid ↓ β-hydroxy-isobutyric acid
Rizza et al. 2014 [16]	17 major cardiovascular events (MACE) patients versus 50 controls	Mass spectrometry	Serum	↑ medium and long chain acylcarnitines in MACE patients

Table 1: Continued.

Authors	Patients	Methods	Sample	Metabolites results
Kalim et al. 2013 [17]	100 individuals dead of a cardiovascular cause versus 100 controls	Liquid chromatography/mass spectrometry	Plasma	↑ oleoyl carnitine in CV patients
Tenori et al. 2013 [18]	185 heart failure patients versus 111 controls	^{1}H-NMR	Serum, urine	↑ phenylalanine, tyrosine, isoleucine, creatine, TMAO, lipid, formate, lipoprotein, hypoxanthine, proline, urea, dimethylamine, serine, acetate, methanol ↓ valine, choline, arginine, creatinine, dimethylsulfone, Gln+ Gli, alanine, l-dopa, dimethylglycine, citrate, lactate, lysine, uridine, methionine
Desmoulin et al. 2013 [19]	126 acute heart failure (AHF) patients	^{1}H-NMR	Plasma	↑ lactate + ↓ low cholesterol = ↑ short term mortality
Samara et al. 2013 [20]	25 acute decompensated heart failure (ADHF) patients versus 16 controls	Selected ion flow tube mass spectrometry	Breath	↑ acetone, pentane in ADHF
Magnusson et al. 2013 [21]	253 cardiovascular disease patients (CVD) versus 253 controls	Liquid chromatography/mass spectrometry	Plasma	↑ branched and aromatic amino acids in CVD
Bodi et al. 2012 [22]	20 angioplasty induced myocardial ischemia versus 9 controls	^{1}H-NMR	Serum	↑ phosphoethanolamine, lactate, glucose, tyrosine, phenylalanine, glycerol in AIMI
Kang et al. 2011 [23]	15 heart failure patients versus 20 controls	^{1}H-NMR	Urine	↑ acetate, acetone, methylmalonic acid, cytosine, phenylacetylglycine ↓ 1-methylnicotinamide

Table 2: Possible long-term consequences in adulthood to subjects born with extremely low birth weight: suggestion for diagnosis and care.

Possible consequences in adulthood	Risks	Suggestion for diagnosis and care
Increase in the QT interval of ECG in some subjects	Risk of arrhythmia and sudden death	ECG monitoring Avoidance of drugs that increase the QT interval
Reduced vascular elasticity	Risk of hypertension	Blood pressure monitoring
High ADMA levels	Risk of acute cardiovascular problems	ECG and blood pressure monitoring
Increase in microalbuminuria and urinary NGAL, reduction of kidney volume	Risk of chronic kidney insufficiency	Urine stick monitoring, albuminuria, creatinine, and cystatin C in the blood, kidney ultrasound

possible even though to our knowledge there is only one study of metabolomics in pregnancy performed by Bahado-Singh et al. [31]. Their aim was to identify metabolomics markers in maternal first-trimester serum for the detection of fetal congenital heart defect. Serum from mothers of CVD fetuses showed a significant disturbance in acylcarnitine and sphingomyelin and other metabolites related to an abnormal lipid metabolism. These findings may help the future development of devices to be used at the bedside similar to those that are already being sold to assess the cardiac troponin in patients with suspected acute coronary syndrome by just a finger prick of blood [32].

Moving from neonates to infants and young adults (since CVDs have a long latent period), metabolomics could be a useful tool to investigate the actual role of genetic predisposition. It could help understand whether a particular gene mutation is protective or harmful and in which metabolism it is involved or if there are any sex differences. A very interesting study concerning this topic was performed by Klein et al. in 2014 [33]. They studied the SORT1 gene through genomics and metabolomics and were able to determine several effects of the mutations of this gene in young males and females.

4. Metabolomics Cross Talks with Microbiomics Even in the Occurrence of Heart Diseases?

Metabolomics allows not only measuring changes in metabolites concentrations, but also discriminating those of human origin from those of microbial origins; in fact several authors consider this technology as the Rosetta Stone of microbiomics [34]. Indeed there are 3 types of metabolites in humans: those of human origins (produced only by eukaryotic cells); those of microbial origins (produced only by prokaryotic cells); and those of common origins [35]. Among the most studied ones is hippurate which both is a marker of renal function and can be produced by gut microbiota as well [36]. Nevertheless, in most cases, the origin of the molecules might not be unambiguously determined by using only metabolomics, but this technique is unique in its possibility of simultaneously analyzing molecules from both host and microbes in a single measurement. In a review of 2013 written by Russell et al. they highlighted the different fates of choline for the first time [37]. This metabolite, involved in the methionine-betaine-choline cycle, if metabolized by an altered microbiota, is transformed into trimethylamine N-oxide (TMAO). It is found in several peripheral tissues but in particular in the arterial epithelium in case of atherosclerosis. At the beginning of this year, a very interesting study was performed by Feng et al. in which the integrated metabolomics and metagenomics analysis of plasma and urine allowed identifying metabolites of microbial origins in coronary heart disease [7]. These molecules, mannitol and N-acetyl-D-glucosamine-6-phosphate among others, are related to a particular strand of *Clostridium* spp. or *Streptococcus* spp., indicating a possible role of the dysbiosis in the occurrence of this pathology. In November 2016 a review written by Jonsson and Bäckhed was published, concerning all the recent literature about the role of gut microbiota in atherosclerosis [38]. They stated that specific strand of bacteria, Proteobacteria, can be found in the atherosclerotic plaque. This phylum comprises the genera of *Helicobacter* and *Chryseomonas* and it is the most abundant in the plaque. Actinobacteria can be found in the plaque as well, while in patients with coronary artery disease (CAD) the number of Bacteroidetes decreases compared to controls and the ratio of Firmicutes/Bacteroidetes increases. They also proposed 3 possible mechanisms by which microbiota could affect the development of atherosclerotic plaque.

The first one: bacterial infection activates the immune system causing an excessive inflammatory response that may turn out to be dangerous, independently of the site of invasion. The subsequent proatherogenic response could be mediated by Toll-like receptor 4 expressed in macrophages.

The second: the TMAO production could initiate the activation of platelets and foam cells.

The third: the production of noxious molecules such as the previously mentioned TMAO is related to the diet and gut microbiota metabolites.

With metabolomics it is possible to demonstrate that apparently healthy young adults who were born with birth weight < 1000 g present a specific profile compared to apparently healthy young adults who were born at term. That is perinatal programming.

Differences in the two groups were related to the alterations in the arginine and proline metabolism, in the purine and pyrimidine metabolism in the histidine, in beta-alanine metabolism, and in the urea cycle [39].

5. Future Perspectives

The most investigated cardiac pathologies are heart failure, coronary heart disease, and myocardial infarction.

Several studies displayed an alteration of metabolites concerning lipid metabolism, highlighting the energy imbalance as a peculiar feature of such pathologies.

On the other hand, some authors showed different metabolites that indicate an interaction between diet and microbiota.

These findings open up unusual scenarios to the cardiologist and although it is normal to feel some sort of incredulity, they could pave the way to new possibilities of early diagnosis and individualized treatment. Congenital malformations, gut colonization by microbiota, individual genetic arrangement, and its interplay with both behavioral and risk factors, such as drugs assumption, can influence the occurrence of heart diseases. Metabolomics, for its peculiarities, seems to be the most promising technology to investigate the individual predisposition or the eventual long-term prognosis of these pathologies.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Research Article

Parental Genetic Variants, MTHFR 677C>T and MTRR 66A>G, Associated Differently with Fetal Congenital Heart Defect

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Background. Congenital heart defect (CHD) is one of the most common birth defects in the world. The methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) genes are two of the most important candidate genes for fetal CHD. However, the correlations between the two genes and fetal CHD were inconsistent in various reports. Therefore, this study is aimed to evaluate the parental effects of the two genes on fetal CHD via three genetic polymorphisms, MTHFR 677C>T (rs1801133), MTHFR 1298 A>C (rs1801131), and MTRR 66A>G (rs1801394). *Methods*. Parents with pregnancy history of fetal CHD were divided into two subgroups: ventricular septal defect (VSD) (21) and non-VSD groups (78). VSD, non-VSD, and 114 control parents (controls) were analyzed in this study. Genotyping of these genetic polymorphisms was done by sequencing. *Results*. The MTHFR 677C>T polymorphism of either mothers or fathers was independently associated with fetal non-VSD ($P < 0.05$) but not VSD, while the MTRR 66A>G polymorphism was independently associated with fetal VSD ($P < 0.05$) but not non-VSD. No significance was found for MTHFR 1298A>C polymorphism. *Conclusion*. In either maternal or paternal group, the MTHFR 677C>T polymorphism was independently related to fetal non-VSD, while the MTRR 66A>G polymorphism was independently related to fetal VSD.

1. Introduction

Around the world, periconceptional folic acid intake in females is thought to reduce the risk of CHD [1] in the newborn. Therefore, interest in the genetic susceptibility to CHD has led to a growing attention to the study of polymorphisms of genes involved in folate metabolism, especially the two key genes—MTHFR and MTRR.

In recent years, many studies reported that two genetic polymorphisms of the two genes, MTHFR 677C>T and MTRR 66A>G, could cause elevated blood homocysteine (Hcy) level in human [2–6]. In several human studies [7–9], the elevated maternal blood Hcy level or amniotic fluid Hcy level was correlated with CHD in embryo. Moreover, high dosage Hcy injection treatment in avian embryo led to embryonic VSD [10]. Therefore, MTHFR and MTRR deficiency seemed to have adverse effects on fetal CHD development and probably via their effects in affecting Hcy level in both pregnant women and embryos.

Several studies reported that the two MTHFR 677C>T and MTHFR 1298 A>C genetic polymorphisms of both pregnant women and fetuses were related to fetal CHD [11–13]; however, some other studies showed contradictory results. For example, in a small Italian population study [14] and a large population metastudy [7], these two studies reported that both maternal and fetal MTHFR 677C>T and MTHFR 1298 A>C polymorphisms were not related to fetal CHD. Moreover, some studies reported that only maternal MTHFR 677C>T was associated with fetal CHD but not MTHFR 1298A>C [15, 16].

Table 1: Classification of congenital heart disease.

CHD types	PA	DORV	TGA	TOF	UVH	SA	IPDA	AVS	PVS	VSD
CHD groups	*Non-VSD parents (78)*									*VSD parents (21)*
Number	5	2	3	22	7	8	22	4	5	21
%	6.4	2.6	3.8	28.2	9.0	10.2	28.2	5.1	6.4	100

Note: PA: pulmonary atresia, DORV: double outlet of right ventricular, TGA: arteries transposition, TOF: tetralogy of Fallot, UVH; univentricular hearts, SA: single atrium, IPDA: isolated patent ductus arteriosus, AVS: aortic valve stenosis, PVS: pulmonary valve stenosis, and VSD: ventricular septal defect.

Table 2: BMI and age distribution.

		Father (kg/m^2)	P_1	Mother (kg/m^2)	P_1	P_2
BMI	Control	23.308 ± 2.607		21.345 ± 2.210		$1.110E-06$
	Non-VSD	23.165 ± 3.093	0.739	21.761 ± 2.143	0.193	$9.114E-05$
	VSD	23.136 ± 2.245	0.967	21.172 ± 2.275	0.254	0.008
Age	Control	29.789 ± 2.585		27.781 ± 2.205		$9.063E-39$
	Non-VSD	30.256 ± 2.525	0.216	28.077 ± 2.278	0.368	$2.256E-30$
	VSD	30.714 ± 2.305	0.128	28.714 ± 1.953	0.072	$1.082E-07$

Note: BMI value is represented as mean ± SD; P_1 is the 2-tail unpaired T-test within mothers or fathers between control and CHD group; P_2 is the 2-tail paired T-test between mothers and fathers within the same group.

In several transgenic mice model experiments, MTRR gene with a hypomorphic mutation led to both embryo heart defect and hyperhomocysteinemia (hHcy) [17–19]. The hHcy resulting from the hypomorphic mutation in mice was similar to MTRR 66A>G mutation induced by elevated blood Hcy level in human. Therefore, MTRR 66A>G polymorphism in human also seemed to have a relationship with human heart defect. However, in human studies, some reported MTRR 66A>G was related to CHD development [20–22], while the others were contradictory [23, 24].

In a transgenic mice model experiment [19], it revealed that the mothers with a MTRR deficiency had an association with fetal VSD phenotype in mice. This result suggested that maternal MTRR deficiency might strongly be related to fetal VSD rather than other CHD types. In order to investigate whether this hypothetical relationship between maternal MTRR and fetal VSD exists in human and whether this relationship also occurs between father and fetus, and furthermore to analyze whether the parental MTHFR 677C>T and MTHFR 1298A>C polymorphisms' effects on fetal CHD in human were also VSD specific, parents with specific fetal VSD-diagnosed pregnancy history (VSD parents) and other types of CHD pregnancy history (non-VSD parents) were both studied in this experiment.

2. Materials and Methods

2.1. Subjects. Statement of responsibility: the authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Parental subjects with Chinese Han nationality were recruited from Middle China, Henan province, the second most populous province in China.

Study subjects (CHD parents and control parents): from January, 2014, to February, 2016, study subjects were recruited from the Department of Gynaecology and Obstetrics, Cardiac Center, and the Prenatal Diagnosis Center (also called the Medical Genetic Center) in the Henan Provincial People's Hospital. Written informed consent form was obtained from all participants.

CHD parents were divided into two subgroups: VSD parents and non-VSD parents. VSD parents were 21 couples with pregnancy history of CHD that occurred in fetuses or children which was specifically diagnosed as VSD. Non-VSD parents were 78 couples with pregnancy history of other types of CHD rather than VSD that occurred in fetuses or children (CHD classification; see Table 1). The CHD parents were at ages between 24 and 34 years (see Table 2) and had at least once pregnancy of CHD child, with no obvious fat or emaciation, no drug abuse history, no hypertension, no heart defect, no medical treatment during pregnancy, no pregnancy-induced hypertension, no diabetes, no smoking, no alcohol abuse, no family history of any diseases.

Control parents: the control parents were at ages between 20 and 34 years (mean ± SD: mothers 27.781 ± 2.205, fathers 29.789 ± 2.585, Table 2) and had, at least once, normal pregnancy of healthy babies and had no abnormal pregnancy history or had not given birth to abnormal children and had no hypertension, no heart defect, no medical treatment during pregnancy, no diabetes, no pregnancy-induced hypertension, no obvious fat, no drug abuse history, no smoking, no alcohol abuse, and no family history of any diseases.

All the control and CHD subjects recruited in this study and their babies/fetus were all diagnosed in the Prenatal Diagnosis Center (also called the Medical Genetic Center) by karyotype analysis of peripheral blood or amniotic fluid. The exactly genetic causation of CHD is unclear and about

Table 3: Distribution of MTHFR 677C>T, MTHFR 1298A>C, and MTRR 66A>G polymorphisms.

		Control (114)				Non-VSD (78)				VSD (21)			
		Mother	Father	χ^2_1	P_1	Mother	Father	χ^2_2	P_2	Mother	Father	χ^2_3	P_3
MTHFR 677C>T	C	120 (52.6)	121 (53.1)	0.009	0.925	57 (36.5)	63 (40.4)	0.488	0.485	24 (57.1)	25 (59.5)	0.049	0.825
	T	108 (47.4)	107 (46.9)			99 (63.5)	93 (59.6)			18 (42.9)	17 (40.5)		
MTHFR 1298A>C	A	202 (88.6)	195 (85.5)	0.954	0.329	135 (86.5)	131 (84.0)	0.408	0.523	35 (83.3)	36 (85.7)	0.091	0.763
	C	26 (11.4)	33 (14.5)			21 (13.5)	25 (16.0)			7 (16.7)	6 (14.3)		
MTRR 66A>G	A	178 (78.1)	180 (78.9)	0.052	0.820	109 (69.9)	119 (76.3)	1.629	0.202	25 (59.5)	26 (61.9)	0.050	0.823
	G	50 (21.9)	48 (21.1)			47 (30.1)	37 (23.7)			17 (40.5)	16 (38.1)		

Note: χ^2_1 and P_1, χ^2_2 and P_2, and χ^2_3 and P_3 all represent the allele distribution analysis between mother and father within the same subject group.

~50% Down syndrome patients [25] have CHD; therefore, the parents or their babies/fetus who have abnormal karyotypes were excluded from this study.

The controls and cases were all unrelated Chinese Han nationality who lived in the middle of China, Henan province, had no preconceptional intake of folic acid, only with folic acid taken at 400 ng/day~800 ng/day since they knew they were pregnant and it is about more one month later after gestation. The mothers within any group studied here were all under 35 years old. The father and mother had well matched ages and BMI between the two study groups and the father had a generally larger age and bigger BMI value than the mother within each group (Table 2).

Total 232 CHD parents and 259 control parents were randomly selected, but only 21 VSD, 78 non-VSD, and 114 control couples fit the above requirements and were studied in this experiment.

2.2. Sample Collection. DNA was extracted from peripheral blood samples drawn into 2 ml tubes containing EDTA and was stored at −20°C.

2.3. Genotype Analysis. All the three polymorphisms were analyzed by polymerase chain reaction (PCR) followed by Sanger Chain Terminal sequencing method. Genomic DNA was amplified by Eppendorf (Master cycler gradient, Germany).

2.3.1. Determination of MTHFR 677C>T Polymorphism. Primers used were as follows: forward primer 5′-gaagcagggagctttgaggctg3′ and reverse primer 5′-cccatgtcggtgcatgccttc3′ (made by Shanghai Sangon Biotech company, China). The PCR products were subjected to direct sequencing by using the reverse primer.

2.3.2. Determination of MTHFR 1298A>C Polymorphism. Primers used were as follows: forward primer 5′gggaggagctgaccagtgaag3′ and reverse primer 5′ggggtcaggccaggggcag3′ (made by Shanghai Sangon Biotech company, China). The PCR products were subjected to direct sequencing by using the forward primer.

2.3.3. Determination of MTRR 66A>G Polymorphism. Primers used were described as in Gaughan DJ [6] (made by Shanghai Sangon Biotech company, China). The PCR products were subjected to direct sequencing by using the reverse primer.

2.4. Statistical Analysis. Statistical significance of the differences in the frequency of alleles and genotypes using the Chi-square test was applied using the Statistical Package for Social Sciences (SPSS) version 13.0 statistical software (SPSS, Chicago, USA).

Odds ratio (OR) and 95% confidence intervals (95% CI) were calculated when it was appropriate to assess the relative risk conferred by a particular allele and genotype. Hardy-Weinberg equilibrium was tested for goodness-of-fit Chi-square test to compare the observed genotype frequencies among the subjects with the expected genotype frequencies.

A p value less than 0.05 ($P < 0.05$) was considered to be statistically significant.

3. Results

The genotype distributions of the MTHFR 677C>T, MTHFR 1298 A>C, and MTRR 66A>G polymorphisms were all in Hardy-Weinberg equilibrium in both mothers and fathers within either control or the two CHD parental subgroups ($P > 0.05$).

3.1. Allele Distribution in Father and Mother (Table 3 and Table 4). There were no significant differences between mothers and fathers within the same group in both control and CHD parents ($P > 0.05$) (Table 3).

The frequency of MTHFR 677T allele was significantly higher in the non-VSD parents than the controls (mother: non-VSD 63.5% versus control 47.4%, $P < 0.05$; fathers: non-VSD 59.6% versus control 46.9%, $P < 0.05$). Therefore, either the maternal or the paternal MTHFR 677T allele was the independent risk factor for fetal non-VSD (mother: OR = 1.930, 95% CI: 1.272–2.928; father: OR = 1.669, 95% CI: 1.105–2.521; $P < 0.05$) (Tables 3 and 4).

The frequency of MTRR 66G allele was significantly higher in the VSD parents than the control parents (mothers: VSD 40.5% versus control 21.9%, $P < 0.05$; fathers: VSD 38.1% versus control 21.1%, $P < 0.05$). Therefore, either the maternal or the paternal MTRR 66G allele was the independent risk factor for fetal VSD (mother: OR = 2.421,

Table 4: Allele distribution differences of MTHFR 677C>T, MTHFR 1298A>C, and MTRR 66A>G polymorphisms in either mothers or fathers between control and CHD (VSD and non-VSD).

Genes	Alleles	Parent	Non-VSD (78) versus control (114)				VSD (21) versus control (114)			
			χ^2	P	OR	95% CI	χ^2	P	OR	95% CI
MTHFR 677C>T	T versus C	Mother	9.654	0.002	1.930	1.272–2.928	0.290	0.590	0.833	0.429–1.619
		Father	5.973	0.015	1.669	1.105–2.521	0.595	0.441	0.769	0.394–1.501
MTHFR 1298A>C	C versus A	Mother	0.365	0.546	1.209	0.653–2.235	0.916	0.339	1.554	0.626–3.854
		Father	0.174	0.677	1.128	0.641–1.984	0.001	0.975	0.985	0.385–2.520
MTRR66 A>G	G versus A	Mother	3.298	0.069	1.535	0.965–2.442	6.539	0.011	2.421	1.213–4.833
		Father	0.382	0.537	1.166	0.716–1.898	5.696	0.017	2.308	1.147–4.645

Note: for exact number of each allele see Table 3.

Table 5: Genotype distribution differences of MTHFR 677C>T, MTHFR 1298A>C, and MTRR 66A>G polymorphisms between control and CHD (VSD and non-VSD) mothers.

		MTHFR 677C>T			MTHFR 1298A>C			MTRR 66A>G		
		CC	CT	TT	AA	AC	CC	AA	AG	GG
Control		36 (31.6)	48 (42.1)	30 (26.3)	89 (78.1)	24 (21.1)	1 (0.8)	67 (58.8)	44 (38.6)	3 (2.6)
Non-VSD		12 (15.4)	33 (42.3)	33 (42.3)	57 (73.1)	21 (26.9)	0 (0)	37 (47.4)	35 (44.9)	6 (7.7)
VSD		8 (38.1)	8 (38.1)	5 (23.8)	14 (66.7)	7 (33.3)	0 (0)	7 (33.3)	11 (52.4)	3 (14.3)
Non-VSD versus control	χ2	3.288	8.473			0.828	0.638		1.433	3.396
	P	0.070	0.004			0.363	1.000		0.231	0.138
	OR	2.063	3.300			1.366	0.989		1.440	3.622
	95% CI	0.937–4.542	1.454–7.488			0.697–2.679	0.967–1.011		0.792–2.621	0.856–15.330
VSD versus control	χ2	0.278	0.215			1.457	0.157		2.92	8.340
	P	0.598	0.643			0.227	1.000		0.088	0.004
	OR	0.750	0.750			1.854			2.393	9.571
	95% CI	0.257–2.189	0.222–2.535			0.673–5.107			0.862–6.643	1.615–56.736

95% CI: 1.213–4.833; father: OR = 2.308, 95% CI: 1.147–4.645; $P < 0.05$) (Tables 3 and 4).

The parental allele frequencies of MTHFR 1298 A>C polymorphism were not significantly different between control and the two CHD groups (Tables 3 and 4).

3.2. Genotype Distribution in Mother (Table 5) and Father (Table 6). The frequency of MTHFR 677TT genotype was significantly higher in both non-VSD fathers and mothers (mothers: non-VSD 42.3% versus controls 26.3%, $P < 0.05$; fathers: non-VSD 34.6% versus controls 26.3%, $P < 0.05$). The frequency of CT genotype was significantly higher only in non-VSD fathers (fathers: non-VSD 50.0% versus controls 41.2%, $P < 0.05$). In mothers, TT genotype has 3.3 times the risk (OR = 3.300, 95% CI: 1.454–7.488, $P < 0.05$) of CC genotype for causing a non-VSD pregnancy. In fathers, TT genotype has 2.775 times the risk (OR = 2.775, 95% CI: 1.206–6.385, $P < 0.05$) of CC genotype for causing a non-VSD pregnancy, CT genotype has 2.559 times the risk (OR = 2.559, 95% CI: 1.176–5.566, $P < 0.05$) of CC genotype for causing a non-VSD pregnancy.

The frequency of MTRR 66GG genotype was significantly higher in VSD mothers (VSD 14.3% versus control 7.7%, $P < 0.05$) and GG genotype has 9.571 times the risk (OR = 9.571, 95% CI: 1.615–56.736, $P < 0.05$) of CC genotype for causing a VSD pregnancy.

The genotype frequencies of MTHFR 1298A>C polymorphism were unrelated to both fetal VSD and non-VSD in either mothers or fathers.

4. Discussion

In this study, all subjects recruited had no preconceptional folic acid intake and only had folic acid intake after more than 1 month pregnancy; this requirement would significantly help to avoid the folic acid supplement effect on reducing fetal CHD [1]. Therefore, the conclusion made from this experiment should be meaningful.

4.1. Parental MTHFR 677C>T Polymorphism Causes Fetal Non-VSD but Not VSD. Both MTHFR 677C>T [2] and 1298 A>C [26] polymorphisms have been expressed and confirmed to affect MTHFR enzyme activity. 677TT enzyme had a 70% activity reduction, 677CT enzyme had a 35% activity reduction, and 1298CC enzyme had a 40% activity reduction. However, only MTHFR 677C>T and not 1298A<C polymorphism was associated with hHcy [27]. In human, the MTRR 66A>G polymorphism was also related to hHcy [6, 28, 29], similar as observed in MTRR deficient transgenic mice [17–19].

Hcy can be harmful to cells because it evokes oxidative stress through the production of reactive oxidative stress

Table 6: Genotype distribution differences of MTHFR 677C>T, MTHFR 1298A>C, and MTRR 66A>G polymorphisms between control and CHD (VSD and non-VSD) fathers.

		MTHFR 677C>T			MTHFR 1298A>C			MTRR 66A>G	
	CC	CT	TT	AA	AC	CC	AA	AG	GG
Control	37 (32.5)	47 (41.2)	30 (26.3)	82 (71.9)	31 (27.2)	1 (0.9)	68 (59.6)	44 (38.6)	2 (1.8)
Non-VSD	12 (15.4)	39 (50.0)	27 (34.6)	55 (70.5)	21 (26.9)	2 (2.6)	47 (60.2)	25 (32.1)	6 (7.7)
VSD	8 (38.1)	9 (42.9)	4 (19.0)	16 (76.2)	4 (19.0)	1 (4.8)	7 (33.3)	12 (57.1)	2 (9.5)
Non-VSD versus control	χ^2	5.778	5.931		0.001	0.855		0.389	3.553
	P	0.016	0.015		0.976	0.741		0.533	0.130
	OR	2.559	2.775		1.010	2.982		0.822	4.340
	95% CI	1.176–5.566	1.206–6.385		0.527–1.936	0.264–33.689		0.444–1.522	0.839–22.441
VSD versus control	χ^2	0.052	0.544		0.484	1.575		3.782	6.221
	P	0.820	0.461		0.487	0.313		0.078	0.013
	OR	0.886	0.617		0.661	5.125		2.649	9.714
	95% CI	0.311–2.519	0.169–2.247		0.205–2.133	0.305–86.248		0.968–7.247	1.179–80.024

through the production of reactive oxygen species, binds to nitric oxide, or leads to the accumulation of its precursor, S-adenosylhomocysteine, a protein inhibitor of biological transmethylation. A study [9] reported that the maternal hHcy was correlated with fetal CHD; therefore, maternal MTHFR 677C>T and MTRR 66A>G polymorphisms, which would induce high blood level of Hcy, should have effects on fetal CHD development. However, in this study, the independent maternal and paternal MTHFR 677C>T polymorphism but not the MTRR 66A>G polymorphism was associated with fetal non-VSD; this suggested that maternal and paternal hHcy might not be a major risk factor for fetal non-VSD. There might exist another important mechanism pathway via MTHFR deficiency causing consequence results rather than hHcy alone. This hypothesis could be supported by the finding observed in the chicken embryo study where Hcy injection into chicken embryo led to majorly (83%) subarterial VSD [30].

Therefore, the effect of MTHFR 677C>T polymorphism on the development of fetal non-VSD observed in this study was probably via impaction on the synthesis of purine, thymidylate, or DNA methylation pathway rather than Hcy alone implicated in the folate metabolic pathway.

Mice lacking the MTHFR gene [31] displayed delayed development, impaired growth, and increased morbidity and mortality in the early postnatal period. In women, the 677C>T mutation was a risk factor for fetal neural tube defects (NTD), fetal Down syndrome, and recurrent embryo loss. Therefore, female MTHFR 677C>T polymorphism might have an impact not only on fetal survival but also on quality of embryo probably via impact on female germ cell development. Moreover, severe MTHFR deficiency in male mice resulted in abnormal spermatogenesis and infertility [32]. In human males, the aberrant promoter hypermethylation of MTHFR gene [33, 34] and the MTHFR 677C>T polymorphism [34–36] were both believed to be strongly associated with male infertility; MTHFR 677C>T polymorphism was also believed to be associated with abnormal spermatogenesis [34], semen quality (motility and morphology) [32, 34], and embryo heart defect [20]; MTHFR might control the DNA integrity and the function of sperm in human males [37, 38]. Therefore, MTHFR 677C>T polymorphism might also affect germ cell development in males. Thus, the effect of MTHFR 677C>T polymorphism on fetal CHD might have its impact on both female and male germ cell development.

However, the MTHFR 677C>T polymorphism only resulted in fetal non-VSD but not VSD observed in the study was a new proposal among the current world studies and the reasons or mechanisms for this were unclear. This proposal could explain why the relationship between MTHFR 677C>T and fetal CHD was varied among different studies due to different proportion of non-VSD subjects included in different population studies. In this study, as the MTHFR 677C>T polymorphism effect on fetal non-VSD was maternal and paternal independent, thus, either the maternal or paternal MTHFR 677C>T polymorphism was the risk factor for fetal non-VSD.

4.2. Parental MTRR 66A>G Polymorphism Causes Fetal VSD but Not Non-VSD. Studies in chicken embryos showed 83% subarterial VSD after injection of 30 μM Hcy into the neural tube lumen [30]. This Hcy concentration resembled mild hHcy in humans. In the transgenic mice model, maternal MTRR gene with a hypomorphic mutation led to hHcy [18] and almost VSD phenotype in the mice embryo as well [19]. Therefore, maternal MTRR deficiency seemed to be strongly associated with fetal VSD phenotype, and this was probably due to hHcy effect resulting from MTRR deficiency. In this study, either the maternal or paternal MTRR 66A>G polymorphism was related to fetal VSD but not fetal non-VSD; this observation was consistent with the hypothesis that the maternal MTRR 66A>G polymorphism was strongly associated with fetal VSD. However, no animal experiment has been done to support the observation found in this experiment where the paternal MTRR 66A>G polymorphism was also strongly associated with fetal VSD rather than non-VSD. The mechanism underlining this is unclear.

4.3. Conclusion. Overall, in this study, the MTHFR 677C>T polymorphism of either mother or father was independently

associated with fetal non-VSD but not VSD; fetal VSD was linked with the independent maternal or paternal MTRR 66A>G polymorphism. These findings made from this study could help to explain why the relationship between the two polymorphisms (MTHFR 677C>T and MTRR 66A>G) and CHD were varied among different studies due to different proportion of VSD and non-VSD subjects included in those different population studies.

Furthermore, a metastudy with selection of a larger sample size on VSD and non-VSD parents if possible could be considered in the future to further confirm the conclusion made from this study.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Qian-nan Guo contributed to the experimental design, data acquisition, analysis, and interpretation and drafted the manuscript. Jian-gang Long and Shi-xiu Liao contributed to the experimental design and provided technical support. Hong-dan Wang assisted in analysis and the manuscript. Li-zhen Tie, Tao Li, and Hai Xiao contributed to sample collection and DNA extraction. All the authors approved the final manuscript to be submitted.

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Clinical Study

Postnatal Growth in a Cohort of Sardinian Intrauterine Growth-Restricted Infants

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Recent studies have shown that infants with intrauterine growth restriction (IUGR) undergo catch-up growth during infancy. The aim of our study was to evaluate the postnatal growth in a cohort of IUGR infants born in a tertiary-level Obstetric University Hospital of Northern Sardinia. An observational retrospective study was conducted on 12 IUGR (group A) and 12 control infants (group B) by measuring the anthropometric parameters of weight (W), length (L) and head circumference (HC) from birth to the 3rd postnatal year. At birth, significant differences were found between group A and group B with regard to all the auxological parameters (W, mean 1846.6 versus 3170.8 g, $p < 0.0001$; HC, 30.1 versus 34.4 cm, $p < 0.0001$; L, mean 43.4 versus 49.4 cm, $p < 0.0001$). During the 1st year, 8 of 12 (70%) IUGR infants exhibited a significant catch-up growth in the 3 anthropometric parameters and a regular growth until the 3rd year of follow-up. The majority but not all infants born with IUGR in our series showed significant postnatal catch-up growth essentially during the first 12 months of life. An improved knowledge of the causes of IUGR will help to develop measures for its prevention and individualized treatment.

1. Introduction

A combination of environmental, genetic, and epigenetic factors, still partially unknown, can be responsible for a condition in which a fetus is unable to reach its genetically determined growth potential: this condition is defined as intrauterine growth restriction (IUGR) [1, 2]. The IUGR fetus begins to lose its growth potential during the first trimester of pregnancy, mainly as a result of uterine hypoperfusion often associated with thin umbilical cord [1, 2]. The causes can be maternal, fetal, or placental. Preeclampsia, pathologic conditions of the umbilical arteries, maternal smoking, and unbalanced diet are known risk factors of IUGR [3–6]. It is essential to diagnose IUGR by ultrasound scan before the 28th week of gestation and to monitor its evolution throughout pregnancy. In this regard, Doppler flow measurement of the fetal vessels (namely, umbilical artery, ductus venosus, and middle cerebral artery) has been found to be particularly helpful [7]. The circulatory status of the fetus is assessed especially in the middle cerebral artery, to determine the appropriate timing of delivery, that needs to be neither too early nor too late for a better outcome and prognosis [7, 8].

Clinical studies have shown that IUGR is a condition associated not only with an increased perinatal mortality, but also with significant morbidity later in life, including short stature, metabolic syndrome, and neurocognitive impairment [9–11]. The "symmetric" form of IUGR, defined by significant reduction of all anthropometric parameters including a small head circumference for gestational age, is associated with a worst prognosis compared to the "asymmetric" form of IUGR, in which the head circumference is within the normal range, and a favorable, complication-free postnatal course is generally observed [12, 13]. Among term infants, morbidity and mortality are 5-30-fold higher in low

Table 1: Main parameters of the enrolled newborns, both group A and B.

Group A infants	Sex	Delivery	G.A.	APGAR at 5′	*W* (g)	*W* (centile)	*L* (cm)	*L* (centile)	CC (cm)	CC (centile)
A1	F	CS	32	7	1115	3rd	37,0	3rd	27,0	3–10th
A2	F	CS	32 + 4	8	1300	10th	36,0	<3rd	28,0	10th
A3	M	CS	33	8-9	1400	3rd	38,0	<3rd	28,0	3rd
A4	F	CS	34	9	1405	<3rd	44,0	50th	27,5	<3rd
A5	M	CS	35	9	1583	<3rd	45,0	25th	31,5	25th
A6	F	CS	36	9	1950	3rd	42,5	3rd	31,0	3rd
A7	F	CS	36 + 3	9	2400	25th	44,5	10th	32,5	25th
A8	M	CS	36 + 6	9	1930	<3rd	43,0	<3rd	31,0	10th
A9	M	CS	37	9	2040	<3rd	43,5	<3rd	32,0	10th
A10	F	CS	37	9	1510	<3rd	42,0	<3rd	28,0	<3rd
A11	F	CS	37 + 2	9	2450	10–25th	45,3	10th	32,0	10–25th
A12	F	CS	37 + 2	9	2300	3–10th	46,0	10–25th	32,5	10th
Group B infants	Sex	Delivery	G.A.	APGAR at 5′	*W* (g)	*W* (centile)	*L* (cm)	*L* (centile)	CC (cm)	CC (centile)
B1	F	VD	38	9	2900	50th	49,0	50th	33,5	50th
B2	F	VD	38	9	3000	50th	50,0	50–75th	33,5	50th
B3	M	VD	38 + 2	9	2900	10–25th	49,5	50th	33,0	25th
B4	M	VD	39	9	3400	75th	47,0	10th	36,0	90th
B5	F	CS	39 + 2	9	2900	10–25th	48,5	25–50th	33,0	25th
B6	M	CS	40 + 2	9	3600	50th	49,0	10–25th	35,0	50th
B7	F	VD	40 + 4	9	2900	25–50th	48,5	25th	34,0	50th
B8	F	VD	41	9	2800	10th	49,5	25–50th	34,0	50th
B9	F	VD	41	9	2800	10th	48,3	10th	35,0	75th
B10	F	VD	41	9	3350	50th	50,5	50–75th	35,0	75th
B11	M	VD	41 + 1	9	3500	25–50th	51,5	50th	35,0	50th
B12	F	VD	42	9	4000	>90th	51,0	50–75th	35,5	75th

birth weight infants (LBWI) compared to infants with birth weight within the normal range (10th–90th centile) [12, 13].

The postnatal catch-up growth begins as soon as infants move to a more favorable environment and becomes evident during the first months of life. However, not all IUGR infants exhibit a postnatal catch-up growth, likely depending on the underlying causative factor/s and genetic diversity [14].

The present study reports results from a 3-year follow-up of a cohort of Sardinian IUGR infants with special emphasis on the postnatal catch-up growth.

2. Study Population and Methods

2.1. Study Population. In the year 2013, a total 27 IUGR diagnoses were made among infants born in the Gynecologic and Obstetric Clinic of the University of Sassari, Italy. Gestational age (GA) was defined on the basis of ultrasonographic estimation (Voluson E8 ultrasound system, GE Healthcare, Fairfield CT, USA) performed at the time of the first scan, as recommended (SIEOG Italian guidelines), and at about 20, 28, and 36 weeks' gestation [15]. Distributions of all measurements were similar to previously reported reference cohorts (data not reported). At the 20-week scan, details about medical history and demographic characteristics of the pregnant women were collected retrospectively. At this time, women were also informed about fetal anatomy and biometric measurements, as well as uterine and umbilical artery Doppler flow velocimetry data [15]. Ultrasonographic measurements of fetal biparietal diameter, head circumference, abdominal circumference, and femur length were performed according to standard techniques. The Hadlock equations and reference standard were used to calculate the fetal weight (EFW) centile, and EFW values less than the 10th centile defined the IUGR [15]. At the 36 week scan, pregnant women were informed about previously undiagnosed placenta praevia, severe oligohydramnios, a previously undiagnosed fetal abnormality, or noncephalic presentation [15]. Women were selected for additional, clinically indicated scans in the third trimester of pregnancy as per routine clinical care, using local and national guidelines (e.g., SIEOG guidelines). The indications for cesarean section (CS) included a not reassuring cardiotocography (85%) and a reversed end diastolic flow of umbilical artery at ultrasound evaluation (15%).

As 13 families moved out of the Sassari province and 2 newborns unfortunately deceased, the access to postnatal data was available for 12 IUGR infants (F : M = 8 : 4), enrolled as group A. Twelve term infants with a birth weight greater than 2,500 g (F : M = 8 : 4) were enrolled as a control group (group B). The parents of all the infants enrolled in this study provided informed consent.

Group A and group B newborns' main parameters are shown in Table 1, ordered by GA (column 4). Among group

Table 2: Weight centile categories of IUGR (group A) infants at 12 months of postnatal life.

	Male	Female	*Total*
<3rd centile	1	3	4
3rd–50th centile	2	3	5
>50th centile	1	2	3
Total	4	8	12

Table 3: Length centile categories of IUGR (group A) infants at 12 months of postnatal life.

	Male	Female	*Total*
<3rd centile	1	3	4
3rd–50th centile	2	4	6
>50th centile	1	1	2
Total	4	8	12

Table 4: Head Circumference centile categories of IUGR (group A) infants at 12 months of postnatal life.

	Male	Female	*Total*
<3rd centile	0	3	3
3rd–50th centile	3	3	6
>50th centile	1	2	3
Total	4	8	12

A, 3 of 12 infants (25%; Table 1, A1–A4) were born preterm and with a very low birth weight (VLBW), ranging from 1115 to 1400 g. The remaining group A, namely, 5 (41,6%) late preterm (Table 1, A5–A8) and 4 (33,3%) at term infants (Table 1, A9–A12), were all but one born with low birth weight (LBW), ranging from 1510 to 2450 g, and one with VLBW (1405 g).

All group A but only 2 group B infants had CS births (Table 1, B5-B6).

2.2. Methods. This is an observational study conducted by retrospective collection of the measures of weight (W), length (L), and head circumference (HC), at birth and at 3-month intervals during the first year, then annually in the second and third years of follow-up (W and L). All values were plotted and recorded in the growth charts as follows: (1) weight to age, (2) length to age, and (3) head circumference to age (Center for Disease Controls, Atlanta, GA, USA).

2.3. Statistical Analysis. Student's t-test was used to compare groups, considering significant a value of $p < 0.05$.

3. Results

At birth, significant differences were found between group A and group B infants with regard to all anthropometric parameters considered in this study (W, mean 1846.6 versus 3170.8 g, $p < 0.0001$; HC, 30.1 versus 34.4 cm, $p < 0.0001$; L, mean 43.4 versus 49.4 cm, $p < 0.0001$).

During the first year of life, a significant catch-up growth led to cover the differences in L (mean 72.6 versus 76.5 cm, p = ns) and to reduce those in W (mean 7861.0 versus 9165.0 g, $p = 0.02$) and HC (mean 43.5 versus 45.7 cm, $p = 0.04$) between the two study groups. At the age of 1 year, 8 (70%) group A infants were comparable to group B infants with respect to the 3 anthropometric parameters (Figure 1). However, analysis of data from individual patients revealed that 4 of 12 (30%) IUGR infants (Table 1, A2, A6, A8, and A10) did not exhibit catch-up growth during the first postnatal year with minimal improvement during the second and third years of follow-up (Figure 1). Categories of centiles for weight, length, and head circumference of IUGR infants at 12 months of life are reported in Tables 2, 3, and 4.

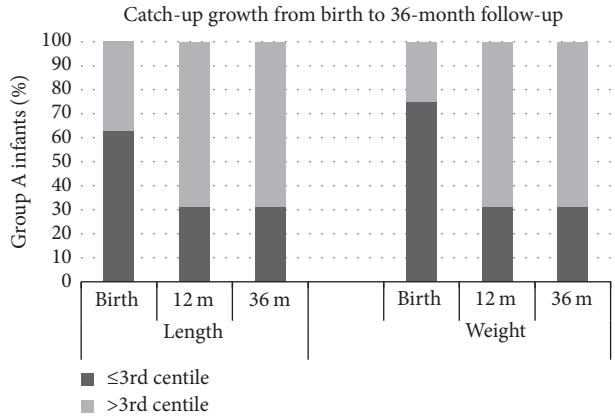

Figure 1: Percentage of group A infants below and above the 3rd centile cut-off for length (left panel) and weight (right panel) at birth, at 12 months (12 m) and 36 months (36 m) of age.

It deserves a note that among those who did not show postnatal catch-up growth, the only IUGR infant born at term (A10, Table 1) was discovered to be affected by the rare Pallister-Killian syndrome, caused by tetrasomy of chromosome 12p which is characterized by facial dysmorphism, rhizomelic limb shortness, and small hands and feet, along with corpus callosum hypoplasia. Moreover, during the postnatal years of follow-up, one of the IUGR preterm infants (A2; GA = 32 + 4; Table 1) showed failure to thrive, and it is currently under pediatric endocrinology evaluation.

4. Discussion

The majority of IUGR infants in our series showed significant postnatal catch-up growth during the first 12 months of life, and regular growth until 3 years of age.

Several studies in literature have reported on the postnatal catch-up growth in preterm IUGR and SGA infants, but only a few studies exist on term IUGR infants [1, 10–12].

One study conducted in North America (USA) on 42 IUGR infants has calculated growth velocity, which was significantly higher in IUGR infants compared to the control group (3.58 kg/m^2 versus 2.36 kg/m^2) during the first 12 months of life [13].

Another study, conducted in North Europe on 73 IUGR newborns, found catch-up growth in up to 90% of cases during the first year of life; 7% of infants among those who did

not have significant catch-up growth exhibited neurological and cognitive impairment [3].

This study was not a clinical trial and was also limited by both its retrospective, observational design and the small sample size. Even with these limitations of the study, our results further confirm those reported by others. All the term IUGR infants but the one affected by Pallister-Killian syndrome exhibit a catch-up growth. More than half of the preterms IUGR did show also a significant catch-up growth, and it was significantly greater during the first 12 months of life [9].

It was not possible for us to determine for each single case whether maternal or fetal factors played a role in the development of IUGR, as well as the role played by genetic, epigenetic, and environmental factors, or likely the complex combination of multiple factors on the catch-up growth and outcome during the postnatal life.

Interesting, the recent personalized medicine approach through the Newborn Individualized Developmental Care and Assessment Program (NIDCAP) has been the focus of a study conducted on preterm infants born with severe IUGR by a multidisciplinary research working group of Harvard University [16, 17]. The NIDCAP was shown to be effective in ameliorating the neurobehavior, electrophysiology and brain structure outcomes compared to IUGR controls [16, 17]. At least 2/3 of our IUGR infants required special assistance at the Newborn Intensive Care Unit (NICU). We can therefore speculate that also our infants compromised by severe IUGR who showed postnatal catch-up growth might have had significant benefit from an individualized developmental care approach during NICU stay.

Moreover, methods of infant feeding (breast-feeding versus formula feeding) and other nutritional factors (including iron, zinc, and vitamins) might play a critical role in the catch-up growth during the first months of life [18, 19] and would deserve further, more extensive, investigation.

Abbreviations

CS:	Cesarean section
EFW:	Estimated fetal weight
GA:	Gestational age
HC:	Head circumference
IUGR:	Intrauterine growth restriction
L:	Length
LBWI:	Low birth weight infants
NIDCAP:	Newborn Individualized Developmental Care and Assessment Program
NICU:	Newborn Intensive Care Unit
PROM:	Premature rupture of the membrane
SGA:	Small for gestational age
VD:	Vaginal delivery
VLBW:	Very low birth weight
W:	Weight.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

The first three authors (Maria Grazia Clemente, Giampiero Capobianco, and Paolo Mattia Galasso) contributed equally and wrote the first draft of the paper. Francesco Dessole, Giuseppe Virdis, and Giampiero Capobianco selected IUGR infants for the study, did the prenatal ultrasound evaluation, and analyzed the gestational auxological data, Maria Grazia Sanna and Mauro Giorgio Olzai did the perinatal physical evaluation of the study population, and Maria Grazia Clemente, Paolo Mattia Galasso, and Lino Argiolas collected and analyzed the perinatal and postnatal clinical records and anthropometric data. Salvatore Dessole and Roberto Antonucci were co-senior authors. All participated in writing the paper. The final version was approved by everyone.

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Research Article

The Role of Interleukin-17, Interleukin-23, and Transforming Growth Factor-β in Pregnancy Complicated by Placental Insufficiency

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Aim. The aim of the study was to evaluate the role of Interleukin-17 (IL-17), Interleukin-23 (IL-23), and transforming growth factor-β (TGF-β) in pregnancy complicated by placental insufficiency and in normal pregnancy. *Material and Methods.* The study comprised 34 patients with pregnancy complicated by fetal growth restriction (FGR) associated with preeclampsia (PE), as well as 35 healthy pregnant women. The concentrations of IL-17, IL-23, and TGF-β in sera from maternal peripheral blood were determined by an immunoenzymatic assay. *Results.* There were higher concentrations of IL-17 in the study group when compared to the controls. In the group of patients with placental insufficiency, the levels of IL-17 positively correlated with systolic blood pressure ($R = 0.42$, $p < 0.01$). The study obtained comparable concentrations of IL-23 in both studied groups. The concentrations of TGF-β were significantly lower in pregnancy complicated by the insufficiency of placenta when compared to the controls. *Conclusions.* It seems possible that the increased concentrations of IL-17 and the deficiency of TGF-β in pregnancy complicated by FGR and PE can be responsible for the activation of inflammatory response observed in PE cases.

1. Introduction

Hypertension occurs in 5–10% of all pregnancies and together with postpartum hemorrhage and infections creates a deadly triad of pregnancy complications, responsible for the majority of maternal deaths [1]. The presence of hypertension in pregnancy is dangerous to the fetus, leading to fetal growth restriction (FGR), premature abruption of the placenta, and hypoxia, often causing stillbirth. We should keep in mind that the only effective treatment of preeclampsia is the termination of pregnancy, which makes this complication one of the main causes of iatrogenic prematurity [2]. Every year, due to preeclampsia or eclampsia, over 40,000 women and as many as 500,000 children die. This means that 110 women and over 1600 children die each day [3]. Currently, however, there are more and more indications that preeclampsia is a disease of immune etiology and that immune factors are responsible for both impaired trophoblast implantation and the cascade of events leading to placental insufficiency and FGR in the course of preeclampsia [4–6].

In recent years, in order to clarify the immunological mechanisms responsible for the proper implantation process, the Th1/Th2 paradigm has been extended to the Th1/Th2/Th17 and regulatory T cells (Treg) paradigm [7]. Th17 cells have been recently discovered as a subpopulation of T cells, whose cytokine profile is different from Th1

one and Th2 cells [8]. The main task of Th17 helpers is the production of Interleukin-17. Many studies found an increased proportion of Th17 subpopulations in pregnancies complicated by miscarriage, preterm birth, and preeclampsia [9–11]. Interleukin-17 (IL-17, also known as IL-17A) is a major, strongly proinflammatory cytokine produced by Th17 helper cells [12]. Interleukin-17, a cytokine with potent proinflammatory properties, has a proven role in the development of inflammatory processes, acute immunological graft rejection, and autoimmune diseases. It has also been shown that IL-17 affects the maturation of dendritic cells and inhibits the response from the regulatory T cells (Treg), responsible for the phenomenon of immune tolerance [12].

Interleukin-23, which is produced, among others, by macrophages and dendritic cells, is an important component of the inflammatory response. Together with TGF-β1 it stimulates the differentiation of $CD4^+$ T cells into Th17 cells [13]. In addition, IL-23 increases the local concentration of matrix metallopeptidase 9 (MMP-9) and stimulates angiogenesis, which makes it an extremely important element in a proper implantation. However, clinical studies showed increased expressions of IL-23 in patients with recurrent pregnancy loss [14]. Its role has been shown in the spread of malignant tumors, as well as in autoimmune diseases. Studies in mice found that animals which are devoid of genes responsible for the production of a subunit of the receptor for IL-23 are definitely more prone to multiple sclerosis and inflammatory bowel diseases [15].

Transforming growth factor-β (TGF-β) released, among others, by macrophages, neutrophils, platelets, and lymphocytes acts primarily to inhibit the proliferation of B and T lymphocytes and NK cells, reduces the release of proinflammatory cytokines, and inhibits the expression of major histocompatibility complex MHC class II on the antigen-presenting cells [16]. In addition, TGF-β is involved in the processes of angiogenesis, wound healing, and repair processes, as well as regulation of the entry of cells onto the apoptotic pathway [17]. The best known protein from the TGF-β protein family is TGF-β1, which is produced by dendritic cells, white blood cells, and NK cells. It was found that TGF-β1 has an immunosuppressive effect on T and B lymphocytes, and the lack of this cytokine may predispose patients to more frequent development of autoimmune diseases, such as systemic lupus or scleroderma [18, 19].

The purpose of our study was to estimate the role of IL-17, IL-23, and TGF-β in pregnancy complicated by fetal growth restriction associated with preeclampsia as well as in normal pregnancy.

2. Material and Methods

Our study comprised 34 patients with pregnancy complicated by fetal growth restriction associated with preeclampsia admitted to the Department of Obstetrics and Perinatology of the Medical University in Lublin. The diagnosis of preeclampsia was made according to the criteria of *American College of Obstetricians and Gynecologists*. The diagnosis of fetal growth restriction was made when less than 10th percentile fetal weight for gestational age was found during ultrasound examination of patients with preeclampsia. Peripheral blood samples from the study group were taken from pregnant patients before starting a therapy. The control group comprised 35 healthy pregnant women with uncomplicated pregnancy. The study was approved by the Ethics Committee of the Medical University of Lublin.

The immunoenzymatic assays were used to determine sera concentrations of IL-17, IL-23, and TGF-β. The assays used kits were produced by the Diaclone Company (Besancon, France). The statistical differences between groups were estimated using Mann-Whitney U test, chi-squared test, and Fisher's exact test. Differences were defined as statistically significant at the level of $p < 0.05$. For the correlation analysis Spearman's rank correlation test was performed. Two-tailed p values less than 0.05 were considered as statistically significant. STATISTICA 7.1 software (StatSoft Poland, Krakow, Poland) was applied to statistical analysis.

3. Results

The concentrations of IL-17 in sera of patients with pregnancies complicated by FGR and preeclampsia were significantly higher when compared to healthy pregnant normotensive women (IL-17: median, 3.9 pg/ml; interquartile ranges, 2.55–5.06 pg/ml, versus median, 2.4 pg/ml; interquartile ranges, 1.78–3.11 pg/ml; $p < 0.01$).

In the group of patients with FGR and preeclampsia, the levels of IL-17 positively correlated with systolic blood pressure ($R = 0.42$, $p < 0.01$).

The concentrations of IL-17 in the control group have increased with the progress of pregnancy ($R = -0.45$, $p < 0.05$). This relationship suggests that in normal pregnancy the concentration of IL-17 gradually increases.

The concentrations of IL-23 in sera of patients with pregnancies complicated by FGR and preeclampsia were significantly higher when compared to healthy pregnant normotensive women (IL-23: median, 1.93 pg/ml; interquartile ranges, 1.37–2.68 pg/ml, versus median, 1.95 pg/ml; interquartile ranges, 1.11–2.84 pg/ml; NS).

Among patients with uncomplicated pregnancies, a negative correlation was found between serum IL-23 and the week of pregnancy, when the blood was collected for the testing ($R = -0.45$, $p < 0.05$). This means that in normal pregnancy the levels of IL-23 gradually decrease with the duration of pregnancy.

The concentrations of TGF-β1 in sera of patients with pregnancy complicated by FGR and preeclampsia were significantly lower when compared to the group of healthy women with uncomplicated pregnancy (TGF-β1: median, 15,092 ng/ml; interquartile ranges, 6,801–20,335 ng/ml, versus median, 17,834 ng/ml; interquartile ranges, 12,245–25,395 ng/ml ($p < 0.05$)). The results are presented in Figure 1.

The clinical characteristics of patients from the study and control groups are presented in Table 1.

4. Discussion

The mechanisms aimed at maintaining the balance of Th1/Th2/Th17 and Treg cells conditioning the normal development of the pregnancy are not fully understood.

Table 1

	Placental insufficiency	Normal pregnancy	Statistical significance
Number of cases	34	35	
Age (years)	32.1 ± 4.36	30.7 ± 5.21	NS
First pregnancy	20	14	NS
Another pregnancy	14	21	NS
The duration of gestation (days)	256 ± 33	272 ± 14	0.0002
Time of blood collection (weeks of gestation)	32.05 ± 2.14	32.62 ± 1.63	NS
Vaginal delivery	4	19	0.0018
Caesarean section	30	16	0.0018
Birth weight (g)	2500 ± 1034	3270 ± 453	0.00003
RR systolic (mmHg)	168 ± 14	112 ± 13	0.0001
RR diastolic blood pressure (mmHg)	108 ± 10	72 ± 9	0.0001
Total protein (g/dl)	5.9 ± 0.65	6.3 ± 0.29	0.002
Prothrombin time (s)	10.4 ± 0.5	11.0 ± 0.3	0.00001
Prothrombin index (%)	114.3 ± 7.1	110.2 ± 6.5	0.01
INR	0.87 ± 0.06	0.93 ± 0.06	0.00004
D-dimers (μg/l)	1246 ± 1106	853 ± 600	0.02
Fibrinogen (g/l)	4.8 ± 1.1	5.0 ± 0.9	NS
K (mmol/L)	4.35 ± 0.4	4.0 ± 0.3	0.006
Na (mmol/L)	138 ± 3.4	138 ± 1.6	NS
e-GFR (ml/min/1.73 m^2)	87.8 ± 37.8	100 ± 32.3	0.02
Creatinine (mg/dl)	0.8 ± 0.3	0.7 ± 0.5	NS
Uric acid (mg/dl)	6.4 ± 1.6	3.8 ± 1.1	0.001
Urea (mg/dl)	23.05 ± 15	16.1 ± 3.8	0.001

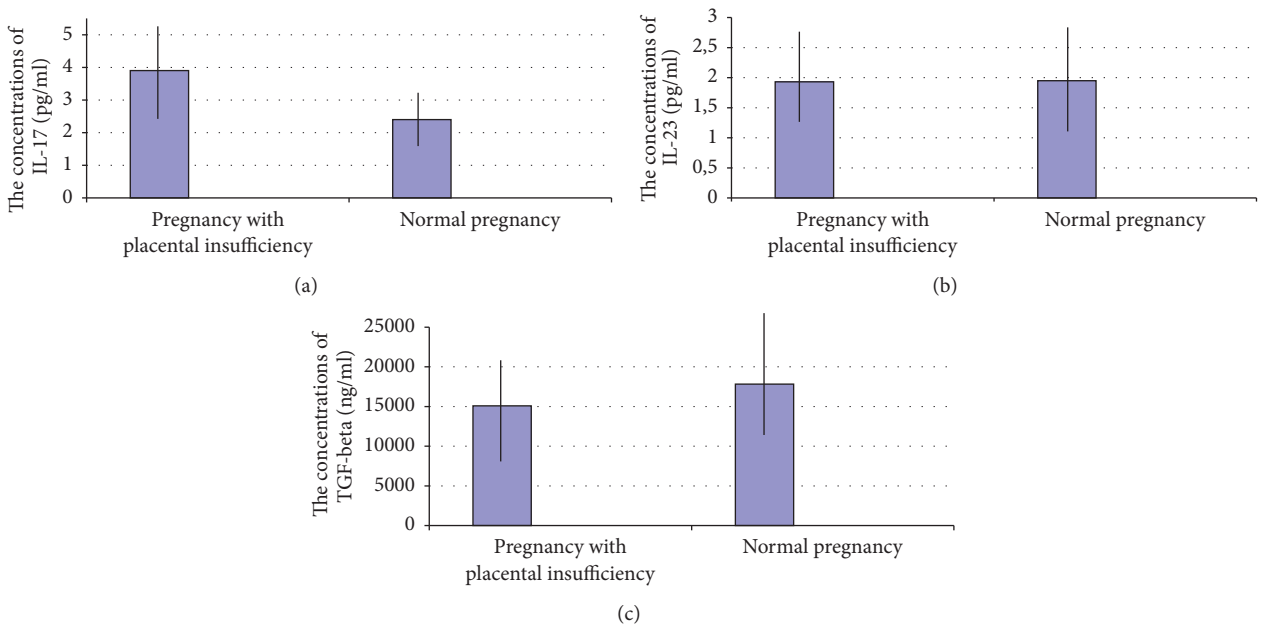

Figure 1: The comparison of (a) IL-17, (b) IL-23, and (c) TGF-β concentrations in sera of patients with pregnancy complicated by placental insufficiency (n = 34) and healthy women with uncomplicated pregnancy (n = 35).

According to Steinborn et al. and Sasaki et al. in pregnancies complicated by placental insufficiency there is a deficit of Treg cells, which supports the expressions of Th17 lymphocytes and the induction of inflammatory response in the fetomaternal interface [20, 21].

The increase in the levels of IL-17 in pregnancy complicated by FGR and PE which was observed in our study confirms the results of our previous study as well as the research conducted by Santner-Nanan et al., who reported a reduction in the number of Treg cells and an increase in the population

of Th17 cells in placental complications of pregnancy [9, 22]. On the other hand, during normal pregnancy, the expansions of Treg cells with decreased expressions of Th17 cells have been observed [9, 23].

Furthermore, the results of our study showed that in normal pregnancy the concentrations of IL-17 gradually increases. Interestingly, Martínez-García et al. also noted an increase in the level of IL-17 in the third trimester of uncomplicated pregnancy. The authors attributed an increase in proinflammatory cytokine release near the term of delivery to the dilation of the cervix and the progress of labor [23].

Moreover, we observed that, in the group of patients with FGR and preeclampsia, the concentrations of IL-17 positively correlated with systolic blood pressure. Similar observations were made by Dhillion et al. The authors observed that the administration of IL-17 to healthy pregnant rats resulted in a statistically significant increase in mean arterial blood pressure. The administration of IL-17 to nonpregnant rats had no effect on blood pressure. Interestingly, the increase of blood pressure in pregnant rats was reversible after the administration of superoxide dismutase or the inhibition of angiotensin II receptor type 1, which can mean that the pressure is generated as a result of oxidative stress and the formation of autoantibodies against angiotensin II receptor type 1 [24].

In our study there were no statistically significant differences in the concentrations of IL-23 in the study and control groups. It was noted, however, that there was a negative correlation between the concentrations of IL-23 and the week of pregnancy in which the blood test was collected, suggesting that the concentrations of IL-23 decrease with the duration of pregnancy. The reduced expressions of IL-23 in late physiological pregnancy may be due to the fact that IL-23 plays a central role in early pregnancy, when dendritic cells present antigens of paternal origin conditioning a proper implantation and invasion of trophoblast. A decrease in the concentrations of IL-23 could also explain the tendency of recurrence of certain autoimmune diseases in pregnancy, such as systemic lupus or inflammatory bowel disease [25–28].

Recent studies suggest that IL-23 is the key proinflammatory cytokine secreted by dendritic cells. Antigen-presenting dendritic cells stimulate or inhibit the proliferation of the relevant T cell subpopulations, deciding on the tolerance or rejection of syncytiotrophoblast cells [29]. The action of IL-23 does not result in the differentiation of progenitor cells into the Th1 cells producing interferon-γ, but it induces the formation and expansion of Th17, which leads to the release of proinflammatory IL-17 [30]. An interesting observation is the lack of receptors for IL-23 on the surface of undifferentiated Th0 lymphocytes, which are the main effector cells for IL-23. It seems that the receptor expressions of IL-23 occur through the activation of Th0 cells by IL-21 secreted T cells in the presence of IL-6 derived from activated dendritic cells and macrophages. This underlines the importance of dendritic cells presenting the antigens in the induction of inflammatory processes via the expression of IL-23 and, consequently, IL-17 [31, 32].

Moreover, in our study we found decreased expressions of TGF-β1 in pregnancy complicated by FGR and PE compared to healthy pregnant women. Contrary to these results, Lygnos et al. observed increased levels of TGF-β1 in pregnant women with hypertension [33]. The increased concentrations of TGF-β1 were also observed in other pregnancy complications associated with the activation of the inflammatory response, such as miscarriages or premature labor [34]. Transforming growth factor-β has immunosuppressive effects and proangiogenic properties. Recently, it has been observed that the conversion of maternal T cells into T $CD4^+FoxP3^+$ regulatory T cells is partially mediated via TGF-β. The authors suggest that TGF-β can contribute to the fetal-maternal tolerance by the increase of the Treg cell population [35, 36]. It seems possible that the decreased concentrations of TGF-β1 observed in pregnancy complicated by FGR and PE can lead to the deficit of Treg cells, Th17/Treg imbalance, and an inappropriate invasion of the trophoblast as well as an abnormal formation of new vessels in the placenta.

5. Conclusions

In normal pregnancy the concentrations of IL-17 increase gradually along with the duration of pregnancy, suggesting an increase of the inflammatory activity with the progress of uncomplicated pregnancy. The reduced expressions of IL-23 in late physiological pregnancy may be due to the fact that IL-23 plays a central role in early pregnancy, when dendritic cells present antigens of paternal origin, conditioning proper implantation and the invasion of trophoblast.

Moreover, the increased concentrations of IL-17 and the deficiency of TGF-β in pregnancy complicated by FGR and PE can be responsible for the activation of the inflammatory response and as a consequence for a placental insufficiency.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the manuscript.

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Review Article

Does Chemotherapy for Gynecological Malignancies during Pregnancy Cause Fetal Growth Restriction?

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Cancer and pregnancy rarely coincide. Gynecological cancers are among the most common malignancies to occur during pregnancy, and chemotherapy with or without surgery is the primary treatment option. The main concern of administering chemotherapy during pregnancy is congenital malformation, although it can be avoided by delaying treatment until after organogenesis. The dose, frequency, choice of chemotherapeutic agents, time of treatment commencement, and method of administration can be adjusted to obtain the best maternal treatment outcomes while simultaneously minimizing fetal toxicity. Use of chemotherapy after the first trimester, while seemingly safe, can cause fetal growth restriction. However, the exact effect of chemotherapy on such fetal growth restriction has not been fully established; information is scarce owing to the rarity of malignancy occurring during pregnancy, the lack of uniform treatment protocols, different terminologies for defining certain fetal growth abnormalities, the influence of mothers' preferred options, and ethical issues. Herein, we present up-to-date findings from the literature regarding the impact of chemotherapy on fetal growth.

1. Introduction

Malignancies rarely coincide with pregnancies; only 1 in 1000 pregnancies occur concurrently with cancer. The most common malignancies diagnosed during pregnancy are breast cancer, cervical cancer, Hodgkin's lymphoma, and leukemia [1].

Small for gestational age (SGA) refers to an infant born with a birth weight below the 10th centile. The centile is adjusted to the maternal characteristics, gestational age, and the sex of the fetus. "Severe SGA" is defined as an estimated fetal weight or abdominal circumference below the 3rd centile. SGA is not synonymous with fetal growth restriction (FGR); the latter refers to a pathological restriction of the genetic growth potential and may manifest as evidence of fetal compromise such as abnormal Doppler findings or reduced amniotic fluid volume. Low birth weight refers to an infant with a birth weight less than the absolute value of 2500 g. Definitions for fetal growth abnormalities may vary in the literature, and the term FGR may even be used inappropriately [2]. Frequently, abnormally restricted growth has been referred to as intrauterine growth restriction (IUGR) in the literature and was previously known as intrauterine growth retardation [3]. Large for gestational age refers to infants with weights above the 90th centile for the gestational age and is not to be confused with macrosomia, which is defined as a fetal weight above an absolute value. Different absolute values have been used in the literature, ranging from 4000 g to 4500 g at birth [4].

The main drawback of administering chemotherapy during pregnancy is the development of congenital abnormalities. However, chemotherapy after the first trimester is not associated with increasing the rates of birth defects above the 3% rate found in the general population [5]. The exact incidence of fetal growth abnormalities caused by chemotherapy has not been established. IUGR is a possible complication of chemotherapy according to the largest medical registry in the literature, in which Cardonick and Iacobucci analyzed 376

fetuses exposed to chemotherapy in utero and showed the following complications: IUGR (7%), spontaneous rupture of membranes or preterm labor (5%), fetal death (5%), and neonatal death (1%). In terms of congenital malformations, 9 of the 11 cases of congenital malformations were attributed to chemotherapy in the first trimester. Most fetal and neonatal deaths were related to maternal hematological malignancies, and 2 deaths were related to idarubicin treatment for breast cancer [6]. In 2010, Van Calsteren et al. presented the results of their analysis of pregnancies complicated by malignancies in 3 European countries. The authors showed that, considering the cytotoxic treatment (chemotherapy and/or radiotherapy), SGA babies were observed significantly more often in 16 of 66 pregnancies (24.2%, $p = 0.001$) versus in 10 of 109 pregnancies (9.2%) without cytotoxic treatment where the association was not significant [7]. Results of the analysis of an American registry of perinatal outcomes describing 152 pregnant women managed with chemotherapy were presented in 2010, where the mean gestational age at delivery for fetuses exposed to chemotherapy was 35.8 ± 2.8 weeks and the mean birth weight was 2647 ± 713 g. Six children (3.8%) were born with a congenital anomaly. One case each of intrauterine fetal death and neonatal death occurred (0.7% of fetuses each). In 12 cases (7.7%), the neonate had SGA. The authors compared their results to those of 67 pregnant women who did not receive chemotherapy during pregnancy and concluded that congenital abnormalities, IUGR, and preterm deliveries were not increased among pregnancies managed with chemotherapy after the first trimester compared to the rates in the general population. However, there was a significant statistical difference in birth weight between the groups, although they may not be clinically consequential [8]. To our knowledge, large for gestational age has not been reported in the literature to be a consequence of chemotherapy.

Chemotherapy can cause maternal and/or fetal side effects [9–11]. In this article, we review the most current literature describing the effects of chemotherapy on fetal growth restriction in pregnant women with gynecological malignancies, including those of the breast, ovary, and cervix.

The investigations of fetal growth abnormalities related to chemotherapy are limited for many reasons. First, malignancy and pregnancy rarely coincide [1]. Most of the evidence in the literature comprises case reports and retrospective studies. Prospective studies are limited because of the possible effect of chemotherapy on fetuses [12]. Intending to deliver the fetus prematurely is one of the methods used to allow more aggressive chemotherapy and/or radiotherapy after labor. This causes delivered babies to risk complications of prematurity rather than of IUGR. Most cases of IUGR occur in the third trimester [3]; therefore, the exact incidence of growth abnormalities can theoretically be underestimated in such studies. The low birth weights reported in systemic reviews of certain chemotherapeutic agents may indicate prematurity with respect to the expected weight rather than pathologically decreased mass. A systemic review of 24 studies of administering platin derivatives to pregnant patients with cervical cancer revealed that the mean delivery weight of newborns was 2213 g [13]. Most other studies analyzed other consequences of chemotherapy such as congenital malformations or neonatal and/or childhood long-term complications [14]. The effect of a single chemotherapy agent can be difficult to analyze since patients can be given multiple agents [13]. The lack of precise uniform protocols of chemotherapy during pregnancy complicates the interpretation of the results. These different protocols involve different times of commencing chemotherapy, multiagent chemotherapy regimens for a single patient, use of chemotherapy alone or combined with surgery, choice of chemotherapeutics (which can be modified), dose of chemotherapy and intervals between cycles, method of administration, maternal wishes, and ethical issues [12–19]. Overlapping of chemotherapy with other factors may further complicate analysis. IUGR can also result from smoking, which is a well-known risk factor for certain malignancies such as cervical cancer [20, 21].

2. Effect of Chemotherapy on a Growing Fetus

The effect of the chemotherapy on a fetus may depend on the amount of the agent transferred to the fetus during pregnancy. Calsteren et al. investigated the transplacental transport of commonly used chemotherapeutics in a pregnant baboon model. The study revealed that fetal plasma concentrations of carboplatin averaged 57.5% of the maternal concentrations. Furthermore, after 3 hours of paclitaxel infusion, fetal tissue concentrations were 15% of those in the maternal tissue. As for docetaxel infusion, the fetus had 5–50% of the maternal tissue concentrations; however, the concentrations were equivalent after 26 hours. Transplacental passage of trastuzumab fell from 85% to 3% at 2 and 26 hours after trastuzumab infusion, respectively [22]. Investigations in human beings by Lanowska et al. examined the level of cisplatin in the amniotic fluid and umbilical cord blood of fetuses whose mothers underwent cisplatin monotherapy for cervical cancer during the second trimester; they found that the cisplatin concentrations in the umbilical cord and amniotic fluid were 31–65% and 13–42% of those in the maternal blood, respectively [23]. Köhler et al. assumed that a placental filtration mechanism of platinum may exist, as platinum concentrations in the umbilical cord blood and amniotic fluid were 23–65% and 11–24% of the maternal blood, respectively [24].

Chemotherapy can act directly on growing fetuses or else indirectly via the placenta [6, 25]. Chemotherapy administered after the completion of organogenesis can affect the eyes, genitalia, hematopoietic system, and central nervous system [6]. Depression of the maternal and fetal bone marrow can also cause anemia [9], which in turn can affect fetal growth [26]. Chemotherapy-induced anorexia can cause maternal nutritional deficiencies that might also contribute to growth abnormalities [2].

3. Role of Chemotherapy during Pregnancy

Both adjuvant and neoadjuvant chemotherapy (NACH) during pregnancy have been reported in the literature [14]. Surgery is one of the methods available for certain malignancies that do not interfere with the continuation of pregnancy,

for example, in breast cancer, where chemotherapy can be administered as an adjuvant treatment [12]. Ovarian or cervical malignancies are more challenging, as total abdominal hysterectomy and bilateral salpingo-oophorectomy are the main treatments but would cause the termination of pregnancy. In such cases, NACH can be a solution for pregnant women, allowing for the treatment of the mother while preserving the life of the fetus [16, 17]. Radical surgery can be performed after delivery or following a preterm elective Caesarean section at a stage when fewer complications of premature birth can be expected [27].

Some malignancies, however, are mainly treated with chemotherapy even in nonpregnant women (mainly lymphomas). In such cases, chemotherapy can be considered for saving the lives of both the mother and fetus [8]. Odelia et al. performed a review of the literature between 1990 and 2014 on chemotherapy for lymphoma in pregnant women. They concluded that, despite a consensus regarding the safety of chemotherapy (except methotrexate) after the first trimester, optimal dosage, central nervous system therapy, timing of delivery, and approach to future pregnancies remain controversial, indicating a need for further collaborative research in this field. In most of the reviewed reports, steroids or vinblastine was suggested to be reasonable "bridging therapies" until the second trimester in patients with Hodgkin lymphoma [28].

4. Timing of Chemotherapy Onset

The time of chemotherapy commencement should be adjusted to increase the survival chances of the pregnant woman while decreasing the harmful effects to the fetus. Most of the observational studies analyzed the effect of chemotherapy in the second and third trimester; however, some studies analyzed the effects of chemotherapy in the first trimester. Avilés et al. investigated the risk of teratogenicity due to chemotherapy in 43 pregnant patients, 19 of whom were treated in the first trimester. However, the physical, neurological, psychological, hematological, and immune function and cytogenetics of the infant postpartum were normal. These results suggest that chemotherapy can be administered even during the first trimester of pregnancy; however, the results should be interpreted with caution because of the low number of patients in the study [15]. Moreover, García-Manero et al. reported that none of 4 pregnant patients with breast cancer who underwent chemotherapy starting in the tenth week of gestation (when organogenesis is completed) had fetuses with congenital malformations [12].

Delivering a baby, even by elective premature labor after the fetus has achieved sufficient gestational growth, can allow for additional chemotherapy and/or radiotherapy [29, 30]. After delivery, more aggressive chemotherapy can be administered to the mothers [31]. Chemotherapy in late pregnancy may not be as safe as previously assumed; a systemic review by Mir et al. suggested that platinum derivatives may cross the placenta during the final weeks of pregnancy and that close neonatal surveillance is therefore recommended in such cases [32].

5. Choice of Chemotherapy

Nonstandard regimens can be used if concerns arise regarding the toxic effects of the chemotherapeutic agent(s) on the fetus. Hubalek et al. reported that nonstandard carboplatin/paclitaxel chemotherapy can be used for the treatment of stage III dysgerminoma in pregnant women instead of the cisplatin, etoposide, and bleomycin (BEP) regimen, which can reportedly cause side effects (especially etoposide) [16]. Furthermore, Picone et al. reported the successful management of advanced (FIGO stage III) endometrioid carcinoma of the ovary that was diagnosed at 22 weeks of gestation; they administered only 2 courses of carboplatin before delivering the infant. After a Caesarean section was performed at 34 weeks of gestation, the therapy was continued with 7 courses of a carboplatin and paclitaxel regimen [33]. However, there is a lack of evidence on whether alternative chemotherapeutic agents can provide better treatment results or have less toxic effects on the fetus. Choosing a suitable chemotherapeutic agent may be challenging, especially when the cancer is advanced or when a relapse occurs during the first trimester. The wishes of the mother should also be considered, and the possible treatment modalities (whether chemotherapy, radiotherapy, and/or surgery) should be discussed with the patient [34]. Multidisciplinary team decision-making and an individualized plan for each case should be considered for all pregnant women with malignancies [35].

6. Dose and Frequency of Chemotherapy

There is insufficient evidence in the literature to ascertain whether dose modification or establishing intervals between chemotherapy cycles can improve the overall outcomes. Doi et al. successfully administered carboplatin therapy with an area under the curve of 3 plus a paclitaxel dose of 120 mg/m^2 biweekly to an ovarian cancer patient; these overall doses were lower than those normally used [18]. However, in a study utilizing an ex vivo human placental perfusion model to predict potential fetal exposure to carboplatin during pregnancy, carboplatin doses up to an area under curve of 7.5 were not associated with significant placental transfer, fetal exposure, or toxicity to the fetus. The investigators thus suggested that it might not be necessary to empirically reduce carboplatin doses in pregnant patients [36]. Similarly, Cardonick et al. retrospectively compared a group of 10 patients who received dose-dense chemotherapy every 2 weeks to a group of 99 pregnant patients who received conventional chemotherapy, with at least 3-week intervals, for breast cancer. They found no significant differences between these groups in terms of infant birth weight and growth rate [37].

7. Chemotherapy for Breast Cancer during Pregnancy

There is no preferred chemotherapy regimen for breast cancer treatment during pregnancy. García-Manero et al. used the classic FAC (5-fluorouracil, doxorubicin, and cyclophosphamide) in 11 patients and found it to be safe; additionally,

4 patients were treated with taxanes with no significant complications in the patients' children postpartum [12].

In another study of 47 patients treated with the FAC regimen, only 1 child born at 29 weeks owing to maternal preeclampsia weighed less than 2000 g at birth. Six infants weighed less than 2500 g at birth, with birth weights ranging between 1389 and 2495 g at gestational ages of 29–40 weeks; 5 of these children were born following at least 38 weeks of gestation [14]. On the other hand, fetal growth abnormalities were reported by Berry et al., who described the effect of chemotherapy on pregnant women with primary or recurrent breast cancer. Chemotherapeutic agents included fluorouracil (1000 mg/m^2), doxorubicin (50 mg/m^2), and cyclophosphamide (500 mg/m^2) administered every 3-4 weeks in the second and third trimesters of pregnancy. Of 24 cases, only 1 infant had a birth weight below the 10th percentile (adjusted for gestational age) [38].

Other protocols have been reported to produce different fetal growth outcomes. Sule and Ewemade reported that doxorubicin and cyclophosphamide administration for breast cancer treatment during the second trimester in a developing country did not affect birth weight [39]. Furthermore, Ring et al. retrospectively described the outcomes of 28 pregnant patients treated with chemotherapy for breast cancer at 5 hospitals in London. One patient received chemotherapy in the first trimester and had a miscarriage; 16 patients were treated with anthracycline-based chemotherapy and 12 received cyclophosphamide, methotrexate, and fluorouracil. Birth weights lower than the 10th percentile (adjusted for gestational age) were not observed among those whose data were available (17 cases); the median birth weight was 3.0 kg (range 1.4–3.5 kg) [40]. In another case study, Gottschalk et al. administered neoadjuvant trastuzumab therapy weekly starting at the 15th gestational week, in addition to 3-weekly carboplatin and docetaxel chemotherapy treatments. They found that trastuzumab caused fetal renal insufficiency. The fetus was delivered by Caesarean section at 34 weeks because of IUGR; prior to delivery, trastuzumab was discontinued after 21 weeks of gestation because of anhydramnios and nonvisualization of the fetal bladder. It is unknown whether trastuzumab itself can cause IUGR directly or indirectly by compromising renal fetal function [41].

8. Chemotherapy for Ovarian Cancer during Pregnancy

There are limited data in the literature regarding the use of chemotherapy for ovarian cancer during pregnancy; no single preferred chemotherapy regimen for this disease has been described for pregnant women. Some investigators used the traditional regimen of a platin derivative with paclitaxel to treat epithelial ovarian cancer. Doi et al. reported that 5 courses of carboplatin and paclitaxel chemotherapy administered during the second trimester (for the treatment of stage IC mucinous cystadenocarcinoma of the ovary diagnosed at 15 weeks of gestation) had no effect on fetal growth [18]. Furthermore, Ruiz Ramos et al. reported that a healthy infant was delivered at 38 gestational weeks after the mother received 6 cycles of neoadjuvant combined chemotherapy (carboplatin and paclitaxel) for stage III ovarian cancer between gestational weeks 16 to 36 [42]. Separately, Ferrandina et al. used only platin-derivative monotherapy for fear of fetal toxicity with multiagent chemotherapy. Their patients received 6 courses of adjuvant monotherapy with cisplatin for ovarian cancer during pregnancy following bilateral salpingo-oophorectomy, omentectomy, and appendectomy at 15 weeks of gestation; a healthy infant (3000 g) was delivered at 36 weeks of gestation [43].

Barut et al. reported administering 3 cycles of carboplatin chemotherapy for ovarian mucinous carcinoma in a patient diagnosed at 22 weeks of gestation. These courses were administered at 25, 28, and 31 weeks of gestation, and the fetus was delivered at 33 weeks weighing 2280 g [44]. A traditional protocol of BEP with a good pregnancy outcome was also reported by Karimi Zarchi et al. where a stage IIIC immature teratoma was diagnosed in a woman at 28 weeks of gestation. After administering BEP (consisting of 2 cycles of 15 mg of bleomycin, 100 mg/m^2 per day of etoposide, and 20 mg/m^2 per day of cisplatin) for 5 days every 3 weeks starting from the 29th week of pregnancy, a healthy 3100 g infant was delivered in gestational week 39 [45].

However, fetal growth abnormalities attributed to chemotherapy have been observed. IUGR developed in 2 of Cardonick et al.'s patients treated in the second and third trimesters with carboplatin and BEP [8]; the IUGR may have been related to the method of administration. In another patient, intraperitoneal carboplatin (area under the curve = 6; days 1 and 4) and paclitaxel (60 mg/m^2; days 1, 8, and 15) on a 28-day cycle were administered to treat stage IIB grade III serous adenocarcinoma diagnosed at 12 weeks of gestation; this regimen was chosen after informing the patient of the lack of data and possible risks. Although 6 cycles were originally planned, the treatment lasted for 4 cycles owing to preeclampsia, thrombocytopenia, and SGA fetal weight. These results should be interpreted with caution since it is unknown whether the growth restriction was a result of preeclampsia or the direct effect of intraperitoneal chemotherapy [19].

The guidelines of the second international consensus meeting of the European Society of Gynecological Oncology (ESGO) recommend NACH in the form of carboplatin and paclitaxel for invasive epithelial ovarian cancer, as is used in nonpregnant patients. Bevacizumab is not recommended, as there is a lack of data regarding the use of this agent in pregnant women. For nonepithelial ovarian cancer, paclitaxel-carboplatin or cisplatin-vinblastin-bleomycin chemotherapy can be used instead of BEP [5].

9. Chemotherapy of Cervical Cancer during Pregnancy

There is no single chemotherapy protocol for the treatment of cervical cancer during pregnancy; most reports describe the use of cisplatin as monotherapy or combined with paclitaxel. Boyd et al. administered 3 courses of cisplatin NACH to a patient with stage IIB high-grade clear cell cervical carcinoma

who was at 25 + 1 weeks of gestation; a healthy neonate was delivered, and no abnormalities in the child were observed during 15 months of follow-up [29]. Another group reported the successful administration of cisplatin as a monotherapy for the treatment of stage IB1 cervical cancer during the second trimester of pregnancy starting at gestational week 17. In this, 6 cycles of cisplatin (with an initial dose of 75 mg/m^2 and a 15% reduction after the 4th cycle) were administered at 10-day intervals; no abnormalities were observed in the infant who was delivered at gestational week 32 [27]. Similarly, neoadjuvant monotherapy for stage IB1 cervical cancer in another pregnant woman (4 cycles of cisplatin 20 mg/m^2) did not impede the normal development of the fetus [35]. This was also the case in other patients [46–48]. The feasibility of combining cisplatin with paclitaxel has also been described [49, 50]. However, despite the abovementioned reports, data remain limited and it remains premature to reach general conclusions on the safety of such regimens.

The second international consensus meeting of the ESGO recommended NACH for stage IB2 and higher cervical cancer. The currently recommended regimen includes platinum-based chemotherapy (cisplatin 75 mg/m^2), preferably with paclitaxel (175 mg/m^2) at 3-week intervals. Carboplatin with an area under the curve of 5-6 can be an alternative option to cisplatin as it is less toxic to the mother [5].

10. Conclusions

There is a lack of national guidelines and consensus regarding the optimal chemotherapy modality during pregnancy. Most of the available literature comprises case reports or retrospective studies that include a small number of patients. Prospective studies to investigate the effect of chemotherapy on intrauterine fetal growth are lacking. Fetal growth abnormalities are recognized sequelae of chemotherapy, and the possibility of fetal growth abnormalities as well as the other side effects of different chemotherapeutic agents should be discussed with the patients. The final decision regarding treatment should consider the type of malignancy and its stage, the use of surgery and/or radiotherapy, the stage of pregnancy, the probability of side effects during treatment, and the patient's own wishes. Centralization of treatment for these patients may help to develop a plan for a prospective study to assess all the associated factors and consequences of chemotherapy during pregnancy, particularly fetal growth abnormalities. Moreover, ethical concerns cannot be ignored. Each patient should ultimately be managed individually with the guidance of a multidisciplinary team.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Review Article

Scientific Evidence for Different Options for GDM Screening and Management: Controversies and Review of the Literature

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Background. Gestational diabetes (GDM) affects up to 7% of pregnant women and is associated with several maternal and perinatal morbidities. International organizations suggest several different recommendations regarding how to screen and to manage GDM. *Objective.* We aimed to analyze the most important and employed guidelines about screening and management of GDM and we investigated existing related literature. *Results.* We found several different criteria for screening for GDM, for monitoring GDM, and for starting pharmacological therapy. When using IADPSG criteria, GDM rate increased, perinatal outcomes improved, and screening became cost-effective. Compared to no treatment, treatment of women meeting criteria for GDM by IADPSG criteria but not by other less strict criteria has limited evidence for an effect on adverse pregnancy outcomes.

1. Introduction

Gestational diabetes (GDM) can be broadly defined as glucose intolerance during pregnancy that affects women without previous diagnosis of diabetes or unknown state. The incidence is about 7% worldwide and this rate has been growing during the last decades and is estimated to increase in the future. The most important risk factors are maternal overweight and obesity, age greater than or equal to 35 years at delivery, hypertension, metabolic syndrome, nonwhite ethnicity, family history of diabetes mellitus, prior unexplained stillbirth, prior infant with congenital anomaly (if not screened during that pregnancy), prior macrosomic infant, history of gestational diabetes, chronic use of steroids, glycosuria, and known impaired glucose metabolism [1].

The importance of GDM is linked to the consequences of pregnancy and also after pregnancy to both mother and newborn. Hyperglycemia in the mother causes abnormal metabolism while in the fetus it causes hyperinsulinemia and its consequences, and incidence of complications is inversely proportional to glucose control. Macrosomia, polyhydramnios, operative delivery, shoulder dystocia, birth injury, perinatal mortality, hypertensive disorders and preeclampsia, congenital malformations (OR: 1.2–1.4), and risk of cesarean delivery are higher in women with GDM; in the long term, women with GDM have a higher risk of developing type 2 diabetes mellitus and cardiovascular diseases; long-term sequelae for offspring are obesity and metabolic syndrome. Approximately 50% of women identified as having GDM will develop frank diabetes within 10 years [2].

To prevent or decrease the risk of GDM, weight loss before pregnancy and cardiovascular exercise could be useful. In fact, aerobic exercise for 35–90 minutes 3-4 times per week during pregnancy is associated with a significantly higher incidence of vaginal delivery and a significantly lower incidence of cesarean delivery, with a significantly lower

incidence of gestational diabetes mellitus and hypertensive disorders [3]. Prompt diagnosis and management are important to reduce worse pregnancy outcomes.

Nonetheless, screening, management, and follow-up of GDM are controversial on international organizations recommendations.

2. Screening Controversies

The aim of screening is to identify asymptomatic pregnant women at high risk of developing GDM. Screening appears to be cost-effective for prevention of obstetrical adverse outcomes and long-term consequences of GDM [4].

Regarding the effect of screening [1] on obstetrical outcomes, there are many controversies:

(a) Indications for screening (who): universal versus selective screening

(b) Timing of screening (when): early screening versus at 24–28 weeks

(c) Type of screening (how): One- versus Two-Step

(d) Criteria for diagnosis: recommendations of international organizations are not standardized

(a) The *population to screen* has not been uniformly identified. There are two possible approaches.

(i) Selective Screening. Only women with risk factor for GDM are offered to be screened, that is, age > 25 years; ethnic origin Hispanic, African, Native American, South or East Asian, or Pacific Islander; BMI > 25; previous personal or family history of impaired glucose tolerance; or history of adverse obstetric outcomes associated with GDM.

(ii) Universal Screening. All women are subjected to screening; in developed countries where overweight and obesity are widespread health problems, this could be the best choice to avoid undiagnosed GDM.

Universal screening is the most commonly adopted method in the USA, while in other countries such as Italy the selective approach is preferred [5].

(b) When identifying the population, it is essential to decide the right *time to screen*.

Women with risk factors and high suspicion of undiagnosed type 2 DM (i.e., obesity, metabolic syndrome) should be screened before pregnancy or at the first prenatal visit (early screening). About 5–10% of women with risk factors have early GDM, and these represent 40% of all women with GDM.

In the absence of early screening or for women negative to early screen, universal screening should be performed at 24 to 28 weeks.

(c) Now we discuss how to screen.

Screening for GDM is somewhat controversial and can be performed either with a One-Step or with a Two-Step approach.

(i) One-Step Approach. GDM screening is performed as an oral 75 g glucose load followed by glucose blood measurement 1 and 2 hours later. A positive result is defined as one value higher than target values. This approach is based on HAPO study [6] and is suggested by IADPSG [7], WHO [8], FIGO [5], and ADA [9]. In fact, HAPO study in 2008 demonstrated a direct correlation between maternal glucose levels and increased birth weight and neonatal hyperinsulinemia.

(ii) Two-Step Approach. GDM is performed as a 50 g one-hour oral glucose load (glucose challenge test, GCT), given to nonfasting women, with a venous glucose measurement one hour later. A positive result is defined as a blood glucose value higher than 130, 135, or 140 mg/dL; the most common value used is 135 mg/dL (ACOG) [4]. Positive screening test is followed by a diagnostic test as an oral glucose tolerance test (GTT) that consists of a beverage with 100 g of glucose, with venous glucose measurement at fasting and after 1, 2, and 3 hours. A positive result is defined as 2 values higher than target values.

(d) Recommendations of international organizations are not standardized.

Table 1 shows the different populations and times to screen and the thresholds used by the most important international organizations worldwide, updated to the latest recommendations [4, 5, 8–12].

We found a large number of studies in international literature comparing One-Step and Two-Step test and different glucose thresholds. When evaluating the best screening method, clinically significant improvements in maternal and neonatal outcomes were analyzed. Two are the most significant studies:

(1) Australian Carbohydrate Intolerance Study in Pregnant Women (ACHOIS) Trial Group: the aim was to determine whether treatment of GDM reduced the risk of perinatal complications.

(2) National Institute of Child Health and Human Development (NICHD) Maternal-Fetal Medicine Units (MFMU) Network Study: the intent was to determine whether treatment of women with mild GDM reduces perinatal and obstetrical complications.

Both trials agree with the rule of IADPSG criteria adoption on reducing fetal birth weight over the 90th percentile and the risk of developing maternal preeclampsia.

Furthermore, to compare the One-Step test to the Two-Step test, several possible study designs have been evaluated in the literature. We can summarize the literature in five groups.

(1) RCTs in which women underwent both the One-Step and the Two-Step tests and the women positive for the One-Step but negative for the Two-Step test were randomized to treatment of GDM versus no treatment: the only such RCT is by Weiss et al. [12], who unfortunately do not report outcomes specific to this group of women.

(2) RCTs of treatment versus no treatment of GDM, focusing on women positive for the One-Step but negative for the Two-Step test: we found 6 RCTs comparing insulin or glyburide to placebo or routine care, and all of them used a Two-Step approach with different glucose thresholds (Table 2) [13–18]. In this group, ACHOIS trial by Crowther

Table 1: Criteria for GDM screening and diagnosis.

	Population to screen	Time to screen	Test	Number of abnormal values required for diagnosis	Fasting glucose (mg/dL)	1 hour after loading (mg/dL)	2 hours after loading (mg/dL)	3 hours after loading (mg/dL)
ACOG 2013 C&C [4]	Selective screening	First visit	Two-Step, 3 h, 100 g	≥2	95	180	155	140
ACOG 2013 NDDG [4]	Selective screening	First visit	Two-Step, 3 h, 100 g	≥2	105	190	165	145
ADA 2015 [9]	Universal screening	24–28 weeks	One-Step, 2 h, 75 g	≥2	95	180	155	Not required
ADA 2015 [9]	Universal screening	First visit	Two-Step, 3 h, 100 g	≥2	95	180	155	140
ADIPS 2013 [52]	Selective screening	24–28 weeks	One-Step, 2 h, 75 g	≥1	92	180	153	Not required
CDA 2013 [10]	Universal screening	First visit	Two-Step, 2 h, 75 g	≥2	95	191	160	Not required
FIGO 2013 [5]	Universal screening	24–28 weeks	One-Step, 2 h, 75 g	≥1	92	180	153	Not required
IADPSG 2010 [7]	Universal screening	24–28 weeks	One-Step, 2 h, 75 g	≥1	92	180	153	Not required
NICE 2015 [11]	Selective screening	24–28 weeks	One-Step, 2 h, 75 g	≥1	101	Not required	140	Not required
WHO 2013 [8]	Universal screening	24–28 weeks	One-Step, 2 h, 75 g	≥1	92	180	153	Not required

ACOG: American College of Obstetricians and Gynecologists; ADA: American Diabetes Association; ADIPS: Australasian Diabetes in Pregnancy Society; CDA: Canadian Diabetes Association; C&C: Carpenter and Coustan; FIGO: International Federation of Gynecology and Obstetrics; IADPSG: International Association of Diabetes Pregnancy Study Group; NICE: National Institute for Health and Care Excellence; NDDG: National Diabetes Data Group; WHO: World Health Organization.

Table 2: RCTs of treatment versus no treatment of GDM, focusing on women positive for the One-Step but negative for the Two-Step test.

Study	Screening test	Diagnostic test	Values for diagnosis	Intervention group	Control group	Primary outcome
O'Sullivan et al., 1966 (USA) [13]	50 g GCT: positive if ≥**130** mg/dl	100 g, 3 h (110-170-120-110)	2 or more values	Insulin	Routine care	LGA
Coustan and Lewis, 1978 (USA) [14]	50 g GCT: positive if ≥**130** mg/dl	100 g, 3 h (95-180-160-135)	2 or more values	Insulin	Routine care	Macrosomia
Thompson et al., 1990 (USA) [15]	50 g GCT: positive if F > 105 mg/dL or 1 h > **140** mg/dL	100 g, 3 h (105-190-165-145)	2 or more values	Insulin	Routine care	Maternal and neonatal morbidity
Crowther et al., 2005 (Australia) [16]	50 g GCT: positive if ≥**140** mg/dl	75 g OGTT (F > 7.8; 2 h 7.8–10 mmol/L)	Both values	Insulin	Routine care	Perinatal complications
Landon et al., 2009 (USA) [17]	50 g GCT: positive if ≥**135** mg/dl	100 g, 3 h (95-180-155-140)	2 or more values but F < 95 mg/dL	Insulin	Routine care	Perinatal outcome
Casey et al., 2015 (USA) [18]	50 g GCT: positive if ≥**140** mg/dL	100 g, 3 h (105-190-165-145)	2 values	Glyburide	Placebo	Birth weight

et al. [16] is included, mentioned before. The main common outcome was lower rate of fetal birth weight over the 90th percentile and macrosomia.

(3) RCTs comparing the One-Step to the Two-Step methods: we found 3 RCTs by Meltzer et al. [19], Sevket et al. [20], and Scifres et al. [21] (Table 3). In each one, there are a study group undergoing One-Step 75 g test and a control group undergoing Two-Step 100 g test. Regarding GDM rate, Sevket et al.'s and Scifres et al.'s RCTs reveal an incidence more than double in the study group with respect to control group (14.5% versus 6%; 4.3% versus 0.0%), while in Meltzer et al.'s RCT, there are no differences (3.6% versus 3.7%). Maternal

Table 3: RCTs comparing the One-Step to the Two-Step methods.

Author (origin)	Study group	Control group (1)	Control group (2)	GDM rate	Primary outcome
Meltzer et al., 2010 (Canada) [19]	One-Step (2 h, 75 g)	Two-Step (50 g, 1 h; 100 g, 3 h)	Two-Step (50 g, 1 h; 75 g, 3 h)	3.6% versus 3.7% versus 3.7%	Costs of screening
Sevket et al., 2013 (Turkey) [20]	One-Step (2 h, 75 g)	Two-Step (50 g, 1 h; 100 g, 3 h)		14.5% versus 6%	Maternal and neonatal outcomes
Scifres et al., 2014 (USA) [21]	One-Step (2 h, 75 g)	Two-Step (50 g, 1 h; 100 g, 3 h)		4.3% versus 0.0%	Maternal and neonatal outcomes

and neonatal outcomes have been analyzed only in 2 studies. Sevket et al.'s RCT reveals that GDM-negative women by IADPSG had better perinatal outcomes than GCT-negative women and GCT-positive women with a negative OGTT; Scifres et al.'s RCT concludes that rates of macrosomia, cesarean delivery, and pregnancy-induced hypertension were also similar between groups.

Interestingly, Meltzer et al.'s RCT analyzed costs of the One-Step compared to the Two-Step test: while the Two-Step test involved the lowest costs, the One-Step test recognized higher GDM rate. The authors' conclusion was in favor of the Two-Step test because the universal glucose screen with 50 g glucose load is an inexpensive, easy-to-administer tool for GDM screening, especially with the use of a lower diagnostic cut-off.

(4) Prospective non-RCTs or retrospective studies comparing incidence of GDM and/or outcomes between the One-Step and Two-Step methods: we found 9 retrospective studies comparing the One-Step approach with IADPSG criteria and Two-Step approach with ACOG criteria (Table 4) [22–30]. Regarding GDM rate, the incidence is higher for women undergoing the One-Step test in all the studies analyzing this issue. Only two studies concluded that IADPSG One-Step approach is useful to avoid worse pregnancy outcomes, in particular LGA and macrosomia [22, 27], while five studies did not find statistically significant differences between the two approaches on outcomes [23–26, 28].

(5) Prospective non-RCTs or retrospective studies reporting outcomes of women meeting criteria for GDM based on the One-Step test but not on the Two-Step test: we found 8 retrospective cohort studies (Tables 5 and 6) [31–38], but no study evaluated whether treatment of women meeting criteria for GDM by IADPSG criteria (One-Step test) but not by other less strict criteria has an effect on adverse pregnancy outcomes compared to no treatment. When analyzing outcomes, macrosomia was more common in women positive on 75 g IADPSG criteria but negative on CDA criteria and positive on 75 g IADPSG criteria but negative on NICE criteria.

3. Conclusion

Despite continuing controversy regarding whether the One-Step test or the Two-Step test should be used for GDM screening, we identified very limited evidence regarding whether treatment of women meeting criteria for GDM by IADPSG criteria (One-Step test) but not by other less strict criteria has an effect on adverse pregnancy outcomes compared to no treatment. Moreover, in none of the included studies was the study group with milder disease treated for GDM (positive for IADPSG criteria, but negative for less stringent criteria). We also found a large variety of different criteria (IADPSG, WHO, NICE, CDA, and C&C) for screening for GDM used in the literature. Therefore, it is not surprising that societies such as IADPSG, WHO, and FIGO recommend the One-Step approach (assuming that identification of women with milder GDM might have benefits for them and their babies), while others such as ACOG still recommend the Two-Step approach for screening.

Only well designed RCTs comparing the One-Step versus the Two-Step approach including huge populations could answer this question.

4. Management Controversies

The aim of management is to reduce the risk of adverse outcomes for the mother and the fetus. Several studies demonstrated that treatment can be effective in reducing adverse outcomes in GDM patients.

Regarding the effect of management on obstetrical outcomes, there are many variables that can play a role; these include

(i) criteria to start therapy after diet alone: once GDM has been diagnosed, patients start nonpharmacological therapy, that is, well balanced diet based on BMI and physical exercise, but it is unclear how long this evaluation period should last before deciding to start pharmacological treatment; a recent systematic review found inconclusive evidence for the threshold value to start medical therapy [4];

(ii) type of initial therapy: insulin and oral hypoglycemic agents are equally effective and can be used as first-line therapy [5];

(iii) dose and frequency of initial therapy: therapy should start at the lower effective dose and then increase based on glucose monitoring;

(iv) frequency of glucose monitoring: when patients start therapy, either diet or pharmacological therapy is important to establish whether glycemic control has been reached; while patients in pharmacological therapy should perform glycemic checks at least four times daily (fasting and after 1 or 2 hours from three main meals: breakfast, lunch, and dinner), there

Table 4: Prospective non-RCTs or retrospective studies comparing incidence of GDM and/or outcomes between the One-Step and Two-Step methods.

Author (origin)	Study design	Two-Step group	One-Step group	GDM rate	Primary outcome
Duran et al., 2014 (Spain) [22]	Retroprospective cohort	ACOG: 50 g 1 h GCT; if >**140** mg/dL followed by 100 g 3 h GTT (C&C)	IADPSG: 75 g 2 h GTT	10.6% versus 35.5%	Pregnancy outcomes
Fuller and Borgida, 2014 (USA) [23]	Retroprospective cohort	ACOG: 50 g 1 h GCT; if >**135** mg/dL followed by 100 g 3 h GTT (C&C)	IADPSG: 75 g 2 h GTT	7.0% versus 11.7%	Maternal and delivery outcomes
Liu et al., 2014 (China) [24]	Retrospective cohort	ACOG: 50 g 1 h GCT; if >**140** mg/dL followed by 100 g 3 h GTT (C&C)	IADPSG: 75 g 2 h GTT	7.0% versus 20.4%	Maternal and perinatal outcomes
Oriot et al., 2014 (Belgium) [25]	Retrospective cohort	ACOG: 50 g 1 h GCT; if >**140** mg/dL followed by 100 g 3 h GTT (C&C)	IADPSG: 75 g 2 h GTT	8.0% versus 23.0%	CS, macrosomia
Wei et al., 2014 (China) [26]	Retrospective cohort	ACOG: 50 g 1 h GCT; if >**135** mg/dL followed by 75 g 3 h GTT (NDDG)	IADPSG: 75 g 2 h GTT	18.3% versus 21.0%	CS, macrosomia
Hung and Hsieh, 2015 (Taiwan) [27]	Retrospective cohort	ACOG: 50 g 1 h GCT; if >**140** mg/dL followed by 100 g 3 h GTT (C&C)	IADPSG: 75 g 2 h GTT	4.6% versus 12.4%	Macrosomia, LGA
Kong et al., 2015 (Canada) [28]	Retrospective cohort	ACOG: 50 g 1 h GCT; if >**140** mg/dL followed by 100 g 3 h GTT (C&C)	IADPSG: 75 g 2 h GTT	7.9% versus 9.4%	Maternal and fetal outcomes
Assaf-Balut et al., 2016 (Spain) [29]	Retrospective cohort	ADA: 50 g 1 h GCT; if >**140** mg/dL followed by 100 g 3 h GTT (C&C)	IADPSG: 75 g 2 h GTT	Not stated	Postpartum disorders
Klara Feldman et al., 2016 (USA) [30]	Retroprospective cohort	ACOG: 50 g 1 h GCT; if >**130** mg/dL followed by 100 g 3 h GTT (C&C)	IADPSG: 75 g 2 h GTT if HbA1c < 5.7%	17.0% versus 27.0%	Pregnancy outcomes

is uncertainty for women in nonpharmacological therapy [5];

(v) target glucose values: RCTs to identify ideal glycemic targets have not been performed, but ADA and ACOG recommend a threshold of 140 mg/dL at 1 hour postprandially or 120 mg/dL at 2 hours postprandially as glycemic targets to reduce the risk of macrosomia [5, 9];

(vi) criteria for pharmacologic therapy dose adjustment: when choosing between tight versus very tight glycemic control, we have to consider risk of hypoglycemia, effects of non-well-controlled GDM, and women compliance;

(vii) criteria for adding or switching pharmacologic therapy;

(viii) fetal monitoring;

(ix) time to delivery: women with GDM with good glycemic control and no other complications can be managed expectantly, while if GDM is not well controlled with therapy, induction of delivery could be considered [5].

We analyzed the literature to figure out which management is the best to follow. When evaluating RCTs [16, 17, 39–51] which included criteria for starting pharmacologic therapy in women with GDM, the most common frequency for glucose monitoring was four times per day (i.e., when fasting and after each main meal). The effect of therapy on GDM was assessed using fasting of 90 (or 95) mg/dL and 2 hours of 120 mg/dL as blood glucose target values. Importantly, we found several different criteria for starting pharmacologic therapy after a period of diet alone, with the majority using very tight criteria of either 1 or 2 values in one- or two-week period higher than the target values, of which

Table 5: Prospective non-RCT or retrospective studies reporting outcomes of women meeting criteria for GDM based on the One-Step test but not on the Two-Step test.

Author (origin)	Study design	GDM screening	50 g GCT criteria	75 g OGTT criteria	100 g OGTT criteria
Lapolla et al., 2011 (Italy) [31]	Retrospective cohort	*Two-Step: 50 g* 1 h; if >140 mg/dL: *100 g 3 h GTT*	≥**140** mg/dL: 100 g 3 h GTT	*Not done*	2 abnormal values of fasting ≥ 95 mg/dL, or 1 h 180 mg/dl; 2 h 155 mg/dL; 3 h 140 mg/dL
Bodmer-Roy et al., 2012 (Canada) [32]	Retrospective cohort	*Two-Step: 50 g* 1 h; if 137–184 mg/dL: *75 g 2 h GTT*	137–184 mg/dL: 75 g GTT; >184 mg/dL: GDM	1 abnormal value of fasting ≥ 96 mg/dL; 1 h: ≥191 mg/dl; 2 h: ≥160 mg/dL*	*Not done*
Benhalima et al., 2013 (Belgium) [33]	Retrospective cohort	*Two-Step: 50 g* 1 h; if ≥140 mg/dL:*100 g 3 h GTT*	≥**140** mg/dL: 100 g 3 h GTT	*Not done*	2 abnormal values of fasting ≥ 95 mg/dL, or 1 h 180 mg/dl; 2 h 155 mg/dL; 3 h 140 mg/dL
Ethridge et al., 2014 (USA) [34]	Retrospective cohort	*Two-Step: 50 g* 1 h; if ≥135 mg/dL:*100 g 3 h GTT*	≥**135** mg/dL: 100 g 3 h GTT	*Not done*	2 abnormal values of fasting ≥ 95 mg/dL, or 1 h 180 mg/dl; 2 h 155 mg/dL; 3 h 140 mg/dL
Liao et al., 2014 (China) [35]	Retrospective cohort	*Two-Step: 50 g* 1 h; if ≥140 mg/dL: *100 g 3 h GTT*	≥**140** mg/dL: 100 g 3 h GTT	*Not done*	2 abnormal values of fasting ≥ 95 mg/dL, or 1 h 180 mg/dl; 2 h 155 mg/dL; 3 h 140 mg/dL
Mayo et al., 2015 (Canada) [36]	Retrospective cohort	*Two-Step: 50 g* 1 h; if 140–184 mg/dL: *75 g 2 h GTT*	If 140–184 mg/dL: 75 g GTT; >184 mg/dL: GDM	1 abnormal value of fasting ≥ 95 mg/dL; 1 h: ≥191 mg/dl: 2 h: ≥160 mg/dL*	*Not done*
Meek et al., 2015 (UK) [37]	Retrospective cohort	*Two-Step: 50 g* 1 h; if >138 mg/dL: *75 g 2 h GTT*	>**138** mg/dL: 75 g 2 h GTT	1 abnormal value of fasting ≥ 110/128 mg/dL; 2 h: ≥140 mg/dL**	*Not done*
Tward et al., 2016 (Canada) [38]	Retrospective cohort	*Two-Step: 50 g* 1 h; if >140 mg/dL: *75 g 2 h GTT*	≥**140** mg/dL: 75 g 2 h GTT	2 abnormal values of fasting ≥ 95 mg/dL; 1 h: ≥191 mg/dl: 2 h: ≥160 mg/dL	*Not done*

*2008 Canadian Diabetes Association criteria (ref.). **WHO 1999 criteria until 2007 (fasting, 148 mg/dL), modified WHO 1999 criteria (fasting, 130 mg/dL).

half used only 1 value and half used 2 values, while any RCT used less tight criteria (i.e., >50% glucose values higher than target values) (Table 7) [16, 17, 39–51].

Finally, when analyzing international organizations guidelines on management of GDM, while there is consensus about glycemic targets, we found different opinions about therapy, monitoring, and time of delivery (Table 8). Moreover, there is limited information regarding other important criteria about dose and frequency of therapy, dose adjustment, and adding or switching pharmacologic therapy.

Moreover, the application of the IADPSG was associated with an increase in GDM prevalence up to 3.5-fold, as well as significant improvements in pregnancy outcomes (gestational hypertension, prematurity, CD, number of LGA and SGA, and 1-minute Apgar scores <7), and was cost-effective. This could be presumably by permitting the treatment of a greater number of women at risk for pregnancy complications [22].

5. Conclusion

There are many unsolved questions concerning GDM management. Analyzing the literature in detail, we found different criteria for screening for GDM, for monitoring GDM, and for starting pharmacological therapy. The hope is to reach universally approved and shared recommendations to improve health care and reduce costs and adverse outcomes for women with GDM and their babies.

Table 6: Continues on the same studies as in Table 5.

Author (origin)	Study group	Control	Primary outcome
Lapolla et al., 2011 (Italy) [31]	*100 g IADPSG-positive, C&C-negative* (fasting: 92–94 mg/dL; 2 h: 153-154 mg/dL; *not treated*) [n = 112]	*IADPSG-negative* (fasting: <92 mg/dL; 1 h: <180 mg/dL; 2 h: <153 mg/dL) [n = 1815]	Perinatal outcomes
Bodmer-Roy et al., 2012 (Canada) [32]	*75 g IADPSG-positive, CDA-negative* (fasting: 92–95 mg/dL; 1 h: 180–190 mg/dL; 2 h: 153–159 mg/dL; *not treated*) [n = 186]	*GCT-negative* (50 g 1 h < 137 mg/dL) [n = 186] Or *IADPSG-negative* (fasting: <92 mg/dL; 1 h: <180 mg/dL; 2 h: <153 mg/dL) [n = 186]	LGA > 90th percentile
Benhalima et al., 2013 (Belgium) [33]	*100 g IADPSG-positive, C&C-negative* (fasting: 92–94 mg/dL; 2 h: 153-154 mg/dL; *not treated*) [n = 160]	*GCT-negative* (50 g 1 h < 140 mg/dL) And *IADPSG-negative* (fasting: <92 mg/dL; 1 h: <180 mg/dL; 2 h: <153 mg/dL) [n = 6345]	Pregnancy outcomes
Ethridge et al., 2014 (USA) [34]	*100 g IADPSG-positive, C&C-negative* (fasting: 92–94 mg/dL; 2 h: 153-154 mg/dL; *not treated*) [n = 281]	*GCT-negative* (50 g 1 h < 135 mg/dL) [n = 6999] Or *IADPSG-negative* (fasting: <92 mg/dL; 1 h: <180 mg/dL; 2 h: <153 mg/dL) [n = 772]	Birth weight and neonatal outcomes
Liao et al., 2014 (China) [35]	*100 g IADPSG-positive, C&C-negative* (fasting: 92–94 mg/dL; 2 h: 153-154 mg/dL; *not treated*) [n = 1314]	*GCT-negative* (50 g 1 h < 140 mg/dL) And *IADPSG-negative* (fasting: <92 mg/dL; 1 h: <180 mg/dL; 2 h: <153 mg/dL) [n = 2662]	Maternal and neonatal outcomes
Mayo et al., 2015 (Canada) [36]	*75 g IADPSG-positive, CDA-negative* (fasting: 92–95 mg/dL; 1 h: 180–190 mg/dL; 2 h: 153–159 mg/dL; *not treated*) [n = 155]	*GCT-negative* (50 g 1 h < 140 mg/dL) [n = 4183] Or *IADPSG-negative* (fasting: <92 mg/dL; 1 h: <180 mg/dL; 2 h: <153 mg/dL) [n = 526]	Not stated
Meek et al., 2015 (USA) [37]	*75 g IADPSG-positive, NICE-negative* (fasting: 92–101 mg/dL; 1 h: ≥153 mg/dL; *not treated*) [n = 387]	*IADPSG-negative* (fasting: <92 mg/dL; 1 h: <180 mg/dL; 2 h: <153 mg/dL) [n = 2406]	Delivery and neonatal outcomes
Tward et al., 2016 (Canada) [38]	*75 g IADPSG-positive, CDA-negative* (fasting: 92–95 mg/dL; 1 h: 180–190 mg/dL; 2 h: 153–159 mg/dL; *not treated*) [n = 99]	*GCT-negative* (50 g 1 h < 140 mg/dL) [n = 1021] Or *IADPSG-negative* (fasting: <92 mg/dL; 1 h: <180 mg/dL; 2 h: <153 mg/dL) [n = 184]	Fetal growth in twins

Table 7: Management of women included in RCTs.

	Glucose monitoring	Target value for glycemic control	Type of diet	Recommendations about exercise	Glucose values used for starting pharmacologic therapy based on target values
Garner et al., 1997 [39]	4 times daily[A]	F: <4.4 mmol/l (80 mg/dL); 1 h: <7.8 mmol/l (140 mg/dL)	35 kcal/kg IBW/day	Not stated	2 or more values higher in 2 weeks
Langer et al., 2000 [40]	7 times daily[B]	F: <5.0 mmol/l (90 mg/dL); preprandial: <5.3 mmol/l (95 mg/dl) 2 h: <6.7 mmol/l (120 mg/dL)	(i) 25 kcal/kg BW/day for obese women (ii) 35 kcal/kg BW/day for nonobese women (iii) 3 meals and 4 snacks (iv) 40–45% of calories from carbohydrates	Not stated	1 or more preprandial or 2 h values higher in 1 week
Mecacci et al., 2003 [41]	9 times daily[C]	F: <5.0 mmol/l (90 mg/dL); 1 h: <6.7 mmol/l (120 mg/dL)	ADA recommendations*	Not stated	More than 50% values higher after 1 week
Schaefer-Graf et al., 2004 [42]	6 times daily[D]	Intervention group: F: <4.5 mmol/l (80 mg/dL); 1 h: <6.1 mmol/l (110 mg/dL) Control group: F: <5.0 mmol/l (90 mg/dL); 1 h: <6.7 mmol/l (120 mg/dL)	(i) 25 kcal/kg BW/day for overweight women (ii) 30 kcal/kg BW/day for normal weight women	Exercise after meals	Intervention group: (i) AC > 75th $p < 36$ weeks (ii) F ≥ 120 mg/dL (iii) 2 h ≥ 200 mg/dL Control group: (iv) 2 or more values (v) 4 profiles with at least 1 value higher in 2 weeks
Crowther et al., 2005 [16]	4 times daily[E]	F: <5.5 mmol/l (99 mg/dL); 2 h: <7.0 mmol/l (126 mg/dL)	Dietary advice from a qualified dietician	Not stated	(i) 2 values higher in 2 weeks <35 weeks (ii) 2 h >8.0 mmol/l (144 mg/dl) in 2 weeks >35 weeks (iii) 1 value >9.0 mmol/l (162 mg/dl) in 2 weeks
Anjalakshi et al., 2007 [43]	Not specified	2 h: <6.7 mmol/l (120 mg/dL)	Medical Nutrition Therapy (MNT)	Not stated	1 value 2 h higher in 2 weeks
Landon et al., 2009 [17]	4 times daily[E]	F: <5.3 mmol/l (95 mg/dL); 2 h: <6.7 mmol/l (120 mg/dL)	ADA recommendations**	Not stated	(i) >50% values higher between 2 study visits (ii) 1 random value >160 mg/dl (8.9 mmol/l) (iii) 1 F > 95 mg/dl; the patient's caregiver initiated treatment (more or less 7 visits)

Table 7: Continued.

	Glucose monitoring	Target value for glycemic control	Type of diet	Recommendations about exercise	Glucose values used for starting pharmacologic therapy based on target values
Ijäs et al., 2011 [44]	4 times daily[F]	F: <5.3 mmol/l (95 mg/dL); 1.5 h: <6.7 mmol/l (120 mg/dL)	Dietary and lifestyle counselling	Not stated	2 values higher in 2–4 weeks
Balaji et al., 2012 [45]	4 times daily[E]	F: <5.0 mmol/l (90 mg/dL); 2 h: <6.7 mmol/l (120 mg/dL); HbA1c: <6.0 g/dL	Medical Nutrition Therapy (MNT)	Not stated	1 value higher in 2 weeks
Mukhopadhyay et al., 2012 [46]	7 times daily[B]	F: <5.0 mmol/l (90 mg/dL); 2 h: <6.7 mmol/l (120 mg/dL)	(i) 25 kcal/kg BW for obese women (ii) 35 kcal/kg BW for nonobese women (iii) 3 daily meals; 40–45% of calories from carbohydrates	Not stated	1 value higher in 2 weeks
Niromanesh et al., 2012 [47]	4 times daily[E]	F: <5.3 mmol/l (95 mg/dL); 2 h: <6.7 mmol/l (120 mg/dL)	(i) 15 kcal/kg BW for obese women (ii) 22 kcal/kg BW for overweight women (iii) 30 kcal/kg BW for normal weight women (iv) 40 kcal/kg BW for underweight women (v) 45% of calories from carbohydrates, 20% from protein, and 35% from fat (vi) 3 meals and 3 snacks (vii) Calories: 10% breakfast, 30% each lunch and dinner, and 30% snacks	30 minutes of walking per day	2 values higher in one week
Silva et al., 2010 [48]	4 times daily[A]	F: <5.0 mmol/l (90 mg/dL); 1 h: <6.7 mmol/l (120 mg/dL)	(i) 25 kcal/kg BW/day for overweight women (ii) 35 kcal/kg BW/day for normal weight women (iii) 3 full meals and 4 light meals (iv) 35–45% of calories from carbohydrates	Not stated	2 values higher after 1 week
Mesdaghinia et al., 2013 [49]	4 times daily[E]	F: <5.3 mmol/l (95 mg/dL); 2 h: <6.7 mmol/l (120 mg/dL)	Dietary changes***	Not stated	1 value higher in 1 week
Spaulonci et al., 2013 [50]	4 times daily[E]	F: <5.3 mmol/l (95 mg/dL); 2 h: <6.7 mmol/l (120 mg/dL)	(i) 25–35 kcal/kg IBW based on pregestational BMI (ii) 55% carbohydrates, 15% proteins, and 30% fat	30-minute walk 3 times a week	>30% values higher in 1 week

Table 7: Continued.

	Glucose monitoring	Target value for glycemic control	Type of diet	Recommendations about exercise	Glucose values used for starting pharmacologic therapy based on target values
Behrashi et al., 2016 [53]	4 times daily[E]	F: <5.0 mmol/l (90 mg/dL); 2 h: <6.7 mmol/l (120 mg/dL)	Education for lifestyle change (exercise and diet)	Education for lifestyle change (exercise and diet)	1 value higher in 1 week

F: fasting; GA: gestational age; IBW: ideal body weight; BW: body weight; BMI: body mass index.
[A]Fasting and 1 hour after each main meal: breakfast, lunch, and dinner.
[B]Fasting, before lunch and dinner, 2 hours after main meals, breakfast, lunch, and dinner, and at bedtime.
[C]Fasting, preprandial before lunch and dinner, 1 and 2 hours after each main meal: breakfast, lunch, and dinner.
[D]Fasting, preprandial before lunch and dinner, 1 hour after each main meal: breakfast, lunch, and dinner.
[E]Fasting and 2 hours after each main meal: breakfast, lunch, and dinner.
[F]Fasting and 1.5 hours after each main meal: breakfast, lunch, and dinner.
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Table 8: Management of GDM, international guidelines.

	ACOG 2013 [4]	CDA 2013 [10]	ADA 2015 [9]	FIGO 2015 [5]	NICE 2015 [11]
Criteria to start therapy after diet alone	Inconclusive evidence	Glycemic control not achieved after 2 weeks of nutritional therapy alone	NR	NR	Glycemic control not achieved after 1-2 weeks of diet and exercise
Type of initial therapy	Insulin or oral medications	Insulin or oral medications	Insulin or glyburide	Glyburide inferior to both insulin and metformin, while metformin performs better than insulin	Metformin
Dose and frequency of initial therapy	NR	NR	NR	NR	NR
Frequency of glucose monitoring	4 times daily as fasting and either 1 h or 2 h after each meal	4 times daily as fasting and either 1 h or 2 h after each meal	NR	4 times daily as fasting and 2 h after each meal	7 times daily as fasting, premeal, 1 h after each meal, bedtime
Target glucose values	1 h ≤ 140 mg/dL, 2 h ≤ 120 mg/dL	Fasting ≤ 95 mg/dL, 1 h ≤ 140 mg/dL, 2 h ≤ 120 mg/dL	Fasting ≤ 95 mg/dL, 1 h ≤ 140 mg/dL, 2 h ≤ 120 mg/dL	Fasting ≤ 95 mg/dL, 1 h ≤ 140 mg/dL, 2 h ≤ 120 mg/dL	Fasting ≤ 95 mg/dL, 1 h ≤ 140 mg/dL, 2 h ≤ 116 mg/dL
Criteria for pharmacologic therapy dose adjustment	NR	NR	NR	NR	NR
Criteria for adding or switching pharmacologic therapy	NR	NR	NR	NR	NR
Pregnancy monitoring	No consensus	NR	NR	NR	Ultrasound monitoring of fetal growth and AF volume every 4 weeks from 28 to 36 weeks
Time to delivery	Well-controlled: >39 weeks; insufficient data for others; CD if EFW > 4500 g	NR	NR	Consider induction at 38-39 weeks	Delivery no later than 40 + 6 weeks

NR: not reported.

Conflicts of Interest

The authors report no conflicts of interest.

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Research Article

Apolipoprotein E Genotype in Very Preterm Neonates with Intrauterine Growth Restriction: An Analysis of the German Neonatal Network Cohort

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Aim. Cord blood of intrauterine growth restricted (IUGR) neonates displays lipid changes towards atherosclerotic profiles. Apolipoprotein E (ApoE) and its isoforms (e2, e3, and e4) are involved in the regulation of lipid metabolism. Specifically, ApoE e4 has been associated with atherosclerotic diseases, while e2 has a favorable effect. We therefore hypothesized that ApoE e4 haplotype is frequently observed in IUGR neonates and contributes to impaired fetal growth and the association of IUGR with cardiovascular and metabolic diseases later in life. *Methods.* A cohort of 4885 preterm infants (≥22+0 and <32+0 weeks of gestation and birth weight below 1500 g) from the GNN study cohort was analyzed. Neonates were categorized into subgroups of <3rd, 3rd–10th, and >10th birth weight percentile. Analysis of the single nucleotides rs429358 and rs7412, identifying the ApoE genotype, was carried out using TaqMan® SNP genotyping assays. The proportional odds model was used to assess data. *Results.* No association was found between genotype and birth weight percentiles in each of the subgroups. *Conclusion.* ApoE genotype and low birth weight depict two distinct risk factors for cardiovascular disease without being directly associated.

1. Introduction

Inadequate birth weight, compared to population based standards, has a complex pathophysiology not yet entirely understood. Disrupted placentation and consequently insufficient placental vascular supply of nutrients and oxygen to the fetus, among other factors, are believed to be involved in intrauterine growth restriction (IUGR) and to result in small for gestational age (SGA) neonates [1, 2]. As such it shares common pathomechanisms with preeclampsia. Moreover, preeclampsia and IUGR often occur simultaneously especially if the onset is early preterm [3, 4]. Hence, both diseases have been described as "placental syndrome." The clinical outcome of IUGR born babies is worse compared to neonates born with normal weight. Restricted fetal growth is believed to increase the risk for adverse neurophysiologic development, cardiovascular disease, and dyslipidemia in later life [5–9]. Atherogenic fetal serum lipid configurations have been associated with IUGR by previous research. IUGR born babies were found to have lower concentrations of high-density lipoprotein cholesterol (HDL-C), known for having an anti-inflammatory effect and protective properties against the development of atherosclerosis, while triglycerides and oxidized low-density lipoprotein (oxLDL) levels were elevated in samples of umbilical blood compared to adequate weight newborns [5, 10, 11]. The results propose that disrupted cholesterol and triglyceride handling plays a role in causing suboptimal fetal development and exposes the newborn to an atherosclerotic environment early, consequentially giving raise to irreversible damage to vessels [6].

Apolipoprotein E (ApoE) is an important circulating serum protein involved in transporting lipids and cholesterol and regulating lipid levels. Its regulatory functions have been attributed to many biophysiological processes including neuronal growth and modulation of oxidant and inflammatory processes [12, 13]. The ApoE gene has three allelic variants (e2, e3, and e4). The three haplotypes are determined by the SNPs rs429358 and rs7412. Variations in these nucleotides are determined by nucleobases T-T (e2), T-C (e3), and C-C (e4) [14, 15]. Despite their minor structural disparities, the three variants e2, e3, and e4 and their corresponding amino acid polymorphisms exhibit differences in individual binding properties to different receptors and display diverse effects on lipoprotein metabolism [12].

The association of ApoE e4 with the development of dyslipidemia and cardiovascular disease has been described for the past two decades and is now well established [16, 17]. Recent studies provided evidence of isoform e4 being present in individuals afflicted with severe cerebral palsy, promoting the development of Alzheimer's disease, and promoting the development of epilepsy. On the contrary, possibly due to its diverse binding properties, isoform e2 displays a protective effect [13, 18, 19]. In newborns, carrying the ApoE e2 allele has been associated with lower fetal cord blood LDL-C levels and higher levels of HDL-C suggesting a beneficial effect of this genotype on blood lipid configuration [20, 21].

Given the significant impact of ApoE genotype on serum lipid levels and the association of IUGR with altered lipid metabolism, we hypothesized fetal ApoE genotype to be a modulator of fetal growth and severity of IUGR. A link between ApoE genotype and birth weight percentile would prove the role of the APOE gene as modulator of fetal growth and consequently provide an explanation for the fact that impaired fetal development depicts a cardiovascular risk factor. Hence, the aim of the present study was to identify ApoE genotypes in IUGR neonates. We took advantage of a nation-wide genomic study of the German Neonatal Network (GNN) including more than 18000 preterm neonates born before 32 weeks of gestation or with very low birth weight below 1500 g. The neonates were clustered according to birth weight percentiles with the lowest percentiles likely reflecting impaired fetal development. However, IUGR is not uniquely defined and neonates can be born small for their gestational age (SGA) by genetic determination and in the absence of a pathologic process [5, 22]. Hence, in this study different SGA subtypes and scenarios have been considered including different cut-offs of percentiles to define SGA as well as factors associated with IUGR like hypertensive disorders in pregnancy. Identifying ApoE genotypes as an individual risk factor of IUGR or severity of IUGR has the potential to allow for better individual prenatal observation and postnatal treatment especially in preterm born neonates in need of intensive neonatal care.

2. Methods

2.1. Study Cohort. Subjects were enrolled in the GNN cohort between January 2009 and December 2015 by 54 currently participating neonatal intensive care units in Germany. Preterm infants with a birth weight below 1500 g and born <37+0 weeks of gestation were enrolled. The GNN study was approved by the local committee for research in human subjects of the University of Lübeck (Germany), as well as by all of the local committees of participating centers. Written informed consent was obtained from parents with neonates eligible for the research and publication of data. A predefined clinical data set form, containing obstetric and neonatal data, was filled out by the attending physician. Data were then sent to the GNN coordinating center in Lübeck. Parental refusal, early neonatal death, and parents not asked to participate were reasons for nonenrollment. A specialized neonatologist surveyed the data quality by annual visits to the participating centers. Obstetrical and neonatal data collected included maternal origin, maternal age, gestational age, birth weight, fetal sex, singleton versus multiple birth, fetal malformations, presence of pathologic Doppler/IUGR, presence of maternal pregnancy induced hypertension (PIH) or preeclampsia, and presence of HELLP syndrome. Birth weight percentiles were calculated according to Voigt et al. [23]. Ultrasound measurements executed between 8 and 12 weeks of gestation determined fetal age.

2.2. Genotyping. ApoE genotyping was performed on buccal swabs and/or cord tissue transferred to the study center (University of Lübeck) together with the clinical data set form. DNA extraction was carried out by using commercial DNA purification kits (Qiagen, Hilden, Germany). The samples underwent real-time PCR. Genotyping of the APOE rs429358 and rs7412 single nucleotide polymorphisms (SNP), defining the e2, e3, and e4 allele, was performed by using TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, USA) according to the manufacturer's protocol. Genotyping of rs7412 and rs429358 was done in 10211 GNN-infants who were born between 2009 and 2014. Genotyping was successful in 93% of all patients for rs429358 and 96% of all patients for rs7412 (90% for both SNPs).

2.3. Patient Selection. We selected preterm infants with gestational age below 32+0 weeks, a birth weight below 1500 grams, and European origin. Infants from multiple birth and with fetal malformations were excluded (Figure 1). To estimate interactions between ApoE genotype and abnormal fetal growth different scenarios were established: 4885 preterm neonates eligible for analysis were divided into three birth weight percentile groups: >10th percentile (n = 3921), 3rd–10th percentile (n = 625), and <3rd percentile (n = 339). Those groups were further divided into subgroups according to antenatal clinical diagnosis of one of the subsequent features indicating a placental involvement (placental syndrome): (1) presence of maternal pregnancy induced hypertension (PIH including preeclampsia) and/or HELLP syndrome, (2) presence of IUGR and/or pathologic Doppler (maternal and/or fetal side) with/without PIH/HELLP, or (3) absence of criteria (1) or (2) (no placental syndrome). It was assumed that the subgroup with birth weight percentile <3rd plus the clinical indication of IUGR/pathologic Doppler was most severely growth restricted while the subgroup of

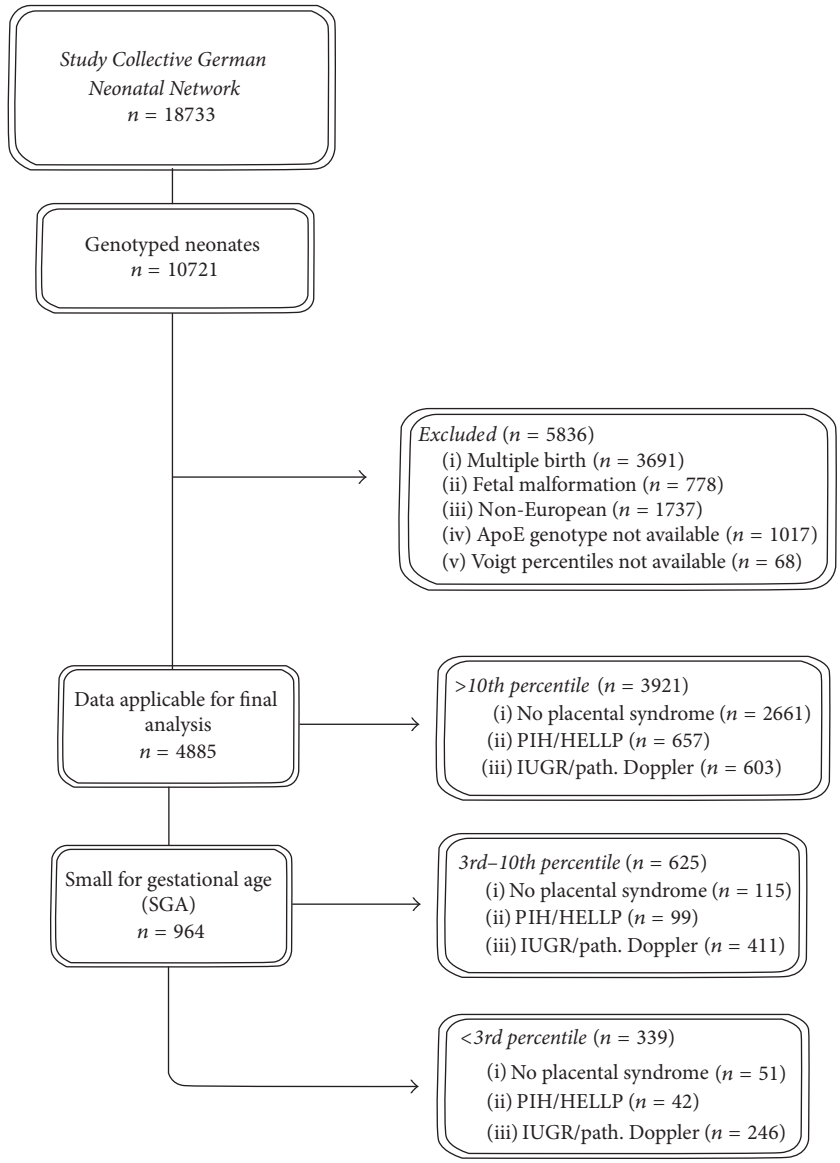

Figure 1: Inclusion/exclusion algorithm of probands of the German Neonatal Network. Multiple reasons for exclusion could apply. PIH includes preeclampsia. Patients with both "PIH/HELLP" and "IUGR/pathological Doppler ultrasound" were grouped into the "IUGR/pathological Doppler ultrasound" subgroup.

patients with birth weight percentile >10th in the absence of (1) or (2) served as the most "pure" control group (Figure 2).

2.4. Statistical Analysis. Data analysis was performed using the SPSS 20.0 data analysis package (IBM, Munich, Germany). In order to evaluate the effect of the ApoE genotype on the birth weight in different SGA subtypes the proportional odds model was used to assess ordinal data. The model analyzes the likelihood to reach a higher ordinal level in relation to other variables. An equal slopes assumption of the data must be met which is tested beforehand. Our null hypothesis was (H_o) = the ApoE genotype not affecting the Voigt birth weight percentile in different subgroups of premature IUGR. The type I error level was set to 0.05. To correct for the multiple testing, nominal p values were adjusted according to the Bonferroni-Holm procedure. Hardy Weinberg equilibrium for three alleles was assessed by chi-square test.

3. Results

Maternal and neonatal patient characteristics for each subgroup are shown in Table 1. The average maternal age was 30.19 years (SD ± 5.92). Gestational age on average was slightly smaller in the >10th percentile weight group (28,28 ± 2.33 weeks) than in the <3rd percentile weight group (29.92 ± 4.25 weeks). Mean birth weight of >10 percentile group was 1075 g (SD ± 275 g), 924 g (SD ± 347 g) in the 10th–3rd, and 839 g (SD ± 421) in the <3rd group. The percentage of female neonates varied from 47.4% to 50.4% among subgroups. From

Table 1: Patient characteristics. Displayed values are based on data available.

	>10P		3–10P		<3P	
	Mean	(SD)	Mean	(SD)	Mean	(SD)
Maternal age (years)	30.20	(5.90)	30.04	(5.94)	30.36	(6.05)
Gestational age at birth (weeks)	28.28	(2.33)	29.74	(3.13)	29.92	(4.25)
Birth weight (gram)	1075	(275)	924	(347)	839	(421)
	frequency	(%)	frequency	(%)	frequency	(%)
Neonatal gender (*n*, % female)	1783	(47.4)	593	(50.4)	325	(48.3)

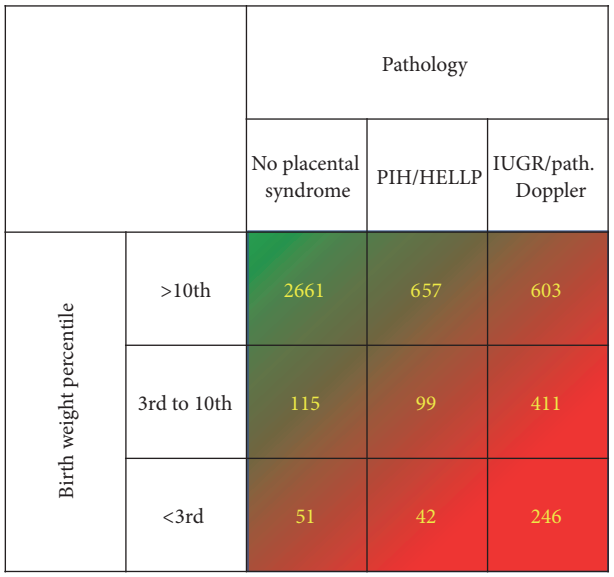

Figure 2: Frequencies of birth weight percentile groups and the presence or absence of PIH/HELLP syndrome or IUGR/pathological Doppler ultrasound as clinically indicated, of the total 4885 preterm neonates included into the analysis. Patients with both "PIH/HELLP" and "IUGR/pathological Doppler ultrasound" were grouped into the "IUGR/pathological Doppler ultrasound" subgroup.

the total group of 4885 probands, close to 95% of values were available for analysis of maternal and neonatal patient characteristics.

The frequencies of birth weight percentiles of neonates born without placental syndrome, in the presence of PIH/HELLP or IUGR/pathological Doppler, respectively, are displayed in Figure 2.

Overall, haplotype frequencies were e2: 7,4%, e3: 79%, and e4: 13,6%, respectively. ApoE genotype combinations found in our study population are shown in Table 2. The genotypes showed a normal distributed Hardy Weinberg equilibrium. Genotype distribution per birth weight percentile and functional subgroup is depicted in Table 3.

Using proportional odds model and cumulative link model, respectively, interactive influence of ApoE genotypes and placental syndromes (three categories) on birth weight centiles have been estimated. As expected the groups

Table 2: Genotyping of the 4885 neonates.

Genotype	e2/e2	e3/e2	e3/e3	e3/e4	e2/e4	e4/e4
n	28	569	3049	1050	94	95
%	0.54	11.63	62.39	21.57	2.01	1.86

PIH/HELLP and IUGR/pathological Doppler showed a statistically significant negative effect on birth weight percentile when compared to neonates born in absence of a placental syndrome (Table 4). None of the neonatal ApoE genotypes could be identified as having an influence on birth weight percentiles when e2/e2 was used as a reference group.

To further investigate a possible correlation between ApoE genotype and birth weight percentile, the ApoE genotypes were pooled in groups containing the haplotype e2 (e2/e2 and e2/e3), haplotype e3 only, and haplotype e4 (e3/e4 and e4/e4). Thus the compiled allele groups did not show a significant effect on the birth weight percentiles in any of the analyzed subgroups compared to the groups e2/e2 and e2/e3 (data not shown).

4. Discussion

To our knowledge, the data set used in this study was the largest analyzed to date. 4885 preterm neonates were included in the analysis with the aim of identifying the effect of the ApoE genotype on proper fetal development. However, we did not find any association of ApoE haplotypes with birth weight percentiles independently of whether or not pregnancy was further complicated by a placental disorder characterized by PIH or IUGR.

Three prior studies investigated the ApoE genotype in IUGR/low birth weight children. Our results are in accordance with Akisu et al. who also found no correlation between the ApoE genotype and IUGR born newborns [24]. However, their results are not entirely applicable to the present study since all infants investigated had completed the 36th week of gestation. Moreover, the study was massively underpowered as it included only 20 cases of IUGR. Another study by Szitanyi et al. retrospectively investigated the ApoE genotypes and birth weight of two groups of 10-11-year-old children. They hypothesized that both intrauterine undernutrition, demonstrated by a lower birth weight, and the ApoE

Table 3: Genotype distribution (frequencies) of preterm neonates subgrouped by birth weight percentile and type of pregnancy related pathology. Patients with both, "PIH/HELLP" and "IUGR/pathological Doppler ultrasound," were grouped into the "IUGR/pathological Doppler ultrasound" subgroup.

Placental syndrome	Birth weight percentile	e2/e2	e3/e2	e3/e3	e3/e4	e2/e4	e4/e4
No placental syndrome	>10	15	321	1644	572	57	52
	3–10	1	17	70	22	3	2
	<3	1	8	28	12	1	1
PIH/HELLP	>10	7	72	417	135	13	13
	3–10	0	10	61	26	1	1
	<3	0	2	29	9	0	2
IUGR/path. Doppler	>10	1	63	399	117	9	14
	3–10	0	52	243	105	5	6
	<3	3	24	158	52	5	4

Table 4: Analysis of interactive effect of ApoE genotype and subgroups with placental syndrome (PIH/HELLP, IUGR/pathologic Doppler) on birth weight percentile groups.

Factor	Estimate	SE	z value	*Nominal P*	*Adjusted P*
e2/e3	0.4891	0.5359	0.9127	0.36	1
e3/e3	0.5667	0.5256	1.0781	0.28	1
e3/e4	0.4305	0.5297	0.8128	0.42	1
e4/e4	0.7221	0.6048	1.1939	0.23	1
e2/e4	0.5635	0.6080	0.9268	0.35	1
PIH/HELLP	−1.2365	0.1224	−10.1034	<0.0001	<0.0001
IUGR	−2.8398	0.0971	−29.2359	<0.0001	<0.0001

Results of analyzing the effect of individual ApoE genotypes with underlying maternal pathology, respectively, on birth weight percentile. Data were compared to reference group e2/e2 for genotype analysis and to no placental syndrome, respectively.
Patients with both, "PIH/HELLP" and "IUGR/pathological Doppler ultrasound," were grouped into the "IUGR/pathological Doppler ultrasound" subgroup. All values are rounded on four decimals. The type I error level was set to 0.05. Adjusted p values are adjusted according to the Bonferroni-Holm procedure.

genotype participate in the development of hypercholesterolemia in childhood [25]. Children were subdivided into tertiles with high and low levels of cholesterol, respectively. While the high-cholesterol group had significantly lower birth weight (0.3 kg) and ApoE e4 had a higher prevalence among this group, no association could be established between ApoE e4 and birth weight alone. Unfortunately, the study did not provide any data on weeks of gestation at birth, actual birth weight, birth weight percentiles, or the presence of IUGR/SGA in their study population of 139 children. Therefore, their results need to be addressed with caution. A third large study by Infante-Rivard et al. (449 newborns) using a family based study design consistently found a significantly reduced transmission of allele e2 to newborns affected with IUGR defined as birth weight below the 10th percentile. The authors concluded that allele e2 is protective against IUGR. However, their diagnosis of IUGR needs to be questioned, since birth weight percentile below the 10th percentile alone is not a reliable definition of IUGR. The authors themselves stated that exclusion of patients with histopathologically confirmed placental infarctions ($n = 10$) results into an even more statistically significant effect. This points to different pathomechanisms for specific IUGR/SGA subgroups [20].

Though we did not primarily aim to analyze the association between ApoE genotype and the incidence of preeclampsia and the HELLP syndrome, our study does partly allow us to draw certain conclusions. Both of the aforementioned diseases are pathologically closely related to IUGR. No association between the clinical diagnosis of pregnancy being terminated in the presence of a hypertensive disorder like preeclampsia or the HELLP syndrome has been observed in this study. The present study therefore contradicts a recent publication by Procopciuc et al. in which the fetal Apo e4 genotype was found to be an independent risk factor for preeclampsia compared to the other ApoE genotypes [26]. The diverging conclusions of their study are probably caused by the small sample-size ($n = 141$ with 47 patients suffering on preeclampsia) with ApoE e4 not being normally distributed in their control group. Other authors did not find associations of ApoE genotype and the risk of preeclampsia when genotyping the mother [27–29] which corresponds with our observations in the fetus.

Despite these findings, there is increasing evidence that ApoE plays a pivotal role in fetal development and lipid metabolism. ApoE is the only apolipoprotein in the fetal circulation in concentrations as high as the mother's [30]. It is highly associated with fetal HDL-C that, in contrast to

adults, is the predominant lipoprotein in the fetus at term [31]. In cord blood of fetuses with IUGR, both HDL-C and ApoE concentrations are largely reduced [32]. Interestingly, maternal ApoE genotype impacts fetal lipid concentration levels and vice versa. While the e2 isoform in newborns is associated with elevated maternal LDL-C and apolipoprotein B (ApoB) levels, the same isoform in the mother is associated with lower LDL-C and ApoB levels and higher HDL-C levels in cord blood [21, 33]. Maternal cholesterol in humans rises during pregnancy to guarantee fetal nutritional supply. Furthermore, cholesterol levels are affected by a number of factors, environmental and genetic [34]. One influencing candidate on cholesterol levels is therefore the *APOE* locus, either through its direct effects on the developing fetus, on the fetal lipoprotein metabolism, or through its effects on maternal cholesterol levels and the maternofetal lipoprotein metabolism. In IUGR fetal serum lipid levels resemble atherogenic profiles, with lower HDL-C concentrations and higher triglyceride levels as well as elevated ratios of oxidized LDL/LDL-C and LDL-C/HDL-C [5, 10, 11]. The relationship is believed to derive from the early susceptibility of tissue to damage while being in a state of plasticity [6, 7].

The physiologic effect of the ApoE genotype on lipoprotein levels and the development of atherosclerotic diseases are well established in humans. Though being born with low birth weight predisposes to cardiovascular disease later in life it is likely that the three haplotypes of the APOE gene and low birth weight depict two independent risk factors on cardiovascular disease, since our study does not suggest that the association between low birth weight and cardiovascular disease is due to ApoE genotype.

Our study is limited by two main aspects. First, preterm birth per se is a pathological condition; hence, our study lacks a valid control group of uncomplicated pregnancies. However, the overall distribution of ApoE genotypes was within the expected range for the German population. Second, the study may be biased due to the vague definition of IUGR in the absence of precise antenatal recorded criteria. Neonates with birth weights less than the 10th percentile of a population are SGA. The term SGA is descriptive and refers not to fetal growth velocity but to the birth weight of an infant. It does not reflect the causes that lead to the low birth weight. Most SGA neonates are born small by constitution and have constant growth patterns [5, 22, 35]. By contrast, IUGR indicates the presence of a pathophysiological process that slows down or inhibits fetal growth at a certain but usually unknown developmental stage during pregnancy. Hence, the diagnosis of IUGR is based ideally on the antenatal observation of a deceleration of fetal growth velocity by serial sonographic measurements showing a "crossing of percentiles" or other antenatal parameters of fetal well-being [22, 36]. Evidence is increasing that IUGR is a heterogeneous disorder that can be subdivided into clinical presentation of concomitant preeclampsia and early onset or late onset IUGR with a cut-off at 32 to 34 weeks of gestation [2, 37]. Both, preeclampsia and increased resistance of the umbilical arteries (as indicated by resistance index/pulsatility index in Doppler sonography) are often associated with severe early onset IUGR. The indication of the presence of PIH, HELLP syndrome, IUGR, and antenatal pathological Doppler parameters in this study relies on rather clinical documentations that has not followed a strict study protocol and is not uniquely defined within the participating centers, since this was not the primary study aim of the GNN study. However, the inclusion criteria in this study are restricted to neonates born very preterm (early onset) making it a rather homogeneous study population. Moreover, the large size of our study population allowed us to investigate subgroups of very-low-birth-weight-infants according to various definitions of IUGR with high accuracy and low chance of error.

5. Conclusion

Genotyping our large cohort of 4885 preterm infants did not reveal an effect or dose-effect relationship of the fetal ApoE isoform on the birth weight percentile in all subgroups investigated. IUGR born infants display atherogenic blood lipid levels. Apolipoprotein E is known to regulate blood lipids and its three haplotypes are associated with distinct blood lipid patterns. Yet, the hypothesis of ApoE genotype affecting birth weight could be rejected since no direct effect was found. The stratification of maternal pathology in our large population size, that in earlier studies might have biased the results, could not alter this conclusion. Cholesterol levels and ApoE genotype depict major risk factors for CVD in later life. Our results, however, suggest that the ApoE genotype and low birth weight represent two synergistic factors exposing the fetus to an atherogenic environment and contributing to intrauterine programming without being directly associated to each other. The maternal genotype, known for affecting fetal blood lipids, could play a role and should be addressed in further studies.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Care-Related and Maternal Risk Factors Associated with the Antenatal Nondetection of Intrauterine Growth Restriction: A Case-Control Study from Bremen, Germany

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Objective. To identify care-related and maternal risk factors for the antenatal nondetection of IUGR. *Methods*. In this hospital-based case-control study we compared antenatally undetected IUGR neonates (cases) to detected IUGR neonates (controls). Data were collected using newborn documentation sheets and standardized personal interviews with the mothers. We calculated antenatal detection rates and used uni- and multivariable logistic regression models to assess the association of antenatal nondetection of IUGR and maternal and care-related factors. *Results*. A total of 161 neonates from three hospitals were included in the study. Suboptimal fetal growth was identified antenatally in $n = 77$ pregnancies while in $n = 84$ it was not detected antenatally (antenatal detection rate: 47.8%). Severity of IUGR, maternal complications, and a Doppler examination during the course of pregnancy were associated with IUGR detection. We did not find statistically significant differences regarding parental socioeconomic status and maternal migration background. *Conclusions*. In our study, about half of all pregnancies affected by suboptimal growth remained undetected. Future in-depth studies with larger study populations should further examine factors that could increase antenatal detection rates for IUGR.

1. Introduction

Intrauterine growth restriction (IUGR) can be described as the inability of a fetus to reach its designated growth potential at any gestational age; pregnancies with IUGR are affected by conditions that restrict the normal growth of the fetus [1]. The term IUGR is often used synonymously with small for gestational age (SGA), defined as a birthweight (BW) or estimated fetal weight (EFW) < 10th percentile for gestational age and sex. Fetuses identified as growth restricted, however, comprise a heterogeneous group regarding causal factors, management, and prognosis [2, 3]. Many fetuses or infants with an EFW/BW < 10th percentile are perfectly normal and simply "constitutionally" small [1]. The American College of Obstetricians and Gynecologists Committee highlights that the distinction between normal and pathological growth in clinical practice is challenging [4].

Approximately 3% to 8% of all infants born in developed countries have been identified as growth restricted [5–8]. IUGR is a prenatal condition and is associated with a higher risk for perinatal morbidity and mortality, with risk increasing with severity of the restriction [1]. A recent population-based study confirmed that IUGR is the single largest risk factor for stillbirth, increasing the stillbirth rate fourfold compared to pregnancies with normally grown fetuses; antenatal nondetection further increases the rate by a factor of two

[9]. An early antenatal detection, choosing the optimal time and method of delivery, and treatment where appropriate could minimize the risks significantly [9–11]. Umbilical artery Doppler examination is the most valuable tool regarding the prediction of perinatal outcome in growth-restricted fetuses [1] and is accepted as the primary assessment tool regarding diagnosis of IUGR [10, 12]. However, low antenatal detection rates of suboptimal fetal growth through routine fetal ultrasonography have been reported [13, 14]. In fact IUGR has been reported to be antenatally detected only in one-third (25% to 32%) of pregnancies with suboptimal fetal growth [15, 16].

Apart from the difficulty to distinguish between healthy SGA fetuses and pathological IUGR cases, reasons for the antenatal nondetection of IUGR have not been well elucidated yet. IUGR is a heterogeneous condition with various underlying maternal, placental, or environmental causes. Antenatal care use and maternal characteristics such as socioeconomic status (SES) and migration background may also play a role [17–19].

The aim of this study was to identify care-related and maternal risk factors for the antenatal nondetection of IUGR and to investigate if there are specific groups with a higher chance of nondetected suboptimal fetal growth.

2. Material and Methods

2.1. Study Design. This hospital-based case-control study was carried out in cooperation with three obstetric units in the federal city-state of Bremen, Germany. Our study design differed from the traditional case-control study design, as we did not compare IUGR cases to healthy controls; instead we compared antenatally undetected IUGR neonates (cases) to detected IUGR neonates (controls). A detailed description of the study design and methods is described in the study protocol [20]. The study region covered a geographical area of some 420 km^2 with 669,915 residents and 6,397 deliveries in 2012. 4,935 of these deliveries took place in the three cooperating hospitals, out of a total of five hospitals with an obstetric unit in the study region. In Germany antenatal and perinatal care is covered by the health insurance system. Health insurance is compulsory and provided by the statutory health insurances (roughly 90% of the German population) or by private health insurances [21].

2.2. Recruitment of Participants. From January 2013 to June 2015 mothers and their newborns with a birthweight <10th percentile in relation to gestational age and sex (SGA) were eligible for the study and invited for participation. Obstetricians and study nurses classified newborns as SGA based on the routinely used population-based percentile values for newborns in Germany by Voigt et al. [22, 23]. Mothers were initially informed about the study by the attending obstetrician during their hospitalization or if they had already left the hospital via postal mail by the study nurses. Study nurses sent reminders three and six weeks after the initial contact, including a nonresponder questionnaire after six weeks. Study materials (i.e., study information, study flyer) were translated into Russian and Turkish language. Where required, the maternal interviews were conducted in one of these languages. The whole study procedure was pretested among a small sample of mothers prior to the recruitment phase.

2.3. Data Collection. We designed a newborn documentation sheet to record basic information, such as birthweight, birth length, head circumference, Apgar score, umbilical cord blood pH, gestational age at birth, complications at birth, and mode of delivery. The basic information was documented by obstetricians at the time of birth or by study nurses based on the birth records. Details on the IUGR diagnosis, such as timing (i.e., antenatal versus at birth) were added by the attending obstetrician or pediatrician in the hospital. For all mothers who declined to participate in the study, the newborn documentation sheet was also filled out by the attending obstetricians or by study nurses (basic information only; no information on IUGR diagnosis).

All mothers who consented to the study were interviewed at home after they were discharged from the hospital. The questionnaire was developed in close cooperation with obstetricians and designed as a standardized CAPI/CATI of approximately 45 mins duration. Aspects covered by the questionnaire were sociodemographic information, medical conditions, and complications/diseases during pregnancy, for example, maternal vascular diseases, infections during pregnancy, preeclampsia, placental anomalies, anomalies of the uterus, risk factors for IUGR during pregnancy such as smoking, alcohol consumption, and illicit drug use, maternal height and weight to determine the maternal prepregnancy BMI, maternal weight gain during pregnancy, and parity and number of pregnancies, as well as use, timing, and content of antenatal care. The interviews were conducted by trained project personnel and medical conditions were confirmed by data of the pregnancy record books.

2.4. Case-Control Definition. Cases were defined as neonates with an IUGR not detected antenatally; that is, the IUGR diagnosis was initially established at the time of birth or during the first medical check-up after birth (newborn documentation sheet) and the mother did not report any IUGR diagnosis in the personal interview.

Controls were defined as neonates whose IUGR was positively identified antenatally; that is, the diagnosis including date either was documented in the newborn documentation sheet or was stated by the mother in the personal interview. Newborns with a suspected (but not confirmed) IUGR diagnosis documented in the newborn documentation sheet were also defined as controls.

2.5. Variables. Maternal migration background was defined as being born in a foreign country and/or having a nationality other than German. Information on household income, education, and occupation were combined into a composite socioeconomic status measure (SES; low, middle, high) as proposed by Winkler and Stolzenberg [24].

Gestational weight gain was calculated based on prepregnancy body-mass-index (BMI) for underweight, normal weight, overweight, and obese women as recommended by *The Institute of Medicine (IOM)* [25] and then categorized into (1) lower than adequate, (2) adequate, and (3) higher than adequate.

Further maternal factors were age (<35 versus ≥35 yrs), parity, number of pregnancies (gravidity), maternal prepregnancy BMI, coffee intake, tobacco consumption, and illicit drug use during pregnancy and maternal complications/diseases during pregnancy, that is, maternal vascular diseases (e.g., hypertension, preeclampsia), infections during pregnancy (e.g., toxoplasmosis), and malformation of the uterus and placental anomalies (e.g., placenta praevia).

The severity of IUGR diagnosis was determined using different cut-off limits for the BW percentile, that is, BW percentile <3rd, ≥3rd–<5th, and ≥5th–<10th, for gestational age and sex. Fetal sex, multiple gestation, and fetal anomalies, for example, trisomy 13, trisomy 18, trisomy 21, and congenital malformations, were included. Further outcome parameters were 1-minute and 5-minute Apgar score (normal: 7–10; minor depression: 4–6; severe depression: 0–3) and umbilical cord blood pH (ideal: >7.3; normal: 7.2–7.29; minor acidification: 7.1–7.19; moderate acidification: 7.0–7.09; severe acidification: <7.0).

We constructed an index to assess adequate antenatal care use [26, 27], by combining gestational age of the first antenatal care visit and the total number of visits, taking the gestational age at birth into account. We used the recommended schedule of the maternity guidelines for Germany as the basis of our index. The values range from 1 to 4 and the index was constructed separately for nulliparae and multiparae. For nulliparae, our index has the following 4 categories of antenatal care use in case of a full-term pregnancy: (1) adequate use—a minimum number of 8 visits and a first visit before gestational age of 12 weeks; (2) less adequate use—less than 8 visits and a first visit before gestational age of 12 weeks; (3) inadequate use—a minimum number of 8 visits and a first visit at gestational age after 12 weeks; (4) more inadequate use—less than 8 visits and a first visit at gestational age after 12 weeks. For the multiparae we used basically the same categories, except that the minimum total visits for a full-term pregnancy were 6 visits. Further care-related factors included the number of routine ultrasonography and Doppler examinations during pregnancy, any hospitalization during pregnancy, number of admissions of newborns to neonatal care unit (NCU), and mode of delivery.

2.6. Statistical Analysis. The incidence of SGA was calculated for the three participating hospitals by dividing all births <10th percentile for gestational age and sex by all recorded births in this period. This calculation was based on the recorded basic information of all births in the participating hospitals during the years 2013 and 2014. Antenatal detection rates of IUGR were calculated by dividing all newborns with antenatally identified IUGR by the whole study sample and stratified by different cut-off limits for IUGR identification (i.e., birthweight <10th, <5th, and <3rd percentile for gestational age and sex). We examined associations between care-related and maternal determinants and nondetection of IUGR in univariate and multivariable logistic regression models, using Odds Ratios (OR) with 95% confidence intervals (CI). Multivariable models were adjusted for maternal migration background, socioeconomic status, maternal age (<35 yrs versus ≥35 yrs), birthweight percentile (<3rd, ≥3rd–<5th, and ≥5th–<10th), complications/diseases during pregnancy, Doppler examination, fetal anomalies, and multiple gestation. In sensitivity analyses we examined differences in the applied method of case-control identification and source of information used, that is, newborn documentation sheet and CAPI. Differences in birth-related characteristics between responders and nonresponders were tested using chi-square tests or t-tests, where appropriate. The study was planned to detect moderate to large differences in terms of risk factors for nondetection of IUGR (OR > 2.0), with a statistical power of 0.8 and a 95% CI with an estimated sample size of $n = 260$.

2.7. Ethics Approval and Consent to Participate. Ethical approval for all study procedures was obtained from the ethics review board of the Bremen Medical Association. All women who delivered an SGA newborn in one of the cooperating hospitals received written and oral information about the study. All participating women had to give written informed consent for data collection.

3. Results

The total number of births during the whole 2.5-year recruitment period was 12,926 in the three participating maternity hospitals. A total of $n = 1{,}087$ (8.4%) newborns had a birthweight <10th percentile for gestational age and sex at the time of birth and were invited for study participation. Fifteen percent of mothers ($n = 163$) participated in the study. We excluded two participants due to a birthweight ≥10th percentile for gestational age and sex and no documented IUGR diagnosis in newborn documentation sheet or maternal survey data. A comparison of neonates' birth characteristics and outcomes between participants and nonparticipants ($n = 926$; basic information of newborn documentation sheet) showed a statistically significant lower birthweight on average for participants as compared to nonparticipants (mean birthweight (gram) 2477.4 ± 544.9 versus 2579.7 ± 432.5; p value: 0.025) (Additional File 1 in Supplementary Material available online at https://doi.org/10.1155/2017/1746146). In total, $n = 51/926$ women who declined to participate in the study filled out the nonresponder questionnaire. The main reasons for nonparticipation were lack of time, language barriers, and no interest in scientific studies in general.

As outlined in Table 1, 20.5% ($n = 33$) of participating mothers had a migration background and only a small number of mothers with low SES participated in our study (high: 51.6%; middle: 41.6%; low: 6.8%). The age distribution among cases and controls was similar, as was the distribution by SES. The proportion of mothers with migration background was

Table 1: Maternal characteristics in total and stratified by cases and controls.

Maternal characteristics	Total (n = 161) % (n)	IUGR detected (controls) (n = 77) % (n)	IUGR undetected (cases) (n = 84) % (n)
Age			
<35 yrs	65.8 (106)	68.8 (53)	63.1 (53)
≥35 yrs	34.2 (55)	31.2 (24)	36.9 (31)
Maternal migration background			
Yes	20.5 (33)	19.9 (13)	23.8 (20)
No	78.3 (126)	81.8 (63)	75.0 (63)
Missing value	1.2 (2)	1.3 (1)	1.2 (1)
Socioeconomic status			
High	51.6 (83)	49.4 (38)	53.6 (45)
Middle	41.6 (67)	44.2 (34)	39.3 (33)
Low	6.8 (11)	6.5 (5)	7.1 (6)
Prepregnancy BMI (kg/m^2)			
Normal	66.5 (107)	62.3 (48)	70.3 (59)
Overweight	18.0 (29)	18.2 (14)	17.9 (15)
Obese	15.5 (25)	19.5 (15)	11.9 (10)
Weight gain during pregnancy			
Higher than adequate	33.5 (54)	37.7 (29)	29.8 (25)
Adequate	32.9 (53)	31.2 (24)	34.5 (29)
Lower than adequate	33.5 (54)	31.2 (24)	35.7 (30)
Parity*			
Nulliparous	55.9 (90)	58.4 (45)	53.6 (45)
Multiparous	44.1 (71)	41.6 (32)	46.4 (39)
Number of pregnancies*			
≤1	79.5 (128)	76.6 (59)	82.2 (69)
2–≤3	17.4 (28)	19.5 (15)	15.4 (13)
≥4	3.1 (5)	3.9 (3)	2.4 (2)
Complications/diseases during pregnancy			
Yes	44.7 (72)	54.5 (42)	35.7 (30)
No	55.3 (89)	45.5 (35)	64.3 (54)
Ultrasound examinations			
<3	0.6 (1)	1.3 (1)	0 (0)
3–8	58.4 (94)	50.6 (39)	65.5 (55)
≥9	41.0 (66)	48.1 (37)	34.5 (29)
Doppler examination			
Yes	80.1 (129)	92.2 (71)	69.0 (58)
No	19.9 (32)	7.8 (6)	31.0 (26)
Index antenatal care use			
Adequate	93.2 (150)	92.2 (71)	94.0 (79)
Less adequate or inadequate	6.8 (11)	7.8 (6)	6.0 (5)
Hospitalization during pregnancy			
Yes	22.4 (36)	27.3 (21)	17.9 (15)
No	77.6 (125)	72.7 (56)	82.1 (69)

Table 1: Continued.

Maternal characteristics	Total (n = 161) % (n)	IUGR detected (controls) (n = 77) % (n)	IUGR undetected (cases) (n = 84) % (n)
Alcohol consumption during pregnancy			
Yes	2.5 (4)	1.3 (1)	3.6 (3)
No	96.9 (156)	98.7 (76)	95.2 (80)
Missing value	0.6 (1)	0 (0)	1.2 (1)
Tobacco consumption during pregnancy			
Yes	10.6 (17)	10.4 (8)	10.7 (9)
No	88.8 (143)	89.6 (69)	88.1 (74)
Missing value	0.6 (1)	0 (0)	1.2 (1)

*Excluding current pregnancy/birth.

slightly higher among cases (23.8%; $n = 20$) than controls (19.9%; $n = 13$) (Table 1). Among controls the proportion of more severe suboptimal fetal growth was higher (<10th percentile: 32.5%; <5th percentile: 14.3%; <3rd percentile: 53.2%) than cases (<10th percentile: 57.1%; <5th percentile: 7.1%; <3rd percentile: 35.7%) (Table 2). None of the mothers stated any illicit drug use during pregnancy.

3.1. Antenatal Detection of IUGR. Suboptimal fetal growth was identified antenatally in $n = 77$ pregnancies (controls) while in $n = 84$ (cases) it remained undetected (antenatal detection rate: 47.8%). The antenatal detection rate was highest in newborns with a birthweight <5th percentile (64.7%) and lowest in newborns with a birthweight <10th percentile (34.2%) in relation to gestational age and sex. Among newborns with a birthweight <3rd percentile the antenatal detection rate was slightly lower (57.7%) as compared to newborns with a birthweight <5th percentile (Table 3).

3.2. Factors Associated with Nondetection of IUGR. In adjusted models, we identified three factors (severity of IUGR, presence of maternal complications/diseases during pregnancy, and Doppler examination during the course of pregnancy) that were associated with the antenatal nondetection of IUGR (Table 4). Newborns with a birthweight <10th percentile for gestational age and sex were about three times more likely to remain antenatally undetected as compared to newborns with a birthweight <3rd percentile for gestational age and sex (OR 2.82; 95%-CI [1.31, 6.10]) (Table 4). The odds for antenatal nondetection of IUGR were markedly reduced for mothers who had any complications/diseases during pregnancy (OR 0.38; 95%-CI [0.18, 0.79]). The use of Doppler examination during the course of pregnancy also reduced the odds of antenatal nondetection of IUGR significantly (OR 0.13; 95%-CI [0.04, 0.40]).

We did not find statistically significant associations between antenatal nondetection of IUGR and maternal SES or migration background in univariate as well as multivariable regression models (Table 4). However, in multivariable analyses, the point estimate indicated that antenatal nondetection of IUGR is about two times more likely in women with a migration background (OR 1.8; 95%-CI [0.68, 4.56]) than nonmigrants, although it was not statistically significant.

4. Discussion

The aim of this paper was to identify care-related and maternal risk factors for the antenatal nondetection of fetal growth restrictions, specifically IUGR. Overall, 8.0% (n = 1,087) of all newborns in the cooperating maternity hospitals during study period were SGA, which is in line with other West European studies [6–8, 28].

In our study suboptimal fetal growth was antenatally identified in less than half of the cases as determined perinatally. As compared to the sensitivities reported in observational studies of the late 1990s and early 2000 (25–32%) [15, 16], our study results indicate that IUGR detection rates did not substantially increase over the last 15 years. In line with our findings, a more recent US study reported similar low antenatal detection rates for IUGR of 25% [29]. However, the detection rates found in our study have to be interpreted cautiously. There is a marked heterogeneity in our study population regarding the severity of IUGR. However, in fact it seemed that the majority of included neonates (54.7%) as compared to nonresponders had more severe growth restrictions (birthweight <5th percentile). Our findings indicate that the antenatal detection rate increases with severity of the growth restriction. However, even among the newborns below the 5th percentile, only approximately half of the cases were identified antenatally, a finding that can be seen as indicating a quality problem in antenatal care.

We identified three factors that influenced IUGR detection. A higher severity of the growth restriction, maternal complications/diseases during pregnancy, and a Doppler examination during the course of pregnancy led to higher antenatal detection rates in our study. Similar to this, findings of a recent US multicenter cohort study including 11,487 births showed that maternal complications, an ultrasonography examination with measurement of EFW within four weeks of birth, gestational age at delivery, and a higher severity of the growth restriction increased antenatal detection rates. Hispanic ethnicity was associated with a higher risk of

Table 2: Newborn characteristics in total and stratified by cases and controls.

Newborn characteristics	Total (n = 161) % (n)	IUGR detected (controls) (n = 77) % (n)	IUGR undetected (cases) (n = 84) % (n)
Sex			
Male	50.9 (82)	50.6 (39)	51.2 (43)
Female	49.1 (79)	49.4 (38)	48.8 (41)
Multiple gestation			
Yes	9.3 (15)	11.7 (9)	7.1 (6)
No	90.7 (146)	88.3 (68)	92.9 (78)
Birthweight percentile			
<10th percentile[1]	45.3 (73)	32.5 (25)	57.1 (48)
<5th percentile	10.6 (17)	14.3 (11)	7.1 (6)
<3rd percentile	44.1 (71)	53.2 (41)	35.7 (30)
Fetal anomalies			
Yes	8.1 (13)	11.7 (9)	4.8 (4)
No	95.0 (153)	88.3 (68)	95.2 (80)
Number of admissions to NCU[2]			
Yes	23.6 (38)	35.1 (27)	13.1 (11)
No	76.4 (123)	64.9 (50)	86.9 (73)
Apgar score			
1 min			
Severe depression	1.2 (2)	2.6 (2)	—
Minor depression	6.2 (10)	6.5 (5)	6.0 (5)
Normal	91.9 (148)	90.9 (70)	92.9 (78)
5 min			
Severe depression	—	—	—
Minor depression	2.5 (4)	3.9 (3)	1.2 (1)
Normal	96.9 (156)	96.1 (74)	97.6 (82)
Umbilical cord blood pH			
Ideal	48.4 (78)	48.1 (37)	48.8 (41)
Normal	35.4 (57)	33.8 (26)	36.9 (31)
Minor acidification	11.2 (18)	13.0 (10)	9.5 (8)
Moderate acidification	1.2 (2)	1.3 (1)	1.2 (1)
Missing value	3.7 (6)	3.9 (3)	3.6 (3)
Mode of delivery			
Vaginal	50.3 (81)	41.6 (32)	58.3 (49)
Cesarean section (elective)	13.7 (22)	22.1 (17)	6.0 (5)
Cesarean section (secondary)	25.5 (41)	29.9 (23)	21.4 (18)
Other	10.5 (17)	6.5 (5)	14.3 (12)

[1]Including n = 4 newborns with a birthweight >10th percentile for gestational age and sex, but with (antenatal) diagnosis of IUGR/SGA.
[2]NCU: neonatal care unit.

antenatal nondetection (RR 2.4; 95% CI [1.4, 4.2]) [29]. Both our study and larger other studies thus indicate that clinical alertness towards maternal and fetal morbidity and the use of Doppler ultrasonography are core factors that can reduce undetected fetal growth restrictions and their consequences. However, it is likely that a suspected IUGR was in many cases the reason for the Doppler examination because the latter is the primary method for diagnosing IUGR. Nonetheless the detected association confirmed that the performance of a Doppler examination during the course of pregnancy is of great value for the detection of suboptimal fetal growth.

Previous studies have reported differences in use and timing of antenatal care between pregnant women depending on SES and migration background which may lead to an

Table 3: Antenatal detection rates according to birthweight percentiles (<3rd, ≥3rd–<5th, ≥5th–<10th percentile for gestational age and sex).

$n = 161$	Participating mothers % (n)
Antenatal detection rate	47.8% (77/161)
≥5th–<10th birthweight percentile	34.2% (25/73)
≥3rd–<5th birthweight percentile	64.7% (11/17)
<3rd birthweight percentile	57.7% (41/71)

*Classification based on combined evaluation of both information sources (newborn documentation sheets, personal interview).

increased risk for adverse pregnancy and birth outcomes for the social disadvantaged [17–19, 30]. Our results showed no statistically significant associations between these variables and antenatal IUGR detection. However, the point estimate indicated twofold increased odds for nondetection of suboptimal fetal growth among women with a migration background. This finding calls for further research with larger study populations and a more differentiated operationalization of migration background. The missing social gradient in the detection rates may be due to the small number of study participants with a low SES.

4.1. Strengths and Limitations. This is one of so far very few studies from Germany explicitly investigating care-related as well as maternal risk factors influencing the (non)detection of suboptimal fetal growth. The interviews were pretested with mothers of neonates who were diagnosed with SGA or IUGR to ensure clarity and feasibility of the interview questions, language, structure, and time needed. A further strength is the relatively high proportion of mothers with migration background included in the study. The main limitation of this study is the low response rate. The recruitment of cases and controls in this study was a particular challenge. Firstly, the incidence of suboptimal fetal growth is relatively low. Secondly, it could be assumed that the severity of suboptimal fetal growth influenced the willingness to participate in the study. Mothers whose newborns had severe growth restriction may have declined to participate in the study as they would want to focus their full attention on their infants. However, our comparison of neonates' birth characteristics of participants and nonparticipants showed that newborns of participating mothers had a lower birthweight on average as compared to newborns of mothers who declined to participate (Supplementary Material). Therefore we believe that this type of selection bias is rather unlikely. Furthermore, the percentage of mothers who smoked during pregnancy in our study (10.7%) was similar to the data presented by Kuntz and Lampert on the percentage of mothers who smoked during pregnancy (12.1%), which are based on the German Health Interview and Examination Survey for Children and Adolescents (KiGGS) [31]. All mothers who delivered an SGA newborn in one of the three participating maternity units had an equal chance to participate in the study. However, we cannot fully rule out that there is some extent of selection bias, due to unique characteristics of the mothers who agreed to participate in the study, as their babies were of significantly lower birthweight as compared to the nonparticipants.

Due to the very limited time for recruitment of individual mothers in the maternity units, the shift work, and extensive work load, obstetricians were not always able to inform the mothers about the study and to invite them to participate. The majority of mothers were exclusively invited to participate by written letters. In an attempt to address these issues we offered three interview options, either a CAPI directly in the hospital, a CAPI after birth at home, or a CATI. Several reminders were sent and study material and the interview were translated in different languages to cater for the main migrant groups living in the study region. Nonetheless, our recruitment aim could not be fully reached such that true associations may have remained undetected in our analyses.

5. Conclusions

IUGR detection rates do not seem to have substantially increased since the late 1990s, as about half of the pregnancies affected by suboptimal fetal growth remain undetected under routine conditions. Several clinical and care-related factors reduce the risk that IUGR remains undetected. A migration background of the mother may increase nondetection odds, but further studies with larger sample sizes are warranted. A direction for future research could be to examine whether a mandatory Doppler examination (at least for some subgroups) increases antenatal detection rates for IUGR. Our study data can feed into ongoing international efforts to investigate antenatal care explicitly addressing IUGR detection and diagnosis [32].

Disclosure

The authors presented parts of the results of this paper (poster presentation) at a conference on national level, 28 August 2016–2 September 2016 (German Society for Epidemiology, DGEpi, and German Association for Medical Informatics, Biometry and Epidemiology, GMDS). The conference website is located at http://www.hec2016.eu/.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Table 4: Association between antenatal nondetection of IUGR and maternal and care-related factors; univariate and multivariable regression analyses.

$n = 161$	Univariate regression model Odds Ratio [95%-CI]	Multivariable regression model[1] Odds Ratio [95%-CI]
Maternal migration background		
Yes	1.54 [0.71, 3.36]	1.76 [0.68, 4.56]
No	Reference	Reference
Socioeconomic status		
High	Reference	Reference
Middle	0.82 [0.43, 1.56]	0.75 [0.35, 1.61]
Low	1.01 [0.29, 3.58]	0.47 [0.10, 2.27]
Maternal age		
<35 years	0.77 [0.40, 1.49]	0.94 [0.44, 2.02]
≥35 years	Reference	Reference
Birthweight percentile		
<10th percentile	**2.62 [1.34, 5.15]**	**2.82 [1.31, 6.10]**
<5th percentile	0.75 [0.25, 2.24]	0.73 [0.22, 2.49]
<3rd percentile	Reference	Reference
Complications/diseases during pregnancy		
Yes	**0.46 [0.25, 0.87]**	**0.38 [0.18, 0.79]**
No	Reference	Reference
Doppler examination		
Yes	**0.19 [0.07, 0.49]**	**0.13 [0.04, 0.40]**
No	Reference	Reference
Fetal anomalies		
Yes	0.38 [0.11, 1.28]	0.21 [0.05, 0.97]
No	Reference	Reference
Multiple gestation		
Yes	0.58 [0.20, 1.72]	0.45 [0.12, 1.61]
No	Reference	Reference
BMI (kg/m^2)		
Normal	Reference	—
Overweight	0.87 [0.38, 1.98]	
Obese	0.54 [0.22, 1.32]	
Weight gain during pregnancy		
Higher than adequate	0.71 [0.0.33, 1.53]	—
Adequate	Reference	
Lower than adequate	1.03 [0.48, 2.22]	
Tobacco consumption		
Yes	1.05 [0.38, 2.87]	—
No	Reference	
Parity[2]		
Nulliparous	Reference	—
Multiparous	1.22 [0.65, 2.27]	
Index antenatal care use		
Adequate	Reference	—
Less adequate or inadequate	0.75 [0.22, 2.56]	
Hospitalization during pregnancy		

Table 4: Continued.

$n = 161$	Univariate regression model Odds Ratio [95%-CI]	Multivariable regression model[1] Odds Ratio [95%-CI]
Yes	0.58 [0.27, 1.23]	—
No	Reference	
Newborn sex		
Male	1.02 [0.55, 1.90]	—
Female	Reference	

[1]Adjusted for maternal migration background, socioeconomic status, maternal age (<35 yrs versus ≥35 yrs), birthweight percentile (<10th–≥5th, <5th–≥3rd, <3rd), complications/diseases during pregnancy, Doppler examination, fetal anomalies, and multiple gestation.
[2]Excluding current pregnancy/birth.
Note. Numbers of entries given in bold indicate a significant association.

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Review Article

Do miRNAs Play a Role in Fetal Growth Restriction? A Fresh Look to a Busy Corner

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Placenta is the crucial organ for embryo and fetus development and plays a critical role in the development of fetal growth restriction (FGR). There are increasing evidences on the role of microRNAs (miRNAs) in a variety of pregnancy-related complications such as preeclampsia and FGR. More than 1880 miRNAs have been reported in humans and most of them are expressed in placenta. In this paper, we aimed to review the current evidence about the topic. According to retrieved data, controversial results about placental expression of miRNAs could be due (at least in part) to the different experimental methods used by different groups. Despite the fact that several authors have demonstrated a relatively easy and feasible detection of some miRNAs in maternal whole peripheral blood, costs of these tests should be reduced in order to increase cohorts and have stronger evidence. In this regard, we take the opportunity to solicit future studies on large cohort and adequate statistical power, in order to identify a panel of biomarkers on maternal peripheral blood for early diagnosis of FGR.

1. Introduction

Fetal growth restriction (FGR) refers to a condition in which there is the stop or the decrease of the genetic determined potential growth of a fetus during pregnancy. FGR is due to different causes, including maternal smoking, undernutrition, infection, and congenital abnormalities; conversely, if it is not possible to individuate a clear cause, it is defined as idiopathic. FGR is often associated with preeclampsia [1] and represents the most common pregnancy complication, accounting for about a third of all preterm births. Usually FGR is diagnosed when, through ultrasound, fetal weight is estimated to be less than the 10th percentile for gestational age using a validated fetal growth scale [2]. The most studied cause of FGR in animal models is maternal calorie restriction during gestation, but while much is known about the consequences of this deprivation, molecular mechanisms that underlie these conditions still remain unclear.

Placenta is the crucial organ for embryo and fetus development and plays a critical role in the development of FGR. In this regard, FGR could be considered as a placentation disorder, derived from a dysregulation in trophoblast invasion with characteristic tissue morphology that leads to uteroplacental insufficiency. This condition would greatly benefit from the availability of early diagnostic tests to give an opportunity for early intervention or prevention, to improve maternal-fetal outcomes, and to substantially contain the public health costs.

There are increasing evidences on the role of microRNAs (miRNAs) in a variety of pregnancy-related complications such as preeclampsia and fetal growth restriction. More than 1880 miRNAs have been reported in humans and most of them are expressed in placenta. This kind of nucleic acid belongs to the family of small noncoding RNAs of on average 22 nucleotides in length, which regulates gene expression at the posttranscriptional level, inhibiting translation or promoting specific mRNAs degradation through interaction with the 3′ untranslated region [3, 4]. In detail, miRNAs seem to modulate cell development, differentiation, and proliferation, cell type-specific function, and are involved in the pathogenesis of many human diseases [5]. In several cases, miRNA expression is tissue-specific and, in addition, is significantly different between physiology and pathological conditions: for these reasons, investigations about miRNAs gained increasing attention for the possibility of future application in clinical diagnostics [6, 7].

Starting from these considerations, we aimed to review the current literature focusing on the role of miRNAs in FGR.

2. Materials and Methods

We performed a selective literature search of articles in English language, published from 2002 to 2017 and indexed in PubMed. We searched the following Medical Subject Headings (MeSH): "MicroRNAs" AND "Fetal Growth Retardation". The initial database screening was performed by three authors (Laganà AS, Vaiarelli A, La Rosa VL), who were blinded to the aim of the study. Subsequently, other three authors (Chiofalo B, Rossetti D, Vitale SG) selected relevant information from the screened literature. We considered eligible all original manuscripts (randomized, observational, and retrospective studies), case series, and case reports. Furthermore, we extracted relevant information from selected reviews.

3. Results

3.1. Placental MicroRNAs. Several studies focused their attention on the expression of different miRNAs in placentas using real-time-PCR. Cindrova-Davies et al. [8] analyzed miRNA-21 expression from placentas of a small cohort ($n = 6$) of early-onset FGR cases and found its significant upregulation. Guo et al. [9] identified a significant downregulation of miRNA-194 in placentas from 26 FGR cases and from 16 preeclamptic women (16), compared to those from 29 normal pregnancies (29). Hromadnikova et al. [10] for the first time explored, in two different experiments, the placental expression profile of miRNAs known to be involved in cardiovascular and cerebrovascular diseases. They found that upregulation of miR-499a-5p is a common feature of all placental insufficiencies such as preeclampsia ($n = 80$), gestational hypertension ($n = 35$), and FGR ($n = 35$); in addition, they demonstrated an upregulation of miR-1-3p in FGR pregnancies with abnormal umbilical fetal flows ($n = 19$); finally, they found downregulation of a series of miRNAs (miR-16-5p, miR-26a-5p, miR-100-5p, miR-103a-3p, miR-122-5p, miR-125b-5p, miR-126-3p, miR-143-3p, miR-145-5p, miR-195-5p, miR-199a-5p, miR-221-3p, miR-342-3p, and miR-574-3p) in FGR requiring the delivery before 34 weeks of gestation.

Other authors studied miRNA-424 and its target gene (mitogen-activated protein kinase) that play a role in endothelial cell proliferation through fibroblast growth factor receptor 1 and regulate vascular endothelial growth factor [11]. According to their data analysis, the levels of this miRNA are increased in placentas from 25 FGR pregnancies compared with 25 placentas from uncomplicated pregnancies, suggesting that miRNA-424 is involved in placental disorders. Another study by Su et al. [12] searched, in a cohort of placentas, the miRNAs that regulate endocrine gland derived vascular endothelial growth factor (EG-VEGF) expression: they concluded that miR-346 and miR-582-3p regulate EG-VEGF-induced trophoblast invasion through repressing metalloproteinases 2 and 9. In addition, FGR placental tissues show an aberrant high expression level of miR-141, suggesting that this miRNA might play important roles in the pathogenesis of the disease by suppressing E2F transcription factor 3 and pleomorphic adenoma gene 1 [13].

To date, many studies focused their attention on chromosome 19 miRNA cluster (C19MC) [14–16]. In detail, C19MC comprises 46 miRNAs and is the largest gene cluster of miRNAs in humans, exclusively expressed in undifferentiated cells and in placenta. In this regard, comparing 14 placentas from FGR pregnancies with 14 from normal pregnancies, it was recently found that hypoxic stress does not affect C19MC miRNA expression, except for downregulation of miR-500c-3p [14]. Similarly, Hromadnikova et al. [17] detected a downregulation of 6 miRNAs (miR-517-5p, miR-518f-5p, miR-519a, miR-519d, miR-520a-5p, and miR-525) in placental tissues of 36 FGR pregnancies: compared to the previous studies, these results seem more robust since that authors investigated more types of miRNAs and used those that were previously demonstrated to be exclusively expressed or highly expressed in placental tissues. The significantly decreased expression of miR-519d, but not of miR-520a-5p and miR-525, was also confirmed by others on a larger cohort (50 healthy pregnancies compared with 45 FGR cases) [15]. Nevertheless, other experiments found that the expression of miR-518b was decreased, whereas miR-519a was significantly increased, in 30 FGR placentas [16]. Some of these miRNAs studied in human placentae were also studied in animal models.

3.2. Circulating miRNAs. During pregnancy, due to a normal extravillous trophoblast invasion, nucleic acids of the placental compartment are released into the maternal circulation: this release occurs through the migration of microvesicles from apoptotic/necrotic cells and active cellular communication system, involving also nanovesicles/exosomes and subcellular fragments [18, 19]. Due to placental continuous remodeling, these extracellular nucleic acids may be detected in maternal blood during the course of gestation and can be measured to monitor placental function and allow early diagnosis of pregnancy complications [20–23].

For these motivations in recent years there has been a trend to develop noninvasive methods for the detection in maternal circulation of cell-free nucleic acids, including miRNAs coming from the embryo-placental compartment [24–43]. Some studies detected FGR-specific miRNA expression changes in placentas, but these differences were not detectable in plasma [15–44]. A significant elevation of several extracellular placenta-specific miRNA levels was recently showed (miR-516-5p, miR-517, miR-518b, miR-520a, miR-520h, miR-525, and miR-526a,) during early gestation in 7 pregnancies with later onset of preeclampsia and/or FGR [44]. According to these data, an early screening (i.e., within the 12th to 16th weeks) of miRNA circulating levels may differentiate between women with normally progressing pregnancies and those who could later develop placental insufficiency–related complications [44]. Nevertheless, recent data showed that C19MC microRNAs might play a role in the pathogenesis of preeclampsia, but not of FGR [45]. Last year, Hromadnikova's group investigated maternal blood levels of specific miRNAs involved in cardiovascular and cerebrovascular diseases, finding a downregulation of miR-100-5p, miR-125b-5p, and miR-199a-5p in 39 patients with gestational hypertension, in 68 with preeclampsia, and in 33 with fetal growth restriction compared with 55 healthy controls; in addition, they showed downregulation of miR-17-5p, miR-146a-5p, miR-221-3p, and miR-574-3p only in FGR pregnancies [46]. In a small-scale analysis, others found that a group of miRNAs that are altered by hypoxia in trophoblasts (miR-27a, miR-30d, miR-141, miR-200c, miR-424, miR-205 and miR-451, miR-491, miR-517a, miR-518b, miR-518e, and miR-524) is elevated in FGR pregnancies ($n = 14$ FGR versus $n = 14$ controls) [47].

Some of these miRNAs, such as miR-141, miR-200c, and miR-205, were studied also in animal models [48, 49]. In particular, it was found that they play important roles in the maintenance of the integrity of the folded trophoblast-endometrial epithelial bilayer in porcine placentas [48].

4. Discussion

Based on the abovementioned data, miRNAs seem to be involved in placental development and consequently in placenta related disorders. As showed in Table 1, controversial results among these studies in placental expression of miRNAs could be due, at least in part, to the different experimental methods used by different groups. Despite the fact that several authors have demonstrated a relatively easy and feasible detection of some miRNAs in maternal whole peripheral blood [44–47], costs of these tests should be reduced in order to increase cohorts and have stronger evidence.

In this regard, we acknowledge that it may be extremely important to address future research directions taking into account the already available data from in vitro experiments and animal models: indeed, accumulating evidence suggests that miR141-3p and miR-200a-3p play a pivotal role for placental development in mouse and regulate the expression of insulin-like growth factor 2 [50]. Interestingly, upregulation of miR-125b was found to reduce significantly ethanol-induced caspase-3 activation and to diminish ethanol-induced growth retardation in mouse embryos [51], suggesting a possible protective role that is worthy of further investigation. Conversely, miR-24 and miR-103-2, which are related to adipocyte development, were both increased in low birth weight male guinea pig pups [52]. Probably this last element could be further confirmed in future studies, since several sex-specific effects were already found to be more pronounced in males with respect to females [53]. Last but not least, recent data showed that FGR is associated with increased lung miR-126-3p levels, which is known to modulate the expression of angiogenic factor, in rats [54]. The importance of angiogenic regulatory pathways was also highlighted by the abnormal upregulated expression of miR-127, miR-21, and miR-16 in placentas of deceased cloned sheep with respect to controls [55]. These data are extremely fascinating, since miR-21 expression was associated with increased vascular resistance also in growth-restricted human pregnancies [8, 56].

As suggested by accumulating evidence, miRNAs play also a pivotal role in epigenetic processes [57, 58]. Epigenetic mechanisms include DNA methylation, imprinting, and RNA transcriptional regulation through RNA molecules, such as miRNAs. These processes are influenced by multiple factors: intrauterine nutrient availability (determined by maternal nutrition and placental function) [59–62], maternal age [63, 64], use of drugs [65, 66], endocrine disruptors [67], toxins, and infectious agents. For this reason, integrated assessment of early pregnancy should evaluate a combination of biomarkers and ultrasound [68–73]. In addition, we take the opportunity to stress how future investigations about miRNA levels in both sera and placentas should evaluate the possible overlapping among preeclampsia, FGR, and gestational diabetes, since they all have in common placental vascular alterations due to angiogenic disbalance [74].

It is however clear that epigenetic information is transmitted, and potentially inherited, across generations through the remodeling of chromatin states. In this regard, selective miRNA expression may be involved in FGR through epigenetic mechanism.

5. Conclusion

Understanding which miRNAs are associated with the onset/progression of FGR seems mandatory to improve early diagnosis and management of the disease. In this regard, we take the opportunity to solicit future studies on large cohort and adequate statistical power, in order to identify a panel of biomarkers on maternal peripheral blood for early diagnosis of FGR.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Table 1: Summary of relevant information from selected studies.

Authors, year	Type of study	Enrolled population	Methods of analysis	Samples	Main findings
Cindrova-Davies et al., 2013 [8]	Case-control	6 FGR, 6 PE-AD, 7 PE-ND, 7 HP (controls)	RT-qPCR	Placentas	Expression of miR-21 was significantly upregulated in PE-AD and FGR cases, but not in PE-ND cases
Guo et al., 2013 [9]	Case-control	26 FGR, 16 PE, 29 HP (controls)	RT-qPCR	Placentas	Downregulation of miR-149 was shown in PE cases; miR-194 was significantly downregulated in FGR and PE cases
Hromadnikova et al., 2015 [10]	Case-control	35 GH, 80 PE, 35 FGR, 20 HP (controls)	RT-qPCR	Placentas	(i) Upregulation of miR-499a-5p in PE, GH, FGR cases; (ii) upregulation of miR-1-3p FGR-AD in PE cases delivering after 34 weeks; (iii) downregulation of miR-26a-5p, miR-103a-3p, miR-145-5p in PE and FGR cases requiring delivery before 34 weeks; (iv) downregulation of miR-16-5p, miR-100-5p, miR-122-5p, miR-125b-5p, miR-126-3p, miR-143-3p, miR-195-5p, miR-199a-5p, miR-221-3p, miR-342-3p, miR-574-3p in FGR cases requiring delivery before 34 weeks
Huang et al., 2013 [11]	Case-control	25 FGR, 25 HP (controls)	RT-qPCR	Placentas	Upregulation of miR-424
Tang et al., 2013 [13]	Case-control	21 FGR, 34 HP (controls)	RT-qPCR	Placentas	High expression level of miR-141 in FGR cases
Donker et al., 2012 [14]	Case-control	14 FGR, 14 HP (controls)	RT-qPCR	Placentas	Hypoxic stress does not affect C19MC miRNA expression, except for downregulation of miR-500c-3p
Higasijima et al., 2013 [15]	Two-step case-control	First step: 45 FGR, 50 HP (controls)	RT-qPCR	Placentas	(i) miR-518b, miR-1323, miR-516b, miR-515-5p, miR-520h, miR-519d, miR-526b were significantly lower in FGR placentas than in controls; (ii) no differences in miR-516a-5p, miR-525-5p, miR-520a-5p levels
		Second step: 10 FGR, 10 HP (controls)	RT-qPCR	Maternal plasma/serum	No differences were found in miR-518b, hsa-miR-1323, hsa-miR-520h, hsa-miR-519d levels between FGR cases and controls
Wang et al., 2014 [16]	Case-control	30 FGR, 30 LGA, 30 NGA (controls)	RT-qPCR	Placentas	Decreased expression of miR-518b and increased expression of miR-519a in FGR cases with respect to NGA and LGA cases
Hromadnikova et al., 2015 [17]	Case-control	21 GH, 63 PE, 36 FGR, 42 HP (controls)	RT-qPCR	Placentas	(i) Downregulation of miR-517-5p, miR-519d, miR-520a-5p, miR-525 in GH cases; (ii) downregulation of miR-517-5p, miR-518f-5p, miR-519a, miR-519d, miR-520a-5p, miR-525 in FGR cases; (iii) downregulation of miR-515-5p, miR-517-5p, miR-518b, miR-518f-5p, miR-519a, miR-519d, miR-520a-5p, miR-520h, miR-524-5p, miR-525, miR-526a in PE cases
Hromadnikova et al., 2012 [44]	Case-control	16 PE, 11 FGR, 5 PE and FGR, 7 normal pregnancies with later onset of PE and/or FGR, 10 NP (controls), 50 HP (controls)	RT-qPCR	Maternal plasma/serum	(i) No differences were found in miR-516-5p, miR-517, miR-518b, miR-520a, miR-520h, miR-525, miR-526a concentrations between pathologic and normal pregnancies; (ii) significant elevation of miR-516-5p, miR-517, miR-518b, miR-520a, miR-520h, miR-525, miR-526a levels during early gestation in pregnancies with later onset of PE and/or FGR
Hromadnikova et al., 2013 [45]	Case-control	63 PE, 27 FGR, 23 GH, 55 HP (controls)	RT-qPCR	Maternal plasma/serum	(i) Plasmatic levels of miR-516-5p, miR-517, miR-520a, miR-525, miR-526a did not differ between FGR, GH, and HP patients; (ii) increased plasmatic levels and high expression of miR-516-5p, miR-517, miR-520a, miR-525, miR-526a were found in PE patients

Table 1: Continued.

Authors, year	Type of study	Enrolled population	Methods of analysis	Samples	Main findings
Hromadnikova et al., 2016 [46]	Case-control	39 GH, 68 PE, 33 FGR, 20 HP (controls)	RT-qPCR	Maternal plasma/serum	(i) Downregulation of miR-100-5p, miR-125b-5p, miR-199a-5p in GH, PE, FGR cases compared with controls; (ii) downregulation of miR-17-5p, miR-146a-5p, miR-221-3p, miR-574-3p in FGR cases; (iii) downregulation of miR-100-5p, miR-125b-5p in PE cases; (iv) downregulation of miR-100-5p, miR-125b-5p, miR-146a-5p, miR-199a-5p, miR-221-3p, miR-574-3p in FGR cases; (v) downregulation of miR-100-5p, miR-125b-5p, miR-199a-5p in patients with GH
Mouillet et al., 2010 [47]	Case-control	14 FGR, 14 HP (controls)	RT-qPCR	Maternal plasma/serum	(i) No differences were found in the level of individual miRNA (miR-27a, miR-30d, miR-141, miR-200c, miR-424, miR-205 and miR-451, miR-491, miR-517a, miR-518b, miR-518e, miR-524) in FGR and HP cases; (ii) When considered as a group, the level of all tested miRNA species was elevated in plasma samples from women with FGR compared to controls

HP: healthy pregnancies; FGR: fetal growth restriction; PE: preeclampsia; -AD: abnormal fetal-Doppler; -ND: normal fetal-Doppler; GH: gestational hypertension; LGA: large for gestational age; NGA: normal for gestational age; NP: nonpregnant; RT-qPCR: real-time quantitative reverse transcription polymerase chain reaction.

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Research Article

First-Trimester Crown-Rump Length and Embryonic Volume of Fetuses with Structural Congenital Abnormalities Measured in Virtual Reality: An Observational Study

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Background. With the introduction of three-dimensional (3D) ultrasound it has become possible to measure volumes. The relative increase in embryonic volume (EV) is much larger than that of the crown-rump length (CRL) over the same time period. We examined whether EV is a better parameter to determine growth restriction in fetuses with structural congenital abnormalities. *Study Design, Subjects, and Outcome Measures.* CRL and EV were measured using a Virtual Reality (VR) system in prospectively collected 3D ultrasound volumes of 56 fetuses diagnosed with structural congenital abnormalities in the first trimester of pregnancy (gestational age 7^{+5} to 14^{+5} weeks). Measured CRL and EV were converted to z-scores and to percentages of the expected mean using previously published reference curves of euploid fetuses. The one-sample t-test was performed to test significance. *Results.* The EV was smaller than expected for GA in fetuses with structural congenital abnormalities (−35% $p < 0.001$, z-score −1.44 $p < 0.001$), whereas CRL was not (−6.43% $p = 0.118$, z-score −0.43 $p = 0.605$). *Conclusions.* CRL is a less reliable parameter to determine growth restriction in fetuses with structural congenital abnormalities as compared with EV. By measuring EV, growth restriction in first-trimester fetuses with structural congenital abnormalities becomes more evident and enables an earlier detection of these cases.

1. Introduction

In the past decade prenatal screening has partly shifted from the second trimester to the first trimester of pregnancy. Because of vast improvements in imaging technology the embryo and fetus in early pregnancy can be evaluated in much more detail, allowing screening for structural abnormalities between 11 and 14 weeks GA [1–5]. A significant proportion of major structural abnormalities can be detected already in this period. In some cases, nonspecific findings, like increased nuchal translucency, may be the first sign for existing structural abnormalities, leading to additional ultrasound examinations [6].

It is well known that first-trimester growth is associated with pregnancy outcome [7–10] and that several factors like maternal factors and dietary pattern influence first-trimester growth [11–13]. Traditionally, first-trimester fetal growth has been documented by two-dimensional (2D) crown-rump length (CRL) measurements. With the introduction of three-dimensional (3D) ultrasound it has become possible to measure embryonic volumes (EV) [14]. Earlier studies show that the relative increment of the EV is much larger than the increment of the CRL during the same period [15]. Using an innovative 3D Virtual Reality (VR) technique, Rousian et al. demonstrated in this study that when the CRL doubles the EV increases 6.5-fold. Volume measurement might therefore enable earlier detection of fetal growth restriction in pregnancy. It is well known that too small CRL is a clinical predictor for miscarriage, chromosomal abnormalities (especially trisomy 18), and fetal growth restriction in the second and third trimester of pregnancy [10, 16–19]. It has been suggested that EV is smaller in aneuploid pregnancies and by using VR

it was proven that, compared with CRL, EV was not only smaller in trisomy 18 pregnancies but also in trisomy 21 and trisomy 13 pregnancies [20, 21]. EV therefore turns out to be a better parameter to detect growth restriction caused by aneuploidy than CRL.

From these observations it is suspected that underlying pathophysiological changes in these cases might influence embryonic and early fetal growth. First-trimester growth might also be impaired in pregnancies diagnosed with a congenital abnormality. An association between the presence of structural congenital abnormalities and second- and third-trimester growth restriction is already known for a long time [22–24].

The aim of this study is to examine the first-trimester growth pattern in embryos and fetuses with structural congenital abnormalities. CRL and EV measurements of pregnancies with structural abnormalities were compared with references values of CRL and EV in uncomplicated pregnancies.

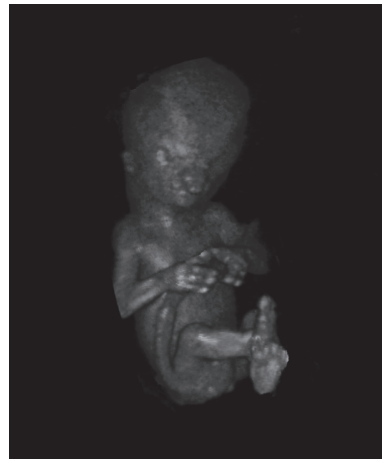

Figure 1: 3D transvaginal ultrasound dataset of a fetus with an ectrodactyly ectodermal dysplasia-cleft (EEC) syndrome visualized in Virtual Reality. Bilateral split hands and split feet are seen as well as bilateral cheilognatoschisis. An overriding aorta with a ventricle septum defect was diagnosed additionally.

2. Methods

Between December 2008 and November 2013 transvaginal three-dimensional (3D) ultrasound volumes were collected of first-trimester pregnancies in which a structural congenital abnormality was diagnosed ($N = 71$). Cases were collected at the department of Obstetrics and Prenatal Medicine at Erasmus MC University Medical Center Rotterdam ($n = 47$) and at Hôpital Princesse Grace Monaco ($n = 15$). Additional cases ($N = 9$) were included from the Rotterdam Predict study [11], a periconception cohort aimed at early pregnancy. Ultrasound scans were performed using the Voluson E8 Expert system (GE Medical Systems, Zipf, Austria) by operators experienced in collecting 3D ultrasound datasets. Structural congenital abnormalities were all confirmed either during the midpregnancy ultrasound scan, postpartum diagnosis, or a pathological investigation after termination of pregnancy.

In spontaneously conceived pregnancies dating was based on the first day of the last menstrual period (LMP). When the menstrual cycle was regular but >3 days different from 28 days the gestational age (GA) was adjusted for the cycle length. In pregnancies conceived by in vitro fertilization (IVF) with or without intracytoplasmic sperm injection (ICSI) GA was calculated from the day of oocyte retrieval plus 14 days. In pregnancies originating from intrauterine insemination GA was calculated based on the LMP or inseminated date plus 14 days. If the first day of the LMP was missing or if the menstrual cycle was irregular, these pregnancies were excluded from this analysis. The GA ranged from 7^{+5} to 14^{+5} weeks.

The 3D volumes were converted to Cartesian volumes, using 3D software (4D View, GE Medical Systems, Zipf, Austria), and transferred to the BARCO (Kortrijk, Belgium) I-Space VR system at the department of Bioinformatics of Erasmus MC University Medical Center Rotterdam. This is a four-walled CAVE™ like VR system in which investigators are surrounded by stereoscopic images. A "hologram" of the ultrasound data is created by the V-Scope [25] volume rendering application (Erasmus MC, Rotterdam, the Netherlands) and polarized glasses enable the viewer to perceive depth and to interact with 3D volumes in an intuitive manner. In the I-Space all 3D ultrasound volumes were evaluated and the best volume for each case was selected based on image quality and completeness of the volume. A fetus with structural congenital abnormalities visualized in Virtual Reality is shown in Figure 1.

CRL and EV were measured in the BARCO I-Space using the V-Scope software. The V-Scope application includes a region-growing segmentation algorithm combined with a neighbourhood variation threshold for semiautomatica volume calculation in selected structures [25]. The procedure for measuring EV is described in detail by Rousian et al. [15] The innovative VR technique has already been successfully applied in various prenatal studies [14, 25].

To include all body parts of the embryo, the omphalocele, physiological or pathological, is included in the EV calculation, as well as hydrops, frequently present in fetuses with structural congenital abnormalities. All measurements were performed by the same investigator (LB). The accuracy and reproducibility of CRL and EV measurements have been proven in previous studies and CRL and EV reference curves have been established [15, 26–28]. *Inter- and intraobserver variability for 3D-VR measurements were very high for CRL (ICC 1.000; 95% CI: 0.999–1.000, resp., ICC 1.000; 95% CI: 0.999–1.000) as well as for EV measurements (ICC 0.999; 95% CI: 0.997–0.999, resp., 0.999; 95% CI: 0.998–0.999) [27, 29].* The data of the present study are compared with these reference curves.

This study has been approved by the Central Committee on Research in The Hague and the Local Medical Ethical and Institutional Review Board of the Erasmus MC (METC2004-227).

2.1. Statistical Analysis. In each pregnancy complicated by a congenital abnormality the observed values for CRL and/or

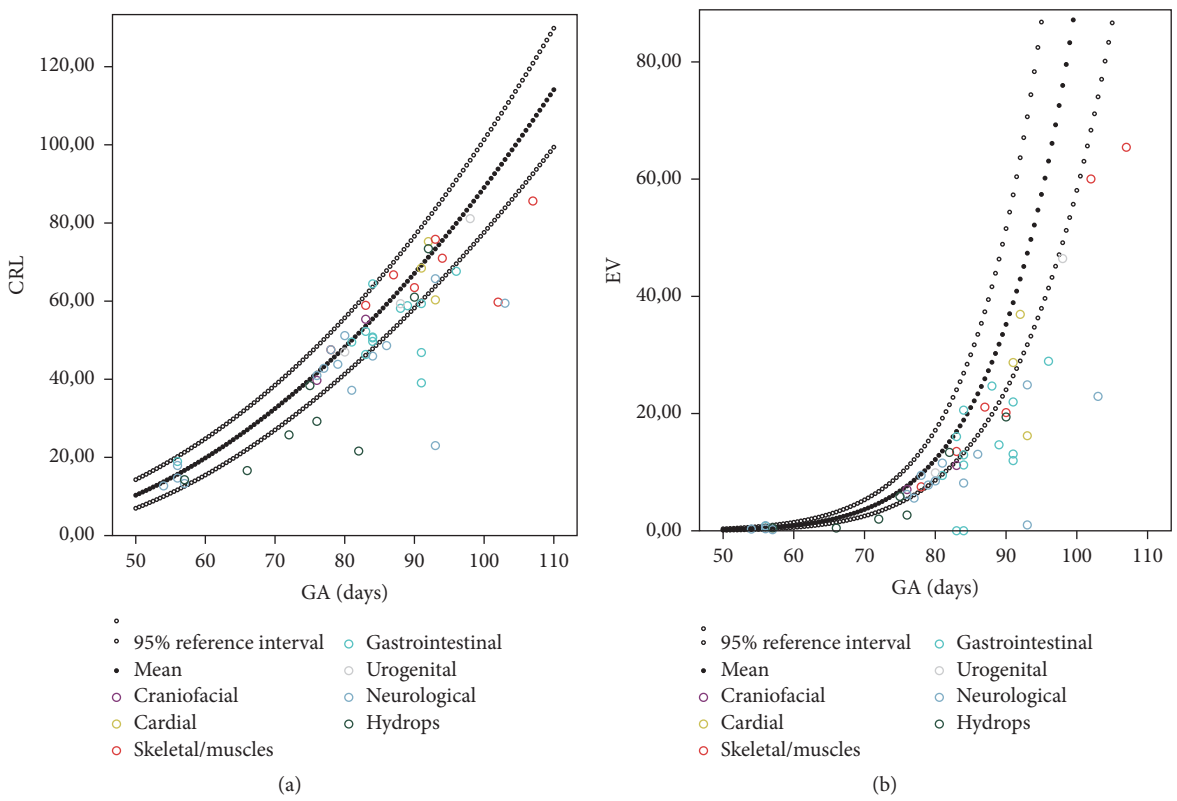

FIGURE 2: CRL ($n = 56$) and EV ($n = 51$) of all cases with structural congenital abnormalities plotted relative to the reference curves for healthy pregnancies.

EV were subtracted from the expected mean of CRL and EV for GA. This expected value was obtained from reference curves published in earlier studies [15, 26–29]. This difference was divided by the standard deviation (SD) for GA of the reference values in order to obtain the z-score. This difference was as well expressed as a percentage of the mean CRL and EV of reference fetuses. When different ultrasound volumes of different GA were present, the dataset of the oldest GA was used. The same analysis was performed when the expected value for EV was corrected for the measured CRL.

The one-sample two-sided t-test was used to test for a statistically significant difference in z-score as compared to the reference value. This analysis was performed in the overall group of cases with structural congenital abnormalities and in the different subgroups of various structural congenital abnormalities.

Data analysis was performed using SPSS v.21 (SPSS Inc., Chicago, IL, USA). A p value < 0.05 was considered statistically significant.

3. Results

Three cases were excluded from the analysis because of uncertain GA and one because of a twin pregnancy. We excluded 11 cases for the measurements of both CRL and EV due to poor image quality caused by an intermediate position of the uterus or movement artifacts ($N = 9$), due to incompleteness of the volume ($N = 1$) and because of absence of heartbeat at the time of the ultrasound scan ($N = 1$). A total of 56 cases remained for analysis of CRL. As in five of these cases the image quality was too poor for performing EV measurement; only 51 cases remained for analysis of EV. Of these 7 of 56 cases were conceived by artificial reproductive techniques, 4 using ICSI, 2 using IVF, and 1 using IUI.

In the overall group of fetuses with structural congenital abnormalities the EV was smaller than expected for GA (−35% $p < 0.001$, z-score −1.44 $p < 0.001$), whereas CRL was not smaller than expected (−6.43% $p = 0.118$, z-score −0.43 $p = 0.605$). In 15 out of the 56 cases (26,8%) with structural abnormalities the CRL was more than two standard deviations below the mean (a z-score > −1,64). In 18 out of 51 cases (35,3%) the EV was more than two standard deviations below the mean. The CRL was significantly smaller in the subgroups with urogenital abnormalities and in the subgroup with hydropic abnormalities. The EV was significantly smaller in the subgroup with cardiac abnormalities, gastrointestinal abnormalities, urogenital abnormalities, neurological abnormalities, and in the group with hydropic abnormalities (Table 1).

In Figure 2 CRL and EV of all cases with structural congenital abnormalities are plotted in the reference curves for pregnancies without structural congenital abnormalities. In the supplemental figures the different groups of structural congenital abnormalities are plotted separately on the references curves for CRL and EV (Supplemental Figure 1 is available online at https://doi.org/10.1155/2017/1953076).

Table 1: Mean percentage difference and z-scores for CRL and EV in both the overall group of structural congenital abnormalities and in the various subgroups.

Variable/congenital abnormality	n	Mean difference in % (95% CI)	p^*	Mean difference in z-score (95% CI)	p^*
CRL					
Overall	56	−6.43 (−14.55, 1.69)	0.118	−0.46 (−2.21, 1.30)	0.605
Craniofacial	2	−0.60 (−50.36, 49.15)	0.903	−0.05 (−6.25, 6.15)	0.931
Cardiac	5	−5.03 (−16.46, 6.39)	0.288	−1.34 (−2.27, 0.90)	0.296
Skeletal/muscles	8	−3.02 (−18.58, 12.54)	0.660	−0.55 (−2.62, 1.51)	0.547
Gastrointestinal	15	−7.02 (−18.05, 4.02)	0.194	−1.08 (−2.44, 0.28)	0.111
Urogenital	5	−4.98 (−7.87, −2.09)	0.009	−0.64 (−1.04, −0.25)	0.010
Neurological	14	0.12 (−30.53, 30.77)	0.993	1.59 (−5.74, 8.94)	0.646
Hydrops	7	−26.89 (−43.26, −8.51)	0.011	−2.94 (−5.18, −0.71)	0.018
EV					
Overall	51	−34.91 (−41.97, −27.85)	<0.001	−1.44 (−1.71, −1.16)	<0.001
Craniofacial	2	−21.22 (−187.3, 144.85)	0.351	−1.00 (−8.73, 6.73)	0.349
Cardiac	5	−31.86 (−56.43, −7.28)	0.023	−1.34 (−2.30, −0.39)	0.017
Skeletal/muscles	6	−26.18 (−60.67, 8.30)	0.108	−0.92 (−2.21, 0.37)	0.127
Gastrointestinal	13	−35.78 (−49.45, −22.11)	<0.001	−1.53 (−2.09, −0.96)	<0.001
Urogenital	4	−16.26 (−25.21, −7.31)	0.010	−0.75 (−1.22, −0.27)	0.016
Neurological	14	−43.24 (−59.20, −27.28)	<0.001	−1.70 (−2.27, −1.13)	<0.001
Hydrops	7	−40.85 (−66.34, −15.36)	0.008	−1.76 (−2.88, −0.64)	0.008

* It is for observed mean difference versus 0.

In Table 2 the percentage difference and z-score for observed versus expected EV after correction for the observed CRL is presented. No statistical differences were found.

4. Discussion

To the best of our knowledge this is the first study that investigates the relationship between EV and first-trimester structural congenital abnormalities. Although overall the CRL was not significantly smaller in fetuses with structural congenital abnormalities, a smaller than expected CRL was observed in hydropic fetuses and fetuses with urogenital abnormalities. In contrast to CRL, EV was statistically significant smaller than expected in the overall group of structural congenital abnormalities. In all subgroups, except for those with craniofacial and skeletal/muscle abnormalities, we found a significantly smaller EV than expected.

The mean difference in EV was more evident than the mean difference in CRL and went up to −43% (z-score −1.70) in fetuses with neurological abnormalities. This can be explained by the fact that a volume is a three-dimensional measurement in contrast to CRL, which is a flat, two-dimensional distance measurement. It was already demonstrated by Rousian et al. that when the CRL doubles EV increases 6.5 times [15]. However, after correcting the EV for the measured CRL significant differences were no longer present, suggesting proportional growth restriction. EV turned out to be a better parameter to detect first-trimester growth restriction as compared with CRL.

From the literature it has recently become evident that a detailed anatomical scan can be successfully preformed at the end of the first-trimester. The majority of major structural congenital abnormalities can therefore be diagnosed between 11 and 14 weeks GA. EV measurements can be performed from 6 weeks GA onwards [15] and may therefore possibly be used as a marker of an underlying abnormality long before an early anomaly scan can be performed. EV measurements in early pregnancy might point a clinician to the increased risk of a congenital abnormality. The effectiveness of EV as a marker for structural congenital abnormalities should be subject of further study.

The combination of early growth restriction and the presence of structural congenital abnormalities might be due to underlying pathological mechanisms. Growth restriction might either occur as a result of a structural congenital

Table 2: The mean percentage differences and z-scores for EV after correction for the observed CRL both in the overall group of structural congenital abnormalities and the subgroups of structural congenital abnormalities.

Variable/congenital abnormality	n	Mean difference in % (95% CI)	p^*	Mean difference in z-score (95% CI)	p^*
EV					
Overall	51	27.86 (−14.34, 70.05)	0.191	0.92 (−0.66, 2.49)	0.247
Craniofacial	2	−6.18 (−266.27, 253.92)	0.813	−0.40 (−11.64, 10.84)	0.730
Cardiac	5	2.90 (−11.97, 17.76)	0.617	−0.10 (−0.71, 0.51)	0.673
Skeletal/muscles	6	29.69 (−71.29, 130.66)	0484	0.88 (−3.07, 4.82)	0.591
Gastrointestinal	13	14.94 (−4.72, 34.60)	0.124	0.47 (−0.38, 1.31)	0.254
Urogenital	4	4.95 (−2.93, 12.82)	0.139	0.008 (−27.34, 28.98)	0.932
Neurological	14	−1.82 (−25.66, 22.03)	0.872	−0.12 (−1.11, 0.87)	0.798
Hydrops	7	150.26 (−211.03, 511.54)	0.348	5.50 (−7.89, 18.89)	0.354

*It is for observed mean difference versus 0.

abnormality or growth restriction and structural congenital abnormalities might have a common etiological factor.

Limitations of the study are the low numbers of included cases with structural congenital abnormalities. Still finding significant differences for EV suggests a strong relationship of first-trimester structural congenital abnormalities and a decreased EV. Therefore, increasing the numbers in future studies will most likely only strengthen this relationship. Pregnancies with known chromosomal abnormalities were not included in the study. As fetal karyotyping was performed in 36 of 56 cases; it may be possible that cases with a chromosomal abnormality in our study group remained unnoticed. However, in all but 5 cases with an increased nuchal translucency, hygroma colli or hydrops fetalis, karyotyping was performed and showed to be euploid. The five cases with an increased nuchal translucency, hygroma colli or hydrops fetalis, were all in "hydrops" group and either spontaneously miscarried or were terminated before karyotyping could be performed.

We included pregnancies conceived by artificial reproductive techniques in our series of cases with structural congenital abnormalities. Recent studies point out that growth trajectories in early pregnancy do not differ between spontaneously conceived pregnancies and pregnancies conceived using artificial reproductive techniques in our population [30].

Furthermore, the BARCO I-Space is too large and too expensive to become a routine method for the measurement of EV. However, a much smaller and more affordable 3D VR desktop system is currently being evaluated and will provide a good alternative, making this technique broadly available to hospitals [31]. Following the introduction of the desktop VR system we foresee implementation of VR as an option in ultrasound machines in the near future.

We are aware that 3D ultrasound and its calculating software, that is, 4D view, are widely available for volume calculations, in contrast to the VR technique. However, using the available software on the ultrasound machine requires delineating the contours of the embryo manually in several different planes, which is subject to individual variation. The semiautomatic approach of the I-Space and its true depth perception allow for more objective volume measurements and prevent incomplete segmentations. Another advantage of the VR technique is that the whole body volume is measured with this technique, whereas when using the manual delineating technique only a head and trunk volume can be calculated, resulting in an underestimation.

5. Conclusions

In conclusion, CRL, the current golden standard for the detection of first-trimester growth restriction, seems a less reliable parameter to detect growth restriction in fetuses with structural congenital abnormalities as compared with EV, being significantly decreased in these pregnancies. By measuring EV, first-trimester growth restriction becomes more evident and might enable an earlier detection of cases at risk for a congenital abnormality.

Conflicts of Interest

No conflicts of interest are declared.

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Research Article

Suspected Fetal Growth Restriction at 37 Weeks: A Comparison of Doppler and Placental Pathology

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Objective. Our objective was determining if abnormal Doppler evaluation had a higher prevalence of placental pathology compared to normal Doppler in suspected fetal growth restriction (FGR) of cases delivered at 37 weeks. *Study Design.* This retrospective cohort study of suspected FGR singletons with antenatal Doppler evaluation delivered at 37 weeks had a primary outcome of the prevalence of placental pathology related to FGR. Significance was defined as $p \leq 0.05$. *Results.* Of 100 pregnancies 46 and 54 were in the abnormal and normal Doppler cohorts, respectively. Placental pathology was more prevalent with any abnormal Doppler, 84.8% versus 55.6%, odds ratio (OR) 4.46, 95% confidence interval (CI): 1.55, 13.22, and $p = 0.002$. Abnormal middle cerebral artery (MCA) Doppler had a higher prevalence: 96.2% versus 54.8%, OR 20.7, 95% CI: 2.54, 447.1, and $p < 0.001$. *Conclusion.* Abnormal Doppler was associated with more placental pathology in comparison to normal Doppler in fetuses with suspected FGR. Abnormal MCA Doppler had the strongest association.

1. Introduction

Fetal growth restriction (FGR) is defined in the antenatal period as an estimated fetal weight (EFW) by ultrasound less than the 10th percentile for gestational age in the United States [1]. International consensus definition of FGR is more comprehensive and incorporates other parameters such as abdominal circumference, gestational age of onset, Doppler indices, and growth deceleration before arriving at the diagnosis of FGR [2]. Doppler evaluation of maternal, fetal, and umbilical vessels has been used in the management of suspected FGR to aid in timing of delivery and theoretically could separate the fetus with a placental problem from the constitutionally small normal fetus. Newborns that are less than the 10th percentile for gestational age are classified as small for gestational age (SGA). Methods to determine whether an SGA newborn also has pathologic growth restriction are imperfect. Obvious physical features of FGR in the SGA infant, uncommon with modern obstetric management, include decreased muscle mass and subcutaneous tissue and skin desquamation [3]. Other observations proposed for diagnosing FGR among SGA newborns include low ponderal index [4] and postnatal catch-up growth [5]. Current management in FGR is designed to avoid stillbirth, incidence 1.1–3.6%, and deliver the most mature baby as possible [6, 7]. Up to 70% of fetuses with suspected FGR may be constitutionally small normal infants and may not be at increased risk for stillbirth, and the remainder (after exclusion of birth defects, congenital infections, and chromosomal abnormalities) will have FGR presumably related to a pathologic placental process [5, 8].

The use of umbilical artery Doppler in management of suspected FGR is associated with a reduction in perinatal

deaths [9]. The relationship of umbilical artery Doppler patterns in FGR to placental pathology is more straightforward when the most severe patterns, absent end diastolic velocity (AEDV) or reversed end diastolic velocity (REDV), are present [10]. In these cases, which are usually delivered markedly preterm because of nonreassuring fetal testing, there is loss of arterial vessels within the villi accounting for the abnormal Doppler patterns. In FGR at later gestational ages the villous vascular tree has a larger capacity and abnormal umbilical artery Doppler patterns are less frequent; the placental pathology is more subtle and the lesions can overlap with normal pregnancies [11]. Late-onset FGR pregnancies with uterine artery and middle cerebral artery (MCA) Doppler abnormalities have been associated with placental lesions of underperfusion [12].

We therefore chose to study the correlation of Doppler abnormalities in fetuses with suspected FGR delivered at 37 weeks' gestation at our institution in order to remove the confounding factor that gestational age has on interpretation of placental pathology and the bias toward more severe placental lesions that are seen in FGR fetuses that require preterm delivery. We hypothesized that the group of suspected FGR fetuses with abnormal Doppler would have a higher prevalence of gross and histopathologic abnormalities found in FGR as compared to the group with normal Doppler.

2. Methods

This was a retrospective cohort study of singleton fetuses with an ultrasound estimated fetal weight less than the 10th percentile delivered at Penn State Milton S Hershey Medical Center at 37 weeks' gestation during the time period 2011–2013. The study was approved by the Research Subjects Review Board at the Penn State Milton S Hershey Medical Center. Cohorts were divided into normal and abnormal Doppler and compared with respect to both the presence and number of gross and histopathologic findings in the placenta that were plausible in their relation to the FGR. Suspected FGR was a standard indication for submission of the placenta to pathology; thus all placentas from this group were expected to have had a pathologic examination. Pregnancies with uncertain dating, multiple gestations, fetuses with major birth defects, or viral or parasitic infections were excluded. Ascertainment of gestational age followed standard clinical and ultrasound guidelines [13].

The subjects were identified by viewing the electronic birth log for all deliveries at 37 weeks' gestation with suspected FGR. The ultrasound reports were reviewed for EFW < 10th percentile within three weeks of delivery. EFW and percentile were calculated by software using biometric parameters [14, 15]. Ultrasound measurements were performed with 2–5 MHZ curvilinear transducers using the iU22 (Philips Medical Systems, Bothell, WA). All sonography was performed by experienced sonographers dedicated to maternal-fetal medicine. EFW and head circumference to abdominal circumference ratio (HC/AC) were recorded for analysis.

Doppler measurements were obtained utilizing standard techniques [16–18]. All subjects had umbilical artery Doppler as part of their ultrasound surveillance at diagnosis of suspected FGR and with serial scans up to the time of delivery. MCA and uterine Doppler evaluation had not been utilized at all for the first year of the study period in the evaluation of suspected FGR. After the first year of the study time period, MCA Doppler was incorporated routinely into the evaluation of suspected FGR by 3 of 4 maternal-fetal medicine faculty members and not at all by one faculty member. Only 1 maternal-fetal medicine specialist also utilized uterine artery Doppler in the evaluation of suspected FGR, but only once during the pregnancy. All Doppler studies were reviewed in the GE PACS system (GE Healthcare, Chicago IL) by one maternal-fetal medicine specialist (WMC). The last measurements performed and recorded prior to delivery were used for analysis. The systolic/diastolic ratio, resistance index, pulsatility index (PI), and peak systolic velocity were calculated using the software on the machine. For the MCA Doppler the cerebroplacental pulsatility ratio (CPR) was calculated by dividing the MCA Doppler PI by the umbilical artery Doppler PI. Abnormal umbilical (PI > 95th percentile), MCA (PI or CPR < 5th percentile), or uterine artery (PI > 95th percentile) Doppler for gestational age was defined by using standard reference charts [16–18]. Subjects who had at least one abnormal Doppler of any type were placed in the abnormal Doppler cohort and subjects who had only normal Doppler were placed in the normal Doppler cohort.

Placentas were examined according to standard protocol [19]. Placental weight and gross characteristics were obtained from the placental pathology report. Placental slides were retrieved from the archive and were reviewed by a single pathologist (KAM) blinded to the Doppler categorization. Placentas that had one or more gross or histopathologic feature that could be considered contributing to FGR were classified as abnormal placentas. Those placentas with no pathologic features were classified as normal. The following gross placental features were considered abnormal: placental weight < 5th percentile for gestational age [20], single umbilical artery, marginal or velamentous cord insertion, bilobed or succenturiate placenta, and circummarginate or circumvallate placenta. Additional gross placental findings that were categorized as abnormal included infarcts, abruption, intervillous/subchorionic thrombi encompassing > 5% of placenta parenchyma, and maternal floor infarction. Histopathologic findings considered contributory to FGR included the following: increased syncytial knots, villous agglutination, increased intervillous fibrin, distal villous hypoplasia, acute atherosis, mural hypertrophy in membrane arterioles, muscularization of basal plate arteries, increased placental site giant cells in decidua basalis, immature intermediate trophoblast in decidua basalis, thin umbilical cord (diameter of the umbilical cord $\leq$ 8 mm), uniformly avascular villi, villous stromal-vascular karyorrhexis, villitis of unknown etiology (VUE) with obliterative fetal vasculopathy, large fetal vessel thrombosis, fetal intimal fibrin cushion, chorangiosis, nucleated red cells in capillaries, and VUE. We followed published guidelines for diagnosis for histopathologic lesions related to FGR [21–23].

Maternal demographic variables collected included age, parity, BMI, race/ethnicity, smoking history, diabetes, hypertension, and mode of delivery. Newborn information collected

Table 1: Abnormal versus normal Doppler antenatal comparisons in suspected FGR.

Variable*	Abnormal Doppler $N = 46$	Normal Doppler $N = 54$	OR (95% CI)	p value
Maternal age	25.2 ± 5.7	27.0 ± 6.2		0.044
Maternal BMI	29.6 ± 6.6	27.4 ± 4.9		0.065
Caucasian	28 (60.9)	40 (74.1)	0.54 (0.26, 1.48)	0.158
Diabetes	4 (8.7)	3 (5.6)	1.62 (0.28, 9.78)	0.700
Hypertension	3 (6.5)	1 (1.9)	3.70 (0.34, 95.8)	0.331
Parity ≥ 1	21 (45.7)	31 (57.4)	0.62 (0.26, 1.48)	0.241
Smoking	16 (34.8)	16 (29.6)	1.27 (0.50, 3.20)	0.582
EFW ultrasound (grams)	2138.4 ± 202.0	2167.8 ± 336.8		0.606
EFW < 3rd percentile	13 (28.3)	12 (22.2)	1.38 (0.51, 3.76)	0.487
HC/AC > 95th percentile	16 (34.8)	20 (37.8)	0.91 (0.37, 2.23)	0.915
Induction of labor	38 (82.6)	43 (79.6)	1.22 (0.40, 3.99)	0.705
Vaginal delivery	33 (71.7)	41 (75.9)	0.81 (0.30, 2.16)	0.634
Cesarean for nonreassuring fetal status	5 (10.9)	1 (1.9)	6.46 (0.69, 152)	0.09
Vacuum/forceps vaginal delivery	3 (6.5)	0		0.425

OR = odds ratio, CI = confidence interval, and FGR = fetal growth restriction.
*Results expressed in mean ± SD or number (%).

included gender, birthweight, ponderal index/ponderal index < 10th percentile, birthweight percentile [4], birthweight to placental weight ratio [24], NICU admission, days in hospital, hyperbilirubinemia requiring phototherapy, hypoglycemia, hypothermia, and oxygen requirement. SGA was defined as birthweight for the gestational age of 37 weeks of <2500 grams [4]. Composite neonatal morbidity was defined as at least one neonatal morbidity, including NICU admission. The data were analyzed by t-tests, chi-square tests, and odds ratios with 95% confidence intervals as appropriate. Statistical analysis was performed using SPSS (Chicago, IL). The primary outcome was the proportion of patients with any placental pathology. Performance characteristics of each Doppler type for identification of placental pathology were calculated. Using data by Dicke et al. [25], in a study of both preterm and term SGA infants that showed 94% with histopathologic placental lesions in the abnormal Doppler group and 64% in the normal Doppler group, a sample size of 28 patients in each cohort was calculated to show this difference with a power of 80% and significance level of 0.05.

3. Results

We identified 177 total patients delivered at 37 weeks' gestation for the indication of suspected FGR; 56 were excluded for EFW > 10th percentile, 8 were with fetal anomalies, and 13 were with multiple gestations, leaving a total of 100 subjects: 54, normal Doppler group and 46, abnormal Doppler group. All 100 subjects were evaluated by umbilical artery Doppler, 68 by MCA Doppler, and 39 by uterine artery Doppler. There were no umbilical artery Doppler patterns of AEDV or REDV. The mean gestational ages of the last Doppler type performed prior to delivery were 36.7±0.5, 36.1±1.2, and 32.9±4.4 weeks for umbilical, middle cerebral, and uterine artery Doppler, respectively.

Antenatal comparisons of the cohorts abnormal versus normal Doppler are given in Table 1. Maternal age was slightly less in the abnormal group. There were no significant differences in any of the other categories. The newborn comparisons are given in Table 2. Newborns in the abnormal Doppler cohort were significantly lighter and more likely to be SGA. There were no differences in any other comparisons. Overall, 20% of newborns were admitted to the NICU and 37% experienced at least one morbidity.

The proportion of placentas with pathologic features compared by Doppler type and cohort is given in Table 3. For any Doppler type utilized, a higher proportion of placental pathology was observed if the Doppler was abnormal, OR = 4.46, 95% CI: 1.55, 13.22. Of the individual Doppler types, only an abnormal MCA Doppler was significantly associated with placental pathology compared to a normal MCA Doppler, OR = 20.7, 95% CI: 2.54, 447.1.

The performance characteristics for Doppler in the diagnosis of placental pathology are given in Table 4. Doppler had both limited sensitivity and NPV for the detection and exclusion of placental pathology, respectively. All Doppler types performed better on specificity and PPV.

Comparison of the numbers of individual placental abnormalities is given in Table 5. Infarcts were significantly more common in the abnormal Doppler group, OR = 3.87, 95% CI: 1.23, 12.67. Lesions belonging to the category of maternal vascular underperfusion [22] were more common in the abnormal Doppler cohort, OR = 3.75, 95% CI: 01.51, 9.41.

An analysis of the data comparing groups with (n = 69) and without placental abnormalities (n = 31) showed birthweights to be lower in the placental abnormality cohort, 2297.7 ± 234.7 versus 2452.3 ± 178.9 grams, $p = 0.002$. There was a higher rate of SGA newborns in the placental abnormality cohort, 42 (60.9%) versus 12 (38.7%), $p = 0.040$. There were no differences in newborn morbidities (data not shown).

Table 2: Abnormal versus normal Doppler newborn comparisons in suspected FGR.

Variable*	Abnormal Doppler $N = 46$	Normal Doppler $N = 54$	OR (95% CI)	p value
Birthweight (g)	2268.5 ± 246.0	2411.2 ± 193.7		0.002
SGA	32 (69.6)	22 (49.7)	3.33 (1.34, 8.34)	0.004
Ponderal index (g/cm^3)	2.37 ± 0.33	2.43 ± 0.33		0.379
Ponderal index < 10th percentile	16 (34.8)	12 (22.2)	1.87 (0.71, 4.96)	0.163
Five-minute Apgar < 7	1 (2.17)	1 (1.9)	0.35 (0.22, 0.44)	1.000
Hospital stay (days)	3 (2–16)	3 (2–21)		0.578
NICU admission	9 (19.6)	11 (20.4)	0.95 (0.32, 2.82)	0.920
Hyperbilirubinemia phototherapy	9 (19.6)	9 (16.7)	0.95 (1.22, 3.78)	0.707
Hypoglycemia	6 (13.0)	3 (5.6)	2.55 (0.52, 13.87)	0.295
Hypothermia	5 (10.9)	7 (13.0)	0.82 (0.21, 3.18)	0.748
Oxygen requirement	4 (8.7)	5 (9.3)	0.93 (1.94, 4.37)	1.000
Composite neonatal morbidity	16 (34.8)	16 (29.6)	1.27 (0.50, 3.20)	0.582
Placental weight (g)	347.4 ± 73.4	361.6 ± 83.0		0.372
Birth/placental weight ratio	6.72 ± 1.17	6.94 ± 1.39		0.400
Umbilical cord diameter (cm)	1.20 ± 0.35	1.19 ± 0.24		0.892
Placental weight < 5th percentile	22 (47.8)	18 (33.3)	1.83 (0.6, 4.47)	0.140

OR = odds ratio, CI = confidence interval, and FGR = fetal growth restriction.
*Results expressed in mean ± SD, number (%), or median (min–max).

Table 3: Prevalence of placental pathology: abnormal versus normal Doppler in *suspected FGR*.

Doppler type	Abnormal Doppler Placental pathology*		Normal Doppler Placental pathology		OR (95% CI)	p value
	Yes	No	Yes	No		
Any	39 (84.8)	7 (15.2)	30 (55.6)	24 (44.4)	4.46 (1.55, 13.22)	0.002
Umbilical	12 (75)	3 (25)	57 (67.1)	28 (32.9)	1.42 (0.38, 5.81)	0.770
MCA	25 (96.2)	1 (3.8)	23 (54.8)	19 (45.2)	20.7 (2.54, 447.1)	<0.001
Uterine	16 (88.9)	2 (11.1)	13 (61.9)	8 (38.1)	4.9 (0.74, 40.90)	0.074

OR = odds ratio, CI = confidence interval, MCA = middle cerebral artery, and FGR = fetal growth restriction.
*Results expressed in number (%).

Table 4: Performance of Doppler in prediction of placental pathology in suspected FGR.

Doppler	Sensitivity	Specificity	PPV	NPV
Any	55.1 (42.6, 67.1)	77.4 (58.9, 90.4)	84.5 (70.5, 93.5)	43.6 (30.3, 57.7)
Umbilical	17.4 (9.3, 28.4)	87.1 (70.2, 96.3)	75.0 (47.6, 92.7)	32.1 (22.4, 43.2)
MCA	52.1 (37.2, 66.7)	95.0 (75.1, 99.9)	96.2 (80.4, 99.9)	45.2 (29.9, 61.3)
Uterine	55.2 (35.7, 73.6)	80.0 (44.4, 97.5)	88.9 (65.3, 98.6)	38.1 (18.1, 61.6)

PPV = positive predictive value, NPV = negative predictive value, MCA = middle cerebral artery, and FGR = fetal growth restriction.
*Results expressed in % (95% confidence interval).

To study the issue whether the MFM specialists may have been biased in selection of subjects for MCA Doppler, we analyzed cohorts for baseline characteristics and outcomes according to whether or not MCA Doppler was performed and also whether or not uterine artery Doppler was performed. These analyses are given in Tables 6, 7, 8, and 9. We found that those subjects who had MCA Doppler had no difference in their antenatal characteristics in comparison to those who did not with the exception of a lower probability of vaginal delivery, the reason for which is unclear but may be random given the number of variables analyzed. Specific ultrasound parameters, the estimated fetal weight, the proportion with EFW < third percentile, and HC/AC > 95th percentile did not differ significantly between these two groups. No differences were observed between the groups that had or did not have uterine artery Doppler. We also analyzed the data including only those subjects that had both umbilical and MCA Doppler data, $n = 68$, and found no differences in baseline characteristics and outcomes and while the overall OR of any abnormal Doppler having placental pathology increased, the results were not statistically significant from when all subjects $n = 100$ were included in the analysis. The results of

Table 5: Occurrence of individual placental abnormality by Doppler cohort in suspected FGR.

	Abnormal Doppler $N = 46$	Normal Doppler $N = 54$
Placental weight < 5th percentile	22	18
Placental configuration abnormality*	4	8
Cord problem**	12	10
Infarcts	15	6
Abruption	1	0
Intervillous thrombus > 5%	1	0
Increased pervillous fibrin	7	4
Subchorionic thrombus excessive	2	1
Increased syncytial knots	1	3
Villous agglutination	3	2
Distal villous hypoplasia	0	1
Decidual atherosis	2	0
Hypertrophy membrane arterioles	0	1
Muscularization of basal plate arteries	2	0
Avascular terminal villi	0	1
Large villus intimal fibrin cushion	0	1
Chorangiosis	1	1
VUE	3	0

FGR = fetal growth restriction.
* includes succenturiate lobe, circummarginate, or circumvallate membrane insertion.
** includes thin cord, marginal or velamentous insertion, and single umbilical artery.

these analyses are given in Tables 10, 11, and 12. Logistic regression was performed on the data in Table 10 with variables included in the model: abnormal umbilical artery Doppler, abnormal MCA Doppler, Caucasian ethnicity, maternal age, maternal BMI, EFW on ultrasound, and EFW < third percentile. An abnormal MCA Doppler was the single variable that predicted the presence of placental pathology, adjusted OR = 45.9, 95% CI: 3.46, 609.6.

4. Discussion

Placental pathology was significantly more common in the group of suspected FGR infants delivered at 37 weeks who had an abnormal Doppler evaluation. There was, however, a high prevalence of placental pathology even in the normal Doppler cohort. This degree of pathology in the group with normal Doppler runs counter to the assumption that the fetus with suspected FGR and normal Doppler is the constitutionally small normal fetus. Our population of FGR fetuses would mainly be considered late-onset FGR, that is, >32 weeks [26]. One partial explanation for the high prevalence of placental disease in the normal Doppler cohort would be that even uncomplicated pregnancies have some histopathologic findings. Parra-Saavedra et al. [27] showed that 78% of late-onset SGA births with normal umbilical artery Doppler had histological placental abnormalities as did 22% of AGA births. McCowan et al. [28] found that abnormal umbilical artery Doppler reflected earlier and more severe growth restriction in small for gestational age fetuses but was not independently associated with newborn morbidity. They concluded that SGA newborns with normal umbilical artery Doppler were not simply constitutionally small normal infants.

We had few abnormal umbilical Doppler patterns in our study and it is well known that in late-onset FGR umbilical artery resistance is uncommonly elevated in this group and has limited sensitivity in detecting neonatal morbidity [29]. In our study umbilical artery Doppler had the lowest sensitivity of the three Doppler types utilized for the detection of placental pathology. It is unclear why the American College of Obstetricians and Gynecologists only recommends the use of umbilical artery Doppler in the evaluation of suspected FGR [1]. Umbilical artery Doppler, in combination with MCA Doppler, detects centralization of blood flow, also known as "brain-sparing," whereby the fetus increases the blood flow to the brain when there is hypoxia. The MCA Doppler, particularly the CPR, has been shown to have improved sensitivity over umbilical artery Doppler in detection of perinatal morbidity and mortality [29]. In addition, in fetuses with suspected FGR, there is an association between an abnormal MCA Doppler and poorer neurodevelopmental outcomes at 2 years of life [30]. An abnormal MCA Doppler in our study was strongly associated with the presence of placental pathology; these findings are in agreement with those of Parra-Saavedra et al. [12].

With respect to newborn outcomes, our study showed the abnormal Doppler group to be of lower birthweight and more likely to be classified as SGA. No difference in neonatal morbidity was noted but our study was not powered to detect differences in secondary outcomes. Overall, 32% of newborns experienced at least one morbidity and there was a 19% admission rate to the NICU. This rate of morbidity appears high and brings up questions regarding the ideal gestational age for delivery in late-onset FGR. The Disproportionate Intrauterine Growth Intervention Trial at Term showed lower neonatal intensive care unit admissions after 38 weeks in comparison to 36 to 37 weeks [31].

The strengths of our study were the uniform delivery gestational age of 37 weeks and an institutional guideline that recommends placental examination for all deliveries with suspected FGR. This allowed comparisons of placental pathology not confounded by gestational age or selection bias. The weaknesses were that this was a retrospective study, not all subjects were evaluated by uterine artery and MCA Doppler, and that the Doppler examinations did not occur at the same gestational age. The timing of the uterine artery Doppler evaluations prior to delivery with an average gestational age of 32.9 weeks, considerably shorter than the timing

Table 6: MCA Doppler versus no MCA Doppler antenatal comparisons in suspected FGR.

Variable*	MCA, yes $N = 68$	MCA, no $N = 32$	OR (95% CI)	p value
Maternal age	25.9 ± 6.1	27.9 ± 5.8		0.114
Maternal BMI	28.9 ± 6.3	27.2 ± 4.5		0.119
Caucasian	46 (67.6)	22 (68.8)	0.95 (0.35, 2.56)	0.912
Diabetes	5 (7.4)	2 (6.3)	1.19 (0.19, 9.47)	1.000
Hypertension	3 (4.4)	1 (3.1)	1.43 (0.12, 37.2)	1.000
Parity ≥ 1	36 (52.9)	16 (50.9)	1.13 (0.46, 2.83)	0.784
Smoking	25 (36.8)	7 (21.9)	2.08 (0.72, 6.18)	0.138
EFW ultrasound (grams)	2164.2 ± 196.5	2133.2 ± 412.0		0.689
EFW < 3rd percentile	18 (23.5)	9 (28.1)	1.27 (0.44, 3.64)	0.621
HC/AC > 95th percentile	23 (33.8)	13 (40.6)	1.34 (0.52, 3.46)	0.509
Induction of labor	54 (79.4)	27 (84.4)	1.40 (0.41, 5.01)	0.555
Vaginal delivery	42 (61.8)	27 (84.4)	3.34 (1.05, 11.35)	0.023

OR = odds ratio, CI = confidence interval, MCA = middle cerebral artery, and FGR = fetal growth restriction.
*Results expressed in mean ± SD or number (%).

Table 7: MCA Doppler versus no MCA Doppler newborn comparisons in suspected FGR.

Variable*	MCA, yes $N = 68$	MCA, no $N = 32$	OR (95% CI)	p value
Birthweight (g)	2340.6 ± 241.6	2356.3 ± 205.0		0.752
SGA	39 (57.4)	15 (46.9)	1.52 (0.60, 3.87)	0.327
Ponderal index (g/cm^3)	2.40 ± 0.33	2.41 ± 0.33		0.862
Ponderal index < 10th percentile	20 (29.4)	8 (25.0)	1.25 (0.44, 3.63)	0.647
Five-minute Apgar < 7	0 (0)	1 (3.1)		0.320
Hospital stay (days)	3 (2–16)	3 (2–21)		0.505
NICU admission	15 (22.1)	5 (15.6)	1.53 (0.45, 5.43)	0.453
Hyperbilirubinemia phototherapy	13 (19.1)	5 (27.2)	1.28 (0.37, 4.61)	0.672
Hypoglycemia	6 (8.8)	3 (9.4)	0.94 (0.19, 5.13)	1.000
Hypothermia	8 (11.8)	4 (12.5)	0.82 (0.21, 3.18)	1.000
Oxygen requirement	6 (8.8)	3 (9.4)	0.93 (0.23, 4.07)	1.000
Composite neonatal morbidity	21 (30.9)	11 (34.4)	0.85 (0.32, 2.29)	0.727
Placental weight (g)	363.1 ± 79.7	337.9 ± 74.6		0.135
Birth/placental weight ratio	6.64 ± 1.15	7.26 ± 1.50		0.024
Umbilical cord diameter (cm)	1.20 ± 0.30	1.20 ± 0.30		1.000
Placental weight < 5th percentile	26 (38.2)	14 (43.8)	0.80 (0.31, 2.04)	0.600

OR = odds ratio, CI = confidence interval, MCA = middle cerebral artery, and FGR = fetal growth restriction.
*Results expressed in mean ± SD, number (%), or median (min–max).

of the umbilical and MCA Doppler, may have been a factor in its underperformance in prediction of placental pathology.

The impact of the missing MCA Doppler data on the primary outcome variable of prevalence of placental pathology is addressed further. Had this data been present it may have strengthened the association of abnormal Doppler overall with the presence of placental pathology but could have resulted in no change or even weakened the association. We had 32 cases that did not have an MCA Doppler; of these 5 already had an abnormal umbilical artery Doppler and 1 had an abnormal uterine artery Doppler leaving 26 cases of normal umbilical artery Doppler with no MCA Doppler. From our data we know that in this group of subjects about 1/3 with a normal umbilical artery will have an abnormal MCA Doppler, so this would give an additional 9 subjects in the abnormal Doppler group. The final numbers in the abnormal Doppler group would become $n = 55$ and $n = 45$ in the normal Doppler group. Assuming all 9 subjects with an abnormal MCA Doppler would have placental pathology, a recalculation of the odds of an abnormal Doppler having placental pathology in comparison to a normal Doppler does not substantially change the results: 48/55 (87.2%) versus 30/45 (66.7%), OR = 3.43, 95% CI: 1.44, 10.63. The data from Table 12, when only the subjects with both umbilical and MCA Doppler ($n = 68$) are analyzed, appear to be in agreement with these calculations.

Table 8: Uterine artery Doppler versus no uterine artery Doppler antenatal comparisons in suspected FGR.

Variable*	Uterine artery Yes $N = 39$	Uterine artery No $N = 61$	OR (95% CI)	p value
Maternal age	25.2 ± 5.8	27.4 ± 6.1		0.079
Maternal BMI	28.5 ± 5.6	28.3 ± 5.0		0.844
Caucasian	22 (43.6)	22 (75.5)	0.42 (0.16, 1.09)	0.052
Diabetes	4 (10.3)	3 (4.9)	2.21 (0.39, 13.4)	0.427
Hypertension	2 (5.1)	2 (3.3)	1.60 (0.15, 16.8)	0.642
Parity ≥ 1	24 (61.5)	28 (45.9)	1.89 (0.77, 4.65)	0.127
Smoking	16 (41.0)	16 (26.2)	1.96 (0.76, 5.04)	0.122
EFW ultrasound (grams)	2122.0 ± 203	2174 ± 322		0.362
EFW < 3rd percentile	11 (28.2)	14 (23.0)	1.32 (0.48, 3.62)	0.554
HC/AC > 95th percentile	11 (28.2)	25 (41.0)	0.57 (0.22, 1.46)	0.194
Induction of labor	30 (76.9)	51 (83.6)	0.65 (0.21, 2.0)	0.406
Vaginal delivery	24 (61.5)	45 (73.8)	0.57 (0.22, 1.47)	0.197

OR = odds ratio, CI = confidence interval, and FGR = fetal growth restriction.
*Results expressed in mean ± SD or number (%).

Table 9: Uterine artery Doppler versus no uterine artery Doppler newborn comparisons in suspected FGR.

Variable*	Uterine artery Yes $N = 39$	Uterine artery No $N = 61$	OR (95% CI)	p value
Birthweight (g)	2338.4 ± 263.0	2350.3 ± 207.6		0.804
SGA	39 (57.4)	15 (46.9)	1.52 (0.60, 3.87)	0.327
Ponderal index (g/cm^3)	2.42 ± 0.35	2.39 ± 0.31		0.658
Ponderal index < 10th percentile	12 (30.8)	16 (26.2)	1.25 (0.47, 3.32)	0.622
Five-minute Apgar < 7	0 (0)	1 (3.1)		0.320
Hospital stay (days)	3 (2–16)	3 (2–21)		0.496
NICU admission	9 (23.1)	11 (18.0)	1.36 (0.45, 4.08)	0.539
Hyperbilirubinemia phototherapy	5 (12.8)	13 (21.3)	0.54 (0.15, 1.85)	0.281
Hypoglycemia	3 (3.5)	6 (9.8)	0.76 (0.14, 3.76)	1.000
Hypothermia	3 (11.8)	9 (14.8)	0.48 (0.10, 2.14)	0.358
Oxygen requirement	5 (12.8)	4 (6.6)	2.10 (0.45, 10.14)	0.306
Composite neonatal morbidity	14 (35.9)	21 (34.4)	1.07 (0.42, 2.69)	0.880
Placental weight (g)	355.4 ± 73.8	354.8 ± 82.1		0.972
Birth/placental weight ratio	6.75 ± 1.19	6.89 ± 1.37		0.594
Umbilical cord diameter (cm)	1.24 ± 0.32	1.18 ± 0.28		0.309
Placental weight < 5th percentile	17 (43.6)	23 (37.7)	1.23 (0.52, 3.14)	0.558

OR = odds ratio, CI = confidence interval, and FGR = fetal growth restriction.
*Results expressed in mean ± SD, number (%), or median (min–max).

In suspected FGR, the presence of placental pathology could be seen as validating a placental cause for the FGR. If we considered the presence of placental pathology as representing "true" or "pathologic" FGR and the absence of placental pathology representing the constitutionally small normal fetus, our study would indicate that the latter population of fetuses (31%) actually represents a minority of suspected FGR delivered at 37 weeks. MCA Doppler had high specificity and positive predictive value for placental disease and theoretically "true" growth restriction; however it had many false negatives and consequently low negative predictive value. Ideally, one would want to exclude the constitutionally small normal fetus, so that this group could be managed with less surveillance and without mandated early term delivery; our study suggests that this separation cannot be accomplished with Doppler; thus all cases with suspected FGR would have to be managed similarly. Having an agreed-upon postnatal reference standard as to what constitutes

Table 10: Abnormal versus normal Doppler antenatal comparisons in suspected FGR data for $n = 68$ subjects with both umbilical artery and MCA Doppler evaluation.

Variable*	Abnormal Doppler $N = 41$	Normal Doppler $N = 27$	OR (95% CI)	p value
Maternal age	25.1.2 ± 5.8	27.0 ± 6.4		0.065
Maternal BMI	29.8 ± 6.8	27.6 ± 5.2		0.065
Caucasian	24 (58.5)	22 (81.5)	0.32 (0.09, 1.14)	0.065
Diabetes	4 (9.8)	1 (3.7)	2.81 (0.27, 70.0)	0.641
Hypertension	3 (7.3)	0		0.271
Parity ≥ 1	19 (46.3)	17 (63)	0.51 (0.17, 1.53)	0.179
Smoking	15 (36.6)	10 (37.0)	0.98 (0.32, 3.03)	0.970
EFW ultrasound (grams)	2120.3 ± 198.9	2230.7 ± 176.1		0.022
EFW < 3rd percentile	13 (31.7)	3 (11.1)	3.71 (0.84, 18.76)	0.079
HC/AC > 95th percentile	15 (36.6)	8 (29.6)	1.37 (0.43, 4.43)	0.553
Induction of labor	34 (82.9)	20 (74.1)	1.70 (0.45, 6.78)	0.337
Vaginal delivery	33 (71.7)	41 (75.9)	0.81 (0.30, 2.16)	0.634
Cesarean for nonreassuring fetal status	5 (12.9)	0		0.144
Vacuum/forceps vaginal delivery	1 (3.6)	0		1.00

OR = odds ratio, CI = confidence interval, and FGR = fetal growth restriction.
*Results expressed in mean ± SD or number (%).

Table 11: Abnormal versus normal Doppler newborn comparisons in suspected FGR data for $n = 68$ subjects with both umbilical artery and MCA Doppler evaluation.

Variable*	Abnormal Doppler $N = 41$	Normal Doppler $N = 27$	OR (95% CI)	p value
Birthweight (g)	2269.0 ± 253.3	2449.2 ± 177.0		0.019
SGA	14 (34.1)	6 (27)	3.13 (1.02, 9.83)	0.025
Ponderal index (g/cm^3)	2.37 ± 0.33	2.43 ± 0.33		0.379
Ponderal index < 10th percentile	14 (34.1)	6 (22.2)	1.82 (0.53, 6,4)	0.291
Five-minute Apgar < 7	0	0		
Hospital stay (days)	3 (2–16)	3 (2–12)		0.570
NICU admission	9 (22.0)	6 (22.2)	0.98 (0.27, 3.71)	0.979
Hyperbilirubinemia phototherapy	9 (22.0)	4 (14.8)	1.62 (0.39, 7.2)	0.464
Hypoglycemia	6 (14.6)	0		0.074
Hypothermia	5 (12.2)	3 (11.1)	0.82 (0.20, 6.58)	1.000
Oxygen requirement	4 (9.8)	2 (7.4)	1.35 (0.19, 11.63)	1.000
Composite neonatal morbidity	16 (39)	5 (18.5)	2.82 (0.79, 10.58)	0.073
Placental weight (g)	353.0 ± 74.4	378.4 ± 88.2		0.210
Birth/placental weight ratio	6.60 ± 1.09	6.70 ± 1.26		0.737
Umbilical cord diameter (cm)	1.20 ± 0.35	1.19 ± 0.21		0.867
Placental weight < 5th percentile	19 (46.3)	7 (25.9)	2.47 (0.77, 8.17)	0.090

OR = odds ratio, CI = confidence interval, and FGR = fetal growth restriction.
*Results expressed in mean ± SD, number (%), or median (min–max).

"true" or "pathologic" fetal growth restriction may allow antenatal separation of the constitutionally small normal fetus from the truly growth restricted fetus in the future.

5. Conclusion

Abnormal Doppler ultrasound was significantly associated with the presence of placental pathology in this group of singleton pregnancies delivered at 37 weeks' gestation for suspected FGR. Of the three Doppler types evaluated, an abnormal MCA Doppler had the strongest association with the presence of placental pathology. This study provides further evidence and support for the use of MCA Doppler in the evaluation of suspected FGR and underscores the limitation of umbilical artery Doppler alone in FGR at later gestational ages. Further investigation and tools for separating the constitutionally small normal fetus from the FGR fetus are needed.

Table 12: Prevalence of placental pathology: abnormal versus normal Doppler in suspected FGR for $n = 68$ subjects with umbilical and MCA Doppler evaluations.

Doppler type	Abnormal Doppler Placental pathology*		Normal Doppler Placental pathology		OR (95% CI)	p value
	Yes	No	Yes	No		
Any	35 (85.4)	6 (14.6)	13 (48.1)	14 (51.9)	6.28 (1.75, 23.5)	0.001
Umbilical	9 (75.0)	3 (25.0)	39 (69.6)	17 (30.4)	1.31 (0.27, 7.02)	1.000
MCA	25 (96.2)	1 (3.8)	23 (54.8)	19 (45.2)	20.7 (2.54, 447.1)	<0.001
Uterine	15 (88.2)	2 (11.8)	13 (61.9)	8 (38.1)	4.62 (0.69, 38.6)	0.136

OR = odds ratio, CI = confidence interval, MCA = middle cerebral artery, and FGR = fetal growth restriction
*Results expressed in number (%).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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