

## Research Article

# I/D Polymorphism Gene ACE and Risk of Preeclampsia in Women with Gestational Diabetes Mellitus

O. P. Dmitrenko <sup>1</sup>, N. S. Karpova <sup>1</sup>, M. K. Nurbekov <sup>1</sup> and O. V. Papysheva<sup>2</sup>

<sup>1</sup>Federal State Budgetary Institution “Research Institute of Pathology and Pathophysiology”, 125315 Moscow, Russia

<sup>2</sup>City Clinical Hospital № 29 named after N.E. Bauman, 123001 Moscow, Russia

Correspondence should be addressed to N. S. Karpova; nataliakarpova.sp@gmail.com

Received 19 August 2020; Revised 27 October 2020; Accepted 13 November 2020; Published 29 December 2020

Academic Editor: Małgorzata Knaś

Copyright © 2020 O. P. Dmitrenko et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Preeclampsia (PE) and gestational diabetes mellitus (GDM) are the most common complications of pregnancy, which result in adverse outcomes for the mother and the fetus. GDM is regarded as a separate independent risk factor for PE development, as evidenced by a higher preeclampsia rate in gestational diabetes mellitus than in the general population. The role the endothelial cell dysfunction plays is considered to be the most reasonable one in the origin of these diseases. The activity of plasma and tissue angiotensin converting enzyme (ACE) is believed to be genetically controlled. The available data suggests that increased ACE activity due to deletion (D)/insertion (I) in the 16th intron of ACE gene, which is called ACE gene I/D polymorphism, is associated with preeclampsia and varies depending on the studied population and the geography. We did not find any literature data that estimates the influence of ACE gene I/D polymorphism on PE rate in pregnant women with GDM. Therefore, the present study aimed to investigate a relationship between ACE gene I/D polymorphism and preeclampsia development in the case of GDM in the Russian population. The study used the genomic DNA derived by phenol-chloroform extraction method from venous blood samples in 137 pregnant women, including samples of 74 women with GDM accompanied with PE and the blood samples of 63 women with GDM w/o preeclampsia. Genotyping of insertion/deletion in the I/D region (16 intron of ACE gene) was conducted by real-time PCR using the TaqMan competing probe technology. The particular features in the frequency array of alleles and genotypes of the ACE gene I/D polymorphism under review, as associated with preeclampsia development risk in pregnant women with GDM, were identified. The acquired data testify to the need to further study of ACE gene I/D region polymorphism association in a large patient sample taking into account the PE and GDM risk factors estimated in the clinical practice.

## 1. Introduction

Preeclampsia (PE) is a multi-system disorder that arises after gestation week 20 and is described by arterial hypertension, combined with proteinuria ( $\geq 0.3$  g/l in daily urine), oedema and multi-organ dysfunction [1–3]. PE develops in 3%–8% pregnant women and is among the Top 5 reasons of maternal morbidity and mortality [4–8]. Data derived in different years points to a much greater preeclampsia rate in the case of gestational diabetes mellitus (7.3%) vs general in the population (4.5%) [9–12]. GDM is diabetes that is first diagnosed in the second or third trimester of pregnancy that is not clearly either preexisting type 1 or type 2 diabetes [13]. According to the International Diabetes Federation (IDF),

gestation diabetes mellitus prevalence in 2019 decreased slightly, to 12.8%, varying depending on the population parameters, screening methods and diagnostic criteria: from 1% to 28% [14–17]. In Russia, GDM is a pregnancy complication in 2%–4% cases [18].

Even though the preeclampsia clinical manifestations and diagnostic criteria do not overlap with those of GDM, certain studies suggested that the same predisposing factors are typical of PE and GDM (mother’s age of over 35 y.o., nulliparity, multiple pregnancy and increased pre-gestation BMI); however, GDM is a separate and independent risk factor of PE development [19–22].

Just as with GDM and with preeclampsia, the main pathophysiological criterion is the vascular endothelial

dysfunction [23–26]. The renin-angiotensin-aldosterone system (RAS) is the main vascular tone regulator activation of which results in the blood pressure increase due to the growth in circulating blood and increase in activity of other vasoconstricting factors. The key role in the functioning of RAS belongs to the angiotensin-converting enzyme (ACE), which catalyzes the cleavage of angiotensin I Decapeptide to angiotensin II octapeptide, by releasing the terminal His-Leu, which improves the angiotensin vasoconstrictor activity. [27–29]. ACE has a significant effect on the production of angiotensin II not only in the circulating blood, but also on the synthesis and interaction of components of tissue RAS, including in the beta-cells of the Langerhans islets and the placenta [30–32]. Local RAS in the pancreas and the placenta are believed to be involved in physiological and pathophysiological processes in pregnancy [33].

The ACE gene is located on locus 17q23.3, in the DNA plus chain, which consists of 26 exons and 25 introns, and encodes an angiotensin-converting enzyme [34, 35]. The insertion/deletion (I/D) polymorphism in the 16th intron of ACE gene, which is described by presence or absence of the 264bps Alu-repeats with a 15bps poly-A-tail, as a partial explanation of inter-individual differences in ACE blood serum levels: the maximum one is detected in D/D genotype individuals, and twice as low level, in I/I type individuals [35–37].

I/D polymorphism of ACE gene is associated with ACE blood level and determines the onset of such diseases as coronary heart disease, left ventricular hypertrophy, arterial hypertension, stroke, diabetic nephropathy, type 2 diabetes mellitus, diabetic nephropathy, diabetic neuropathy, diabetic retinopathy and hypertensive retinopathy, according to different research [35, 38–46]. ACE gene D-alleles are believed to be a risk factor in these diseases.

The results of association studies of I/D polymorphism in the case of PE and GDM are inconsistent [47–58]. Some investigators reported that women residing in different regions had an association between ACE gene D-allele or DD genotype and a high preeclampsia risk [51–56], but others noted there was no such association with PE risk [57–58].

We did not find in the literature any data that estimates the influence of ACE gene I/D polymorphism on PE rate in pregnant women with GDM. Therefore, this study was aimed at investigating the relationship between I/D polymorphism of ACE gene and preeclampsia in the case of GDM.

## 2. Materials and Methods

The study involved DNA samples extracted from the venous blood of 137 GDM pregnant women, including those of 74 pregnant women with combined GDM and PE and those of 63 pregnant women with GDM not combined with preeclampsia, who were followed up and gave birth in 2018/2019 in the Maternity Department of the State Clinical Hospital No. 29 (N.E. Bauman Hospital) of the Healthcare Department of Moscow. All respondents were Russian speakers of indefinite ethnicity (because of the ethical standards of the local medical register) and enrolled in the study voluntarily. The study was approved by the Ethical Commit-

tee of the Research and Development Institute of General Pathology and Pathophysiology.

GDM and PE diagnosis were the study inclusion criteria. The absence of acute and chronic diseases at the exacerbation stage was the exclusion criterion. Pregnant women with autoimmune, nervous and mental diseases and with cancer of any localization were also excluded from the study.

GDM diagnosis was established based on the criteria of the Russian National Consensus and clinical guidelines “Gestational Diabetes Mellitus: Diagnosis, Treatment, Postpartum Follow-Up” [59].

Preeclampsia was diagnosed based on the clinical guidelines “Hypertensive Disorders in Pregnancy, Labor and Post-Partum. Pre-eclampsia. Eclampsia” [1].

DNA was extracted from venous blood with the standard phenol-chloroform extraction method of Maniatis et al. [60]. The blood cell element lysis was conducted with the Kunkel method [61]. The high-molecular DNA was desiccated at the ambient temperature and dissolved in TE buffer, and the resulting DNA was stored at -20°C. All DNA extractions were performed by the single investigator only.

The extracted DNA quality and quantity was estimated by the 260/280 wavelength ratio when measuring DNA concentration in the NanoDrop 1000 spectrophotometer, in the two-chain DNA analysis mode, dsDNA-50. Defective samples were not included into further analysis, just as those with low DNA concentration.

The deletion/insertion in the I/D area (ACE gen 16th intron) was studied by real-time PCR (RT-PCR, q-PCR) using the primers we designed. The set of primers includes 2 forward primers: ACE-I/D-F1: 5'-GGAGACCACTCCCA TCCTTTC-3' and ACE-I/D-F2: 5'-GCCTCAGCCTCCCA AG-3', and 1 reverse primer ACE-I/D-R1: 5'-ATGTGG CCATCACATTCGTCAGAT-3'. The primers were designed so that the ACE-I/D-F1 forward primer and the ACE-I/D-R1 reverse primer amplify the I/D area (ACE gen 16th intron) with insertion, while ACE-I/D-F2 and ACE-I/D-R1 primers, the I/D area (ACE gen 16th intron) with deletion. The ACE-I/D-R1 forward primer is annealed in the insertion area and creates a specific PCR product while the ACE-I/D-F2 primer, in a specific site that is formed in case of the Alu-repeat deletion only. To detect the amplification in real-time with subsequent analysis by the allele discrimination method, the specific Taq-man probes are used: ACE-I: 5'-HEX-CCCGCCACTACGCCGGCTAA-3'BHQ1 to detect an insertion and ACE-D: 5'-FAM-GCTGCCTATACAGTCA CTTTTATGTGGT-3'BHQ1 to detect a deletion. All primers and Taq-man probes were synthetically produced by Syntol LLC, Russia.

The reaction mixture for RT-PCR for one 15 µl sample contained 20 ng DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3 mM MgCl<sub>2</sub>, 0.125 mM dNTP, 0,1 µM ACE-I/D-F1, 0,2 µM ACE-I/D-F2, 0,2 µM ACE-I/D-R1, 0,2 µM ACE-I, 0,2 µM ACE-D, 0,4 µM each of Taq-man probes, 0.25 units of act. TaqDNA-polymerase.

Amplification was carried out in the CFX 96 programmable amplifier (Bio-Rad, U.S.A.) with the subsequent thermocycling parameters: initial denaturation for 5 minutes at 95°C; then 40 cycles including denaturation at 95°C for 20

seconds, at 60°C for 30 seconds and at 25°C for 10 minutes; primer annealing and subsequent elongation at 72°C for 10 minutes, with subsequent fluorescence pickup. The obtained data was examined using the CFX Manager TM software (Bio-Rad).

The results were statistically processed using the Hardy-Weinberg equilibrium tests and the chi2 association test in DeFinetti application (freely available on the website of the Institute of Human Genetics (Germany), <http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>). The trend analysis was carried out using the general, dominant and recessive inheritance models. Differences were regarded as significant at  $p \leq 0.05$ . The association force for the tested attributes was determined by the odds ratio (OR) with the confidence interval (CI) at 95% significance level. The anticipated risk factor was regarded as significant for pathology if OR adjusted by CI was greater than 1.

### 3. Results

The insertion/deletion test in the I/D area (ACE gen 16th intron) enabled to assess the allele frequency of the polymorphic loci genotypes in the studied gen. The distribution of genotype and allele frequencies in the I/D polymorphic locus of the ACE gen matched the one expected in the Hardy-Weinberg equilibrium, both for the pre-eclampsia group and for the control (no preeclampsia) group (Table 1).

The investigation into the distribution of genotype and allele frequencies in the study groups revealed that the D allele frequency came to 48.6% in the group of GDM PE+ pregnant women vs 37.3% in the group of GDM PE-. The frequency of DD homozygous genotype in the GDM PE+ group is higher than that in the GDM PE- group (24.3% vs 11.1%). The association analysis revealed the association between ACE gene I/D polymorphism and preeclampsia (Table 2). So, DD homozygous type in the general inheritance model is a genetic factor predisposing to this pregnancy complication in GDM women, increasing its risk by 2,96 ( $p=0,041$ ).

### 4. Discussion

Preeclampsia and GDM are the most common pregnancy complications that result in an adverse outcome for the mother and the fetus. Despite numerous data on the reasons for these complications in the literature, the key drivers are still unknown; however, the role the endothelial cell dysfunction plays is considered to be the most probable cause of GDM and preeclampsia onset.

The research and clinical studies suggest that an array of genetic factors are involved in PE and GDM pathogenesis. So far, 100 polymorphic gene allelic variants, in particular, those regulating the endothelium, vascular system etc. functions, have been found to be associated with pre-eclampsia. Special focus was on ACE gen, one of the renin-angiotensin system encoding elements, because the Alu-repeat deletion of I/D polymorphism in ACE gen 16th intron leads to increased expression of the gen, which explains the inter-individual differences in angiotensin-converting-enzyme (ACE) blood

TABLE 1: Distribution of alleles and genotypes of ACE gene I/D polymorphism in GDM pregnant women in PE+ and PE- groups.

Genotypes and alleles	PE+, n=74	PE-, n=63
II	20 (27.0%)	23 (36.5%)
ID	36 (48.7%)	33 (52.4%)
DD	18 (24.3%)	7 (11.1%)
I	76 (51.4%)	79 (62.7%)
D	72 (48.6%)	47 (37.3%)

II: insertion genotype; ID: insertion/deletion genotype; DD: deletion genotype; I: insertion allele; D: deletion allele. For example, in the PE+ group II indicates 20 (27%) is the genotype number, and in the parentheses, it indicates the percentage of the alleles frequency.

levels. The available data suggests that persons with D/D genotype have the maximum ACE blood level and those with I/I genotype, twice as low level [55, 62].

The available reference data on the study of the ACE gen I/D polymorphism association with preeclampsia is inconsistent. Our study findings conform to some previous studies that pointed to the high frequency of DD genotype and/or D allele in preeclampsia. For instance, in the Asian region, ACE gene I/D polymorphism is associated with PE development risk in the Korean and Chinese populations [55, 63, 64]. Gürdöl F. et al. (2004), Bereketoglu C. et al. (2012) reported high frequency of D allele in the Turkish population, Salimi S. et al. (2011), in the Iranian population [53, 56, 65]. According to several studies, high frequency of D allele is associated with the pre-eclampsia risk in the European population. For example, Mišković et al. (2008) found a significant association between D allele frequency and preeclampsia recurrence risk and preterm delivery before gestation week 34 [66]. The study of Mandò C et al. (2009) comprising 672 women, including 204 with pre-eclampsia pregnancy complication and 56 with gestation diabetes mellitus, suggested that D allele was much more common among women suffering from mild preeclampsia than it was in the control group [67]. Velloso et al. (2007) suggested that the DD genotype can be used as the marker of predisposition to preeclampsia in pregnant women in the Brazilian population [68].

On the other hand, some studies did not find any differences in the allocation of allele genotypes and frequencies and the association between DD genotype and preeclampsia [48, 58, 69–74]. In the association analysis, Radkov et al. (2012) established that the D heterozygous genotype and D allele were the genetic factors predisposing to preeclampsia in the Russian population residing in Central Russia, increasing the risk by the factor of 1.96 and 1.45, respectively [75]. When studying more than 1,500 polymorphisms (SNP) using high-density microchips, Glotov et al. (2014) identified several SNPs associated with the preeclampsia risk. These polymorphous sites enabled to identify 31 genes, including ACE, that can influence the disorder development. The further comparative analysis between Russian and Central European study groups did not identify any statistically significant differences in ACE gene allele and genotype frequencies [76].

The differences in the data derived from different studies on the association between ACE gene I/D polymorphism and

TABLE 2: Association of ACE gene I/D polymorphism genotypes with PE in GDM pregnant women.

Model of inheritance	Genotypes	PE+, n =74	PE-, n =63	OR (95% of CI)	chi2	P
Codominant	II	20	23	0,338 (0,117-0,975)	4,17	0,041*
	ID	36	33	1,255 (0,585-2,691)	0,34	0,559
	DD	18	7	2,957 (1,026-8,526)	4,17	0,041*
Dominant	II/ID + DD	20/54	23/40	1,552 (0,752-3,207)	1,42	0,233
Recessive	II + ID/DD	56/18	56/7	0,389 (0,151-1,004)	3,98	0,046*

Control (PE-) versus PE+; OR: odds ratio; CI: confidence interval; chi2: chi-square distribution; p: p value definition. \*p ≤ 0.05.

preeclampsia are likely to be due to the size, population features and the ethnicity of the study groups and also depend on the analytical inheritance model selection. The meta-analysis conducted by Medica et al. (2006) on 10 studies with participation of 1,121 patients and 1,361 control group participants demonstrated the statistical significance in consideration of the I/D polymorphism of ACE gene 16th intron in the recessive model: the odds ratio came to 1.51 (95% CI: 1.17-1.94) [77]. Bereketoglu C. et al. (2012) showed the connection between this polymorphism and pre-eclampsia in the dominant model [56].

The principal goal of this study was to investigate into the relationship between I/D polymorphism of ACE gene and the preeclampsia risk in pregnant women with gestational diabetes mellitus in the Russian population. Our study suggested that the frequency of the DD homozygous genotype of ACE gen varied between the groups, and D allele was more frequent in pre-eclampsia women than it was in PE- group. The relationship between this polymorphism and pre-eclampsia was found in the general inheritance model. We discovered a higher frequency of DD genotype in the preeclampsia group as compared with the control group. It is noteworthy that no studies of the association between ACE gene I/D polymorphism and preeclampsia development in GDM pregnant women have been conducted in the Russian population so far.

## 5. Conclusions

Our study revealed a statistically significant association between the DD homozygous type of ACE gene I/D polymorphism and preeclampsia in GDM women in the general inheritance model. A small size of the study groups is the main limitation of this study. Nonetheless, the data we received point to the need for further study of the association between ACE gene I/D polymorphism in a large patient sampling, with the parallel trial of polymorphism in other genes and taking into account PE and GDM risk factors estimated in the clinical practice (mother's age, BMI, glucose and glycated hemoglobin level etc.), which can also affect the development of these disorders. In the future, it will enable to use this genetic marker as the criterion in assessing the individual forecast of preeclampsia development in GDM pregnant women, which will enable to take efficient preventive efforts for timely correction and improvement of the pregnancy outcome.

## Abbreviations

PE: Preeclampsia  
 GDM: Gestational diabetes mellitus  
 IDF: International diabetes federation  
 BMI: Body mass index  
 RAS: The renin-angiotensin-aldosterone system  
 ACE: The angiotensin-converting enzyme.

## Data Availability

Genotyping data used to support the findings of this study is available upon request to the authors of the article.

## Conflicts of Interest

All authors declared no competing interests.

## Acknowledgments

The study was funded by budget subsidies for the implementation of a state task by the Institute of General Pathology and Pathophysiology (NIIOPP).

## References

- [1] G. T. Sukhikh, V. N. Serov, L. V. Adamyan et al., "Hypertensive Disorders during pregnancy, Childbirth, and the Postpartum period," in *Preeclampsia. Eclampsia*, Russian clinical guidelines, Moscow, 2016.
- [2] E. Eiland, C. Nzerue, and M. Faulkner, "Preeclampsia 2012," *Journal of Pregnancy*, vol. 2012, Article ID 586578, 7 pages, 2012.
- [3] American College of Obstetricians and Gynecologists, "Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy," *Obstetrics and Gynecology*, vol. 122, no. 5, pp. 1122–1131, 2013.
- [4] A. Khalil, P. O'Brien, and R. Townsend, "Current best practice in the management of hypertensive disorders in pregnancy," *Integrated Blood Pressure Control*, vol. 9, pp. 79–94, 2016.
- [5] E. A. P. Steegers, P. von Dadelszen, J. J. Duvekot, and R. Pijnenborg, "Pre-eclampsia," *The Lancet*, vol. 376, no. 9741, pp. 631–644, 2010.
- [6] B. Sibai, G. Dekker, and M. Kupferminc, "Pre-eclampsia," *The Lancet*, vol. 365, no. 9461, pp. 785–799, 2005.
- [7] WHO, *Recommendations for prevention and treatment of pre-eclampsia and eclampsia*, World Health Organization, Geneva, Switzerland, 2011.

- [8] L. Duley, "The global impact of pre-eclampsia and eclampsia," *Seminars in Perinatology*, vol. 33, no. 3, pp. 130–137, 2009.
- [9] V. L. Bilano, E. Ota, T. Ganchimeg, R. Mori, and J. P. Souza, "Risk factors of pre-eclampsia/eclampsia and its adverse outcomes in low- and middle-income countries: a WHO secondary analysis," *PLoS One*, vol. 9, no. 3, article e91198, 2014.
- [10] K. A. Nerenberg, J. A. Johnson, B. Leung et al., "Risks of gestational diabetes and preeclampsia over the last decade in a cohort of Alberta women," *Journal of Obstetrics and Gynaecology Canada*, vol. 35, no. 11, pp. 986–994, 2013.
- [11] HAPO Study Cooperative Research Group, "Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) study: associations with maternal body mass index," *BJOG*, vol. 117, no. 5, pp. 575–584, 2010.
- [12] I. Dedov, M. V. Shestakova, and I. Suntsov Yu, *Diabetes in Russia: problems and solutions*, Moscow, 2008.
- [13] American Diabetes Association, "2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018," *Diabetes Care*, vol. 41, Supplement 1, pp. S13–S27, 2018.
- [14] IDF, "Diabetes Atlas Ninth Edition," 2019, <https://www.idf.org/e-library/epidemiology-research/diabetes-atlas/159-idf-diabetes-atlas-ninth-edition-2019.html>.
- [15] A. T. Kharroubi and H. M. Darwish, "Diabetes mellitus: the epidemic of the century," *World Journal of Diabetes*, vol. 6, no. 6, pp. 850–867, 2015.
- [16] Y. Zhu and C. Zhang, "Prevalence of gestational diabetes and risk of progression to type 2 diabetes: a global perspective," *Current Diabetes Reports*, vol. 16, no. 1, 2016.
- [17] L. Yuen, V. W. Wong, and D. Simmons, "Ethnic Disparities in Gestational Diabetes," *Current Diabetes Reports*, vol. 18, no. 9, 2018.
- [18] V. A. Petrukhin and F. F. Burumkulova, "Gestation diabetes mellitus," *Archive of Obstetrics and Gynecology*, vol. 1, no. 1, pp. 48–51, 2014.
- [19] S. Schneider, N. Freerksen, S. Röhrig, B. Hoeft, and H. Maul, "Gestational diabetes and preeclampsia—similar risk factor profiles?," *Early Human Development*, vol. 88, no. 3, pp. 179–184, 2012.
- [20] T. L. Weissgerber and L. M. Mudd, "Preeclampsia and diabetes," *Current Diabetes Reports*, vol. 15, no. 3, p. 9, 2015.
- [21] I. Östlund, B. Haglund, and U. Hanson, "Gestational diabetes and preeclampsia," *European Journal of Obstetrics & Gynecology and Reproductive Biology*, vol. 113, no. 1, pp. 12–16, 2004.
- [22] J. Huet, G. Beucher, A. Rod, R. Morello, and M. Dreyfus, "Joint impact of gestational diabetes and obesity on perinatal outcomes," *Journal of Gynecology Obstetrics and Human Reproduction*, vol. 47, no. 9, pp. 469–476, 2018.
- [23] C. E. Powe, R. J. Levine, and S. A. Karumanchi, "Preeclampsia, a disease of the maternal endothelium: the role of antiangiogenic factors and implications for later cardiovascular disease," *Circulation*, vol. 123, no. 24, pp. 2856–2869, 2011.
- [24] S. M. Heitritter, C. G. Solomon, G. F. Mitchell, N. Skali-Ounis, and E. W. Seely, "Subclinical inflammation and vascular dysfunction in women with previous gestational diabetes mellitus," *The Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 7, pp. 3983–3988, 2005.
- [25] M. F. B. de Resende Guimarães, A. H. F. Brandão, C. A. de Lima Rezende et al., "Assessment of endothelial function in pregnant women with preeclampsia and gestational diabetes mellitus by flow-mediated dilation of brachial artery," *Archives of Gynecology and Obstetrics*, vol. 290, no. 3, pp. 441–447, 2014.
- [26] K. Nerenberg, S. S. Daskalopoulou, and K. Dasgupta, "Gestational diabetes and hypertensive disorders of pregnancy as vascular risk signals: an overview and grading of the evidence," *The Canadian Journal of Cardiology*, vol. 30, no. 7, pp. 765–773, 2014.
- [27] M. Ito, A. Itakura, Y. Ohno et al., "Possible activation of the renin-angiotensin system in the fetoplacental unit in preeclampsia," *The Journal of Clinical Endocrinology & Metabolism*, vol. 87, no. 4, pp. 1871–1878, 2002.
- [28] N. Vitoratos, D. Hassiakos, and C. Iavazzo, "Molecular mechanisms of preeclampsia," *Journal of Pregnancy*, vol. 2012, Article ID 298343, 5 pages, 2012.
- [29] "UniProtKB - P12821 (ACE\_HUMAN)," <https://www.uniprot.org/uniprot/P12821#function>.
- [30] M. Paul, A. Poyan Mehr, and R. Kreutz, "Physiology of local renin-angiotensin systems," *Physiological Reviews*, vol. 86, no. 3, pp. 747–803, 2006.
- [31] K. Y. Lam and P. S. Leung, "Regulation and expression of a renin-angiotensin system in human pancreas and pancreatic endocrine tumours," *European Journal of Endocrinology*, vol. 146, no. 4, pp. 567–572, 2002.
- [32] D. Herr, I. Bekes, and C. Wulff, "Local Renin-Angiotensin System in the Reproductive System," *Frontiers in Endocrinology*, vol. 4, 2013.
- [33] J. J. Spaan and M. A. Brown, "Renin-angiotensin system in pre-eclampsia: everything old is new again," *Obstetric Medicine: The Medicine of Pregnancy*, vol. 5, no. 4, pp. 147–153, 2012.
- [34] C. A. Hubert, P. Houot, P. Corvol, and F. Soubrier, "Structure of the angiotensin I-converting enzyme gene. Two alternate promoters correspond to evolutionary steps of a duplicated gene," *Journal of Biological Chemistry*, vol. 266, pp. 15377–15383, 1991.
- [35] R. Castellon and H. Hamdi, "Demystifying the ACE Polymorphism: From Genetics to Biology," *Current Pharmaceutical Design*, vol. 13, no. 12, pp. 1191–1198, 2007.
- [36] B. Rigat, C. Hubert, F. Alhenc-Gelas, F. Cambien, P. Corvol, and F. Soubrier, "An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels," *The Journal of Clinical Investigation*, vol. 86, no. 4, pp. 1343–1346, 1990.
- [37] H. K. Hamdi and R. Castellon, "A genetic variant of ACE increases cell survival: a new paradigm for biology and disease," *Biochemical and Biophysical Research Communications*, vol. 318, no. 1, pp. 187–191, 2004.
- [38] Z. Rahimi, M. Moradi, and H. Nasri, "A systematic review of the role of renin angiotensin aldosterone system genes in diabetes mellitus, diabetic retinopathy and diabetic neuropathy," *Journal of Research in Medical Sciences*, vol. 19, no. 11, pp. 1090–1098, 2014.
- [39] H. G. Mengesha, P. Petrucka, C. Spence, and T. B. Tafesse, "Effects of angiotensin converting enzyme gene polymorphism on hypertension in Africa: a meta-analysis and systematic review," *PLoS One*, vol. 14, no. 2, article e0211054, 2019.
- [40] Y. Yuan, L. Meng, Y. Zhou, and N. Lu, "Genetic polymorphism of angiotensin-converting enzyme and hypertrophic cardiomyopathy risk: a systematic review and meta-analysis," *Medicine (Baltimore)*, vol. 96, no. 48, article e8639, 2017.

- [41] S. Yadav, N. Hasan, T. Marjot et al., "Detailed analysis of gene polymorphisms associated with ischemic stroke in South Asians," *PLoS One*, vol. 8, no. 3, article e57305, 2013.
- [42] T. Chen, J. Ma, G. Shan, and Y. Zhong, "The polymorphisms of ATOH 7, ET-1 and ACE in non-arteritic anterior ischemic optic neuropathy," *Experimental Eye Research*, vol. 174, pp. 147–151, 2018.
- [43] H. Rasyid, S. Bakri, and I. Yusuf, "Angiotensin-converting enzyme gene polymorphisms, blood pressure and pulse pressure in subjects with essential hypertension in a South Sulawesi Indonesian population," *Acta Medica Indonesiana*, vol. 44, no. 4, pp. 280–283, 2012.
- [44] K. Mokretar, H. Velinov, A. Postadzhiyan, and M. Apostolova, "Association of polymorphisms in endothelial nitric oxide synthesis and renin-angiotensin-aldosterone system with developing of coronary artery disease in Bulgarian patients," *Genetic Testing and Molecular Biomarkers*, vol. 20, no. 2, pp. 67–73, 2016.
- [45] Z. Zhang, G. Xu, D. Liu, X. Fan, W. Zhu, and X. Liu, "Angiotensin-converting enzyme insertion/deletion polymorphism contributes to ischemic stroke risk: a meta-analysis of 50 case-control studies," *PLoS One*, vol. 7, no. 10, 2012.
- [46] Y. Tian, Z. Ge, Y. Xing, Y. Sun, and J. Ying, "Correlation of angiotensin I-converting enzyme gene insertion/deletion polymorphism with rheumatic heart disease: a meta-analysis," *Bio-science Reports*, vol. 36, no. 6, 2016.
- [47] Z. Dostálová, A. J. Bienertová-Vasků, A. Vasků, and R. Gerychová, "Insertion-deletion polymorphism in the gene for angiotensin-converting enzyme (I/D ACE) in pregnant women with gestational diabetes," *Ceská Gynekologie*, vol. 71, no. 5, pp. 369–373, 2006.
- [48] P. Aggarwal, N. Agarwal, N. Das, and K. Dalal, "Association of polymorphisms in angiotensin-converting enzyme gene with gestational diabetes mellitus in Indian women," *International Journal of Applied and Basic Medical Research*, vol. 6, no. 1, pp. 31–37, 2016.
- [49] M. Mirfeizi, M. Hasanzad, M. Sattari et al., "Association of eNOS and ACE gene polymorphisms as a genetic risk factor in gestational diabetes in Iranian women," *Journal of Diabetes & Metabolic Disorders*, vol. 17, no. 2, pp. 123–127, 2018.
- [50] I. A. Khan, P. Jahan, Q. Hasan, and P. Rao, "Angiotensin-converting enzyme gene insertion/deletion polymorphism studies in Asian Indian pregnant women biochemically identifies gestational diabetes mellitus," *Journal of the Renin-Angiotensin-Aldosterone System*, vol. 15, no. 4, pp. 566–571, 2013.
- [51] A. J. Buurma, R. J. Turner, J. H. M. Driessen et al., "Genetic variants in pre-eclampsia: a meta-analysis," *Human Reproduction Update*, vol. 19, no. 3, pp. 289–303, 2013.
- [52] G. Shaheen, S. Sajid, S. Razak et al., "Role of ACE I/D polymorphism in pathological assessment of preeclampsia in Pakistan," *Molecular Genetics & Genomic Medicine*, vol. 7, no. 7, e00799, 2009.
- [53] F. Gürdöl, E. İşbilen, H. Yılmaz, T. İsbir, and A. Dirican, "The association between preeclampsia and angiotensin-converting enzyme insertion/deletion polymorphism," *Clinica Chimica Acta*, vol. 341, no. 1–2, pp. 127–131, 2004.
- [54] G. Mello, E. Parretti, F. Gensini et al., "Maternal-fetal flow, negative events, and preeclampsia: role of ACE I/D polymorphism," *Hypertension*, vol. 41, no. 4, pp. 932–937, 2003.
- [55] H. Choi, J. Y. Kang, H. S. Yoon et al., "Association of angiotensin-converting enzyme and angiotensinogen gene polymorphisms with preeclampsia," *Journal of Korean Medical Science*, vol. 19, no. 2, pp. 253–257, 2004.
- [56] C. Bereketoğlu, M. Kasap, and A. Pazarbaşı, "Studies on angiotensin-converting enzyme insertion/deletion polymorphism and genotype distributions in Turkish preeclampsia patients," *Journal of Pregnancy*, vol. 2012, Article ID 108206, 4 pages, 2012.
- [57] L. Morgan, F. Foster, R. Hayman et al., "Angiotensin-converting enzyme insertion-deletion polymorphism in normotensive and pre-eclamptic pregnancies," *Journal of Hypertension*, vol. 17, no. 6, pp. 765–768, 1999.
- [58] A. O. Galão, L. H. de Souza, B. E. da Costa, R. M. Scheibe, and C. E. Poli de Figueiredo, "Angiotensin-converting enzyme gene polymorphism in preeclampsia and normal pregnancy," *American Journal of Obstetrics and Gynecology*, vol. 191, no. 3, pp. 821–824, 2004.
- [59] I. I. Dedov, V. I. Krasnopolskiy, and G. T. Sukhikh, "Russian national consensus statement on gestational diabetes: diagnostics, treatment and postnatal care," *Diabetes Mellitus*, vol. 15, no. 4, pp. 4–10, 2012.
- [60] T. Maniatis, Fritsch, and E. F. J. Sambrook, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1982.
- [61] L. M. Kunkel, K. D. Smith, S. H. Boyer et al., "Analysis of human Y-chromosome-specific reiterated DNA in chromosome variants," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 74, no. 3, pp. 1245–1249, 1977.
- [62] B. Rigat, C. Hubert, P. Corvol, and R. Soubrier, "PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1)," *Nucleic Acids Research*, vol. 20, no. 6, p. 1433, 1992.
- [63] N. Zhou, P. Yu, J. Chen, H. Huang, and S. Jiang, "Detection of insertion/deletion polymorphism of angiotensin converting enzyme gene in preeclampsia," *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*, vol. 16, no. 1, pp. 29–31, 1999.
- [64] M. Zhu, Y. Xia, and W. Cheng, "Study on a deletion polymorphism of the angiotensin converting enzyme gene in pregnancy induced hypertension," *Zhonghua Fu Chan Ke Za Zhi*, vol. 33, no. 2, pp. 83–85, 1998.
- [65] S. Salimi, M. Mokhtari, M. Yaghmaei, M. Jamshidi, and A. Naghavi, "Association of angiotensin-converting enzyme intron 16 insertion/deletion and angiotensin II type 1 receptor A1166C gene polymorphisms with preeclampsia in South East of Iran," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 941515, 6 pages, 2011.
- [66] B. Misković, J. Sertić, A. Stavljenić-Rukavina, and F. Stipoljev, "Association of angiotensin-converting enzyme insertion-deletion polymorphism with preeclampsia," *Collegium Antropologicum*, vol. 32, no. 2, pp. 339–343, 2008.
- [67] C. Mandò, P. Antonazzo, S. Tabano et al., "Angiotensin-converting enzyme and adducin-1 polymorphisms in women with preeclampsia and gestational hypertension," *Reproductive Sciences*, vol. 16, no. 9, pp. 819–826, 2009.
- [68] E. P. Velloso, R. Vieira, A. C. Cabral, E. Kalapothakis, and R. A. S. Santos, "Reduced plasma levels of angiotensin-(1-7) and renin activity in preeclamptic patients are associated with the angiotensin I-converting enzyme deletion/deletion genotype," *Brazilian Journal of Medical and Biological Research*, vol. 40, no. 4, pp. 583–590, 2007.

- [69] T. Tamura, G. L. Johanning, R. L. Goldenberg, K. E. Johnston, and D. B. MB, "Effect of a7ngiotensin-converting enzyme gene polymorphism on pregnancy outcome, enzyme activity, and zinc concentration," *Obstetrics and Gynecology*, vol. 88, no. 4, pp. 497–502, 1996.
- [70] K. Nałogowska-Głońska, B. Lacka, M. Zychma et al., "Lack of relationship between angiotensinogen gene m235t polymorphism and gene insertion/deletion (I/D-intron 16) and Pst I RFLP (P/M-intron 7) polymorphisms of the angiotensin I converting enzyme(ACE) gene and the development of H-gestosis. Preliminary results," *Polskie Archiwum Medycyny Wewnętrznej*, vol. 100, no. 1, pp. 19–26, 1998.
- [71] J. T. M. Heiskanen, M. M. Pirskanen, M. J. Hiltunen, A. J. Mannermaa, K. R. A. Punnonen, and S. T. Heinonen, "Insertion-deletion polymorphism in the gene for angiotensin-converting enzyme is associated with obstetric cholestasis but not with preeclampsia," *American Journal of Obstetrics and Gynecology*, vol. 185, no. 3, pp. 600–603, 2001.
- [72] Y. J. Kim, M. H. Park, H. S. Park, K. S. Lee, E. H. Ha, and M. G. Pang, "Associations of polymorphisms of the angiotensinogen M235 polymorphism and angiotensin-converting-enzyme intron 16 insertion/deletion polymorphism with preeclampsia in Korean women," *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, vol. 116, no. 1, pp. 48–53, 2004.
- [73] C. B. Roberts, L. Rom, J. Moodley, and R. J. Pegoraro, "Hypertension-related gene polymorphisms in pre-eclampsia, eclampsia and gestational hypertension in Black South African women," *Journal of Hypertension*, vol. 22, no. 5, pp. 945–948, 2004.
- [74] N. C. Serrano, L. A. Díaz, M. C. Páez et al., "Angiotensin-converting enzyme I/D polymorphism and preeclampsia risk: evidence of small-study bias," *PLoS Medicine*, vol. 3, no. 12, article e520, 2006.
- [75] O. V. Radkov, L. S. Logutova, M. N. Kalinkin, V. V. Zavarin, and V. K. Dadabayev, "Association of ADD1 and ACE gene polymorphisms with clinical and pathogenetic features of pre-eclampsia," *Rossiyskiy vestnik akushera-ginekologa*, vol. 12, no. 2, pp. 22–25, 2012.
- [76] A. S. Glotov, Y. S. Vashukova, O. S. Glotov et al., "Study of the population frequencies of gene polymorphisms, associated with preeclampsia," *Russian Journal of Genetics: Applied Research*, vol. 4, no. 5, pp. 388–396, 2014.
- [77] I. Medica, A. Kastrin, and B. Peterlin, "Genetic polymorphisms in vasoactive genes and preeclampsia: a meta-analysis," *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, vol. 131, no. 2, pp. 115–126, 2007.