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Scientific Poster Presentations

P1
HIGH-RESOLUTION ARRAY BASED CGH TECHNOLOGY WELL DISTINGUISH TWO CLASSES OF PRIMARY MELANOMA: WITH AND WITHOUT METASTASIS

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Introduction. Melanoma is one of the most common tumors among Caucasian population. Histopathology is the current gold standard for the diagnostic classification of melanocytic neoplasms. At diagnosis, it remains unclear if the original tumor has started a undetected program of metastasis. In fact, skin cancer is among the more common causes of "metastatic cancer of unknown primary".
Methods and Materials. We aimed to characterize new genomic alterations in primary melanocytic lesions by using a high resolution CGH array platform. We included 20 samples of melanoma tissues obtained at initial diagnosis from patients that could be segregated according to the development of metastasis at the 3rd years of follow-up. All samples contained >60% of tumour cells as assessed by pathological analysis. aCGH was performed in a 44K oligonucleotide Human Genome CGH Microarray platform from Agilent Technologies. CGH-Analytics 3.2.25 and InSilicoCGH were used for array analysis. Cluster analyses were performed using "Cluster" and "Tree View" for visualization by Eisen Laboratory.
Results and Conclusions. DNA copy number aberrations (CNA) were observed in all cases. The most frequent changes were partial or complete losses of chromosome 9 (13 cases, 65%) and 10 (8 cases, 40%), partial gain or trisomy of chromosome 7 (11 cases, 55%); followed by monosomy 19 (6 cases, 25%). Losses were more frequent than gains. All CNA were compiled to define the smallest overlapping region of imbalance (SORI). Seventy four SORI were delimited and used as variables into unsupervised clustering. This analysis segregated the series in two separated genomic groups. These naturally occurring subgroups fitted with the metastatic condition of the cases. Major differences were: (i) 35 CNA range 11-60, in the non-metastatic versus 48 CNA, range 32-73, in metastatic. (ii) After the supervised two different genomic profiles were delineated that could be used to predict the development of metastasis.

P2
MOLECULAR PROFILE OF PARANEOPLASTIC PERIPHERAL NEUROPATHY: A MICROARRAY ANALYSIS

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Paraneoplastic peripheral neuropathy (PPN) is a clinical and immunological heterogeneous disorder. In the majority of paraneoplastic neurological disorders, circulating autoantibodies directed against neurons have been found in the serum and/or the CSF suggesting disimmunity in the pathophysiology of these diseases. The aim of the present study was to investigate the molecular profile of PPN. We performed a microarray analysis in 4 patients with definite PPN according to PNS Euronetwork criteria (2 patients with sensory neuronopathy with anti-Hu and anti-amphiphysin antibodies respectively; 1 patient affected by lung cancer with sensory neuronopathy and anti-Hu antibodies; 1 patient with sensory-motor neuropathy and osteosclerotic myeloma) and in 2 normal subjects; 3 patients with axonal type CMT were studied as disease controls. Microarray experiments were performed using amplified RNA isolated from cryostat sections of sural nerve biopsy. A GeneChip microarrays panel of cDNA human Gene array containing a whole human genome was used. We found different clusters of greatly activated genes including heat shock protein, chaperone binding protein, MHC I and II protein, nuclear factor of activated T cell (NTAT) protein binding, tau protein and cell development protein (neuron differentiation, regulation of cell differentiation, vasculogenesis). Other gene clusters appeared downregulated such as blood vessel development and neurite regeneration proteins. Our preliminary data of microarrays gene profile suggest the involvement in PPN of molecules implicated in

inflammatory, oxidative stress and DNA damage responses. Microarray analysis could be an effective tool for identifying genes differently involved in PPN and for better understanding of the pathogenetic mechanisms of PPN.

P3
A DELETION IN CHROMOSOME 7Q21.13 DETECTED IN A PATIENT PRESENTING WITH DYSMORPHIC FACIAL FEATURES, REDUNDANT SKIN ON HANDS AND FEET, AND ANTENATAL CARDIAC ABNORMALITIES

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A male patient (aged 15 months) was referred for molecular karyotyping using a BAC array of ~1Mb resolution. G-banded chromosome analysis and MLPA for subtelomeric abnormalities had previously detected no abnormalities. Antenatal ultrasound had previously detected bilateral superior vena cava and a variant Dandy-Walker malformation. However, a postnatal echocardiogram detected no major cardiac abnormalities. The patient has unusual, distinctive facial features and redundant skin on the dorsum of his hands and feet, as well as brachydactyly. Array analysis detected a single probe deletion (RP5-998H4) which maps to 7q21.13 (88.6 – 88.7Mb). Adjacent clones indicating normal copy number delineated the maximum deletion interval as 87.9 – 89.3Mb. This patient therefore has monosomy for between 0.1 and 1.4Mb of material. This region contains a single gene (ZNF804B) with a putative zinc finger domain, but currently of unknown function. In situ hybridisation using the same BAC confirmed this finding. There are no previously reported copy number variants corresponding to this region. The results of inheritance studies and higher resolution array analysis using oligo arrays will be reported. If this finding represents a de novo abnormality, it seems likely that it is contributing to the patient's phenotype. As this was the only abnormality detected by the 1Mb BAC array, other large scale chromosome imbalance is unlikely. The implications of this finding in light of the inheritance and higher resolution investigations will be discussed.

P4
AN EXPERIMENTAL LOOP DESIGN IMPROVES THE DETECTION OF CONSTITUTIONAL CHROMOSOMAL ABERRATIONS BY ARRAY CGH

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Introduction. Comparative genomic hybridization microarrays (array CGH or molecular karyotyping) for the detection of constitutional chromosomal aberrations is the application of microarray technology that is coming fastest into routine clinical application. Through genotype-phenotype association, it is also an important technique towards the discovery of disease causing genes and genowewide functional annotation in human. When using a two-channel microarray of genomic DNA probes for array CGH, the basic setup consists in hybridizing a patient sample against a normal reference sample and detecting copy number variations through the deviation of fluorescent signal intensity between patient and normal reference.
Two major disadvantages of this setup are (1) the use of half of the resources to measure a (little informative) reference sample and (2) the possibility that deviating signals are caused by benign copy number variation in the "normal" reference instead of a patient aberration.
Results. We propose a new experimental loop design that compares three patients in three hybridizations (Patient 1 vs. Patient 3, Patient 3 vs. Patient 2, and Patient 2 vs. Patient 1). We develop and compare two statistical methods (linear models of log ratios and mixed models of absolute measurements). In an analysis of data from 27 patients seen at our genetics center, this new setup together with the linear model analysis significantly overcomes the limitations of the classical setup. Furthermore, we observed that the linear models of the log-ratios had a higher signal-to-noise ratio than the mixed models of the absolute intensities.
Conclusions. The improvements of the loop design are important to guarantee a maximal efficiency of array CGH in a clinical setting and will therefore contribute to its quick adoption as a routine diagnostic tool. The method is implemented as a web application and is available at www.esat.kuleuven.be/loop.

P5

MOLECULAR CHARACTERIZATION OF THE 437 CODON OF PFDHPS IN GAMETOCYTES EMERGING AFTER TREATMENT WITH SULFADOXINE-PYRIMETHAMINE, AMODIAQUINE AND THE COMBINATION SP/AQ

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The high mortality of Plasmodium falciparum is attributed to the emergence and the spread of resistant parasites. The transmission of the disease requires the presence of gametocytes in the peripheral blood. In this study, patients attending the district hospital of Etoug-Ebe (Cameroon) with uncomplicated falciparum malaria in and who fulfilled the criteria as recommended by WHO, were randomly assigned to receive sulfadoxine/pyrimethamine plus placebo; amodiaquine plus placebo, or amodiaquine plus sulfadoxine/ pyrimethamine. Patients were followed up for 28 days and children whose blood contains gametocytes in absence of trophozoites during the follow up were considered for the molecular studies. The presence or absence of mutation was investigated by PCR-RFLP. 13.6 %(36/260) of the patients recruited were gametocyte-positive during the study and the majority came from the group treated with SP (SP: 19/85; AQ: 8/89; SP/AQ: 9/86) p=0, 0208. We observe gametocyte during all the follow up in the group treated with SP, in the group treated with AQ, gametocytes were present only on day 3 and 7 and in the group, which received the combination, we observe gametocytes on days 3, 7 and 14. This study also revealed that most the gametocyte-positive samples carry the mutated allele (dhps-437G), and the majority once again came from the group treated with SP. (SP: 53,3 % ; AQ : 13,3 % ; SP/AQ : 13 ,3 %) p=7,823x10. 8 .
These results show that SP enhances the production of gametocyte, and could further contribute to the spread of the resistant allele. Amodiaquine could be a good candidate to reduce the transmission of malaria in this area.

P6

GENOTYPING MICROARRAY FOR DIAGNOSIS IN 199 FAMILIES AFFECTED WITH AUTOSOMAL RECESSIVE RETINITIS PIGMENTOSA

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Introduction. Retinitis pigmentosa (RP), is a genetically heterogeneous disorder characterized by progressive loss of vision. The disease can be inherent as an autosomal-dominant (adRP), autosomal-recessive (arRP), X-linked (xlRP), and rare mitochondrial and digenic forms. Although arRP is the most common form, it accounts for 40% of cases. Genetic heterogeneity is extensive, 24 genes have been identified (18 cloned, 6 mapped).
Patients and Methods. 199 unrelated Spanish families affected with arRP were studied. The methodology consists of a genotyping microarray (Asperbio), for screening mutations in a number of genes associated with arRP: CERKL, CNGA1, CNGB1, MERKT, PDE6A, PDE6B, PNR, RDH12, RGR, PLBP1, SAG, TULP1, CRB1, RPE65, USH2A, USH3A.
Results. We report 199 unrelated Spanish families, 78 of which had at least one mutated allele (39%). The allele frequencies were: CERKL 3,1% (12/398), CNGA1 1% (4/398), PDE6A 1,8% (7/398), PDE6B 1% (4/398), RGR 4,5% (18/398), RLBP1 0,5% (2/398), SAG 0,25% (1/398), CRB1 0,75% (3/398), USH2A 16,8% (67/398).
Discussion. The preliminary mutation frequency rate found was: 121/398 (30%) of mutated alleles and 39% of mutated families. By analysing the results we have corrected our previous figures, eliminating the possible polymorphisms: 42 families (42/199) 21% with at least one mutation and 61/398 (15%) mutated alleles with the following allele frequencies, respectively: 3,1% (12/398), 1% (4/398), 1,8% (7/398), 0,5% (2/398), 0% (0/398), 0,5% (2/398), 0,25% (1/398), 0,75% (3/398), 7,5% (30/398).
Conclusions. The genotyping microarray offers a fast and efficient method of screening for mutations in most recessive RP genes and should be the first step in the molecular diagnostic of families affected with arRP. The study has to be completed with: Family haplotype analysis and complete candidate gene screening. The differences found bring the need to do studies on a

control Spanish population, the purpose being to determine the possible pathological implications of changes described.

P7
PRIORITIZATION OF CANDIDATE GENES BASED ON OVER-REPRESENTED FEATURES

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Array CGH technology allows to detect chromosomal rearrangements in the whole human genome and is a crucial step in identifying genes responsible for congenital diseases. However, given the size of such chromosomal events, researchers still face lists of hundreds of genes that may be causing the investigated disease. Accurate candidate genes prioritization is therefore of utmost importance to help researchers focus on the best candidates. We present improvements to gene prioritization accuracy in the Endeavour system [Aerts et al. 2006] based on feature enrichment profiles. The principle is to provide the system with a set of known disease-related genes (training set), which will be used to build several models that allow to rank the candidate genes based on their similarity to the training set. Enrichment profiles are built for the Gene Ontology annotations, the KEGG pathways, the protein domains (InterPro) and the EST localization (Ensembl ESTs). To measure the enrichment in a given feature, the hypergeometric distribution is used as a dissimilarity index [Barriot et al. 2004]. Then, the False Discovery Rate [Benjamini and Hochberg 1995] is applied to account for multiple testing. We performed cross validation (leave one out) on 29 human disease training sets by applying different FDR thresholds to prioritize the whole genome. Interestingly, the best performance was obtained for an FDR threshold of 50%. This can be explained by the fact that we use the enrichment score to rank the candidates (Fisher's omnibus meta analysis). Thus, keeping low similarity features in the enrichment profiles helps to better discriminate the candidates. We also stress out that the whole genome is prioritized, which includes genes causing the disease that are still unknown and that may rank higher than the left out gene. The accuracy obtained is thus a pessimistic estimate of the real performances of our method.

P8
CHROMOSOMAL IMBALANCES IN ANAEMIC AND NON-ANAEMIC HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC)

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Chromosomal imbalances have been identified in 68 anaemic and 49 non-anaemic HNSCC by conventional CGH. Statistical analyses revealed significant differences between the two cohorts in the frequency and appearance of DNA gains on chromosomes 1p, 2q, 3q, 4q, 5q, 11q, 12q, 13q, 19p and of DNA losses on chromosomes 3p, 4p, 4q, 10p, 16q, 17p, 17q, 18q, 20q, and 21q. Additionally, chromosomal aberrations correlating with a poor prognosis have been identified for anaemic cases. Chromosomal gains on 14q, 16q and 18p correlate with a significantly reduced survival after radiotherapy in anaemic HNSCC while no chromosomal prognostic markers were observed in the non-anaemic cases. To identify candidate genes within these altered chromosomal regions in anaemic cases array CGH was performed. Array CGH detected gains on 14q12-13, 16q22 and 16q23-24 as well as on 18p11.2-11.3. This led to the identification of several candidate genes such as PAX9, FOXA1, TTF-1 and SSTR1 on 14q, HAS3, TRF2, CDH3, CES-2, ARC, E2F4, BCAR1, MAF, TUBB3, GAS8 and FANCA on 16q and VAPA, RAB31/12, KNTC2, TYMS, ADCYAP1 and CETN1 on 18p. Various BAC clones indicating gained regions on chromosome 16q have been used as FISH probes on paraffin-embedded tissue sections from one anaemic HNSCC case. FISH confirmed amplifications for BAC clones mapping TUBB3, GAS8 and FANCA. FANCA is of special interest since it belongs to the central part of the Fanconi's Anaemia (FA)/BRCA pathway in homology-directed DNA repair. Disruptions of genes in this pathway result in chromosome instability, cellular hypersensitivity to DNA cross-linking reagents and cancer progression. Thus, it is likely that alterations of the FA/BRCA pathway are involved in mechanisms leading to a poor prognosis of a subset of anaemic HNSCC. The generation of a gene specific BAC array for the FA pathway is on the way to enable the analyses of copy number changes of these genes in one experiment. Furthermore, alterations of candidate genes

have been verified using Multiplex Ligation-dependent Probe Amplification (MLPA). The results indicate a specific pattern of alteration in anaemic and non-anaemic HNSCC. The identification of genes involved in chromosomal gains on 14q, 16q and 18p may help to investigate mechanisms responsible for the poor prognosis of anaemic cancer patients in more detail.

P9
DETECTION OF GENOMIC COPY NUMBER CHANGES IN PATIENTS WITH IDIOPATHIC MENTAL RETARDATION BY HIGH-RESOLUTION X-ARRAY-CGH: FREQUENT INCREASED GENE DOSAGE OF KNOWN XLMR GENES

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A tiling X-chromosome-specific genomic array with a theoretical resolution of 80 kb was developed. We first validated the X-array with aberrations previously detected at low resolution in 5 MR patients. This allowed for delineation of the location and extent of the aberration at high resolution and subsequently, more precise genotype-phenotype analyses. Next, we screened a cohort of 108 patients with idiopathic MR consisting of 57 patients suspected of X-linked mental retardation (XLMR), 26 probands of brother pairs, and 25 sporadic patients. In this screened population, we identified 16 copy number changes in 15 patients (13.9%). These include 2 deletions and 14 duplications ranging from 0.2 - 2.7 Mb. The aberration most likely is associated with the phenotype in 5 patients (4.6%) based on absence in normal control individuals, de novo aberration, segregation with the disease in the family, involvement of a known or candidate MRX(S) gene, and/or skewed X-inactivation in carrier mothers. Presumed causal aberrations include 2 deletions and 3 duplications that contain known MRX(S) genes. In addition, nine novel apparent benign variants on the X chromosome are described. One interesting copy number change found in 3 cases is a 0.3 Mb MRX gene containing duplication that might act as a susceptibility factor for MR. Taken together, our data strongly suggest that not only deletions but also duplications on X might contribute to the phenotype more often than expected, supporting our increased gene dosage mechanism for deregulation of normal cognitive development, as reported for MECP2.

P10
MOLECULAR CHARACTERISATION OF PAEDIATRIC AND ADULT GLIOMA CELL-LINES

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Paediatric high-grade gliomas (HGG) have the highest mortality of all brain tumours, with 5 year survival rates of less than 20%. Although advances in our understanding of the molecular genetics of adult HGG are providing rationale for new targeted therapies, the corresponding data is lacking in children. Adult and paediatric HGG are histologically similar, but they appear to be distinct genetic entities. One factor hampering work in the paediatric setting is the relative lack of cell lines derived from paediatric HGG. It is currently unclear whether the well-established adult lines are representative of the underlying molecular genetics of childhood tumours. The aim of this project was to characterize 13 cell-lines, derived from paediatric low-grade gliomas (LGG) and HGG and adult gliomas, in order to identify differences between paediatric and adult gliomagenesis. We have employed traditional karyotyping, copy number and SNP analysis on 500K Affymetrix arrays, oligonucleotide array CGH, expression profiling on U133 2.0Plus chips, and specific target gene/protein characterisation. Supervised analysis was able to generate a list of 169 genes that was able to discriminate between LGG and HGG cell lines, and between adult and paediatric cases. The paediatric HGG lines were found to harbour changes in DNA copy number (>70 chromosomes) including gains of 4p, 16p and 17q and losses of 3p, 6q and 11p. A key amplification at 12q14 was observed in one paediatric line (SF188), which led to overexpression of CDK4 and PIKE, and may provide insight into differential drug sensitivity in this model. Mutations in EGFR and PTEN, common in adults, were not found in the paediatric lines. Although this work is ongoing, the characterization of glioma cell-lines provides insight in the differences of

glioma development in adults and children, and it may aid in the development of new drugs for the treatment of paediatric HGG.

P11
MOLECULAR PROFILING OF RADIATION-ASSOCIATED BREAST TUMORS

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Introduction. Women who received radiation treatment for Hodgkin's Lymphoma (HL) have an increased risk of developing breast cancer. It has been estimated that approximately 90% of the breast carcinomas in these patients is a result of their radiation treatment, which makes this series extremely appropriate to determine a potentially radiation-associated genomic profile.
Methods and Materials. We have used array-CGH and gene expression profiling (GEP) technology to assess the molecular changes in these radiation-associated breast tumors. For genomic profiling we used a human 3.5K BAC array and for GEP a 37K oligo microarray (NKI-CMF). DNA and RNA from breast tumors of HL patients (BfHL) and from breast tumor controls were subsequently hybridized to the arrays and statistical microarray analysis was performed.
*Results.*With array-CGH we have analyzed the genomic profile of 20 BfHL cases and 22 breast tumor controls. Frequency plot analysis revealed several distinct chromosomal aberrant regions in the radiation-associated BfHL tumors compared to the control breast tumors. With GEP we have analyzed the RNA profile of 23 BfHL cases and 20 breast tumor controls. Hierarchical clustering of all samples was performed using 5031 significant oligo's, which resulted in a clustering of the so-called radiation-associated tumors apart from the breast tumor controls. Furthermore we performed supervised classification strategies using SAM and PAM and identified respectively differentially expressed genes and gene classes distinguishing the cases from the controls. Existing gene profiles (70-gene prognosis profile, CIN profile) were applied on our data set and correlations were calculated. Ingenuity and Gene Set Enrichment Analysis (GSEA) were used to identify specific networks and genes that were significantly regulated, and a nearest mean classifier was build. These data will be presented.
Conclusion. Our preliminary results indicate that radiation-induced breast tumors can be distinguished from breast tumor controls on the basis of their genomic and expression profile.

P12
GENE ALTERATION IN PAPILLARY THYROID CARCINOMAS IDENTIFIED BY ARRAY CGH

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Introduction. RET/PTC rearrangements are frequent alterations in papillary thyroid carcinomas (PTC), however, recent studies have shown that these typical changes appear heterogeneously distributed within tumour tissues. Thus, it is likely that additional gene alterations are present in these tumours. Moreover, RET/PTC negative tumours should exhibit alternative changes.
Methods and Materials. We therefore investigated 25 papillary thyroid carcinomas (12 adult tumours, 13 infantile, post-Chernobyl tumours) with known RET/PTC status (RET/PTC positive: 5 adult, 10 infantile cases, RET/PTC negative: 7 adult, 3 infantile cases) by array CGH to uncover such unknown gene alterations in PTC.
Results. Overall, array CGH revealed most frequent imbalances (>30% of cases) on chromosomes 1, 6, 9, 10, 11, 13, 20, 22 (DNA losses) and on chromosomes 10, 12, 16, 19, 20, 21 (DNA gains). Array CGH profiles also indicated distinct aberration patterns of adult and childhood tumours as well as of RET/PTC positive and – negative tumours. This finding could be supported by statistical analysis (maximum permutation t-test) which revealed significant differences between RET/PTC+ adult and RET/PTC+ infantile cases on chromosome 1p and between adult RET/PTC+ and adult RET/PTC-negative cases on chromosome 1p, 19, 20p and 20q. Losses on chromosome 1p appear to be specific for adults whilst losses on chromosome 1q are specific for adult RET/PTC+ cases. Gain on chromosome 19 is specific for RET/PTC+ cases in common whilst losses on chromosome 19 are specific for adult RET/PTC-cases. Deletion of chromosome 13 was shown to be specific for RET/PTC+ infantile cases. A hierarchical

cluster analysis employing the correlations between the array CGH profiles demonstrated that 6 adult RET/PTC negative cases are apart from all other cases which show frequent co-alterations (gains on 19, 21 or losses on 1pq, 6, 9, 13, 20) In contrast, lost chromosomal regions on chromosomes 7q and 22 are most frequently co-altered in the 6 adult RET/PTC negative cases. These alterations revealed by array CGH have been validated by FISH on FFPE tissue sections of the investigated cases. Candidate genes have been identified (e.g., ROBO1, PTK6, RBAK, TGFBR3 and FOXO3A on 12q) and will be tested for altered expression levels (RNA and protein).
Conclusions. Our findings suggest that dependent on the RET/PTC status of tumours additional gene alterations are present in papillary thyroid tumours which may point to alternate pathways involved in tumour development.

P13
SCREENING FOR GENE DOSE IMBALANCES OF AUTISM CANDIDATE GENES IN PATIENTS WITH AUTISM SPECTRUM DISORDERS (ASD) USING TWO-COLOR MLPA

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impairments in communication and social interaction, accompanied by repetitive and stereotyped behaviors and interests. It's a highly heritable and heterogeneous disorder with a complex genetic etiology. The prevalence for autism is in the order of 10 in 10.000, and as high as 60 in 10.000 for all forms of autism [1]. Despite extensive investigations, involving mutation screening along with linkage and association studies, mutations have only been detected in a very few cases and no definite disease genes have yet been identified. However, cryptic chromosome abnormalities, among them maternally derived duplications/triplications of chromosome 15q11-13 have been reported in patients with ASD. In addition, recent high resolution array-CGH studies of syndromatic ASD patients revealed submicroscopic deletions or duplications in >20% of the cases [2]. We hypothesized that microdeletions or microduplications of autism candidate genes, which escape detection by array-CGH and mutation screening, might be involved in the cause of ASD. Therefore, we designed a multiplex ligation-dependent probe amplification (MLPA) synthetic probe set consisting of ~60 probes to screen for gene dose imbalances of candidate genes in patients diagnosed with ASD. By using two-color MLPA we were able to investigate ~30 specific nucleic acid sequences in a single reaction. DNA from 150 syndromatic and nonsyndromatic ASD patients has been collected for screening. The results of this ongoing study will be presented.

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P14
GENOCENSUS, A PROGRAM FOR THE ANALYSIS AND VISUALISATION OF GENOMIC COPY NUMBER VARIATION

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GenoCensus is a stand-alone Java program for Unix or Windows that processes files of numerical data generated from analysis of microarray images. Typical input files are gpr files produced by GenePix (Axon), but GenoCensus can treat any text-formatted data that includes array feature names, image data of spot and background intensities, and other values that characterise the spots, such as flags for quality filtering. IntegraGen has developed BAC arrays (IntegraChips) of about 5000 clones and by default the program uses annotations of chromosomal locations that are specific for these BAC clones. However, GenoCensus can be configured to treat any set of features for any microarray. The program invokes BioConductor R modules for some tasks. Normalisation options include block Loess with trimming. Users can specify several processing parameters to adjust stringency for filtering clones based on various quality criteria. Dye swap or trio designs are

managed and genomic or chromosomal profile views include individual arrays or pairs of arrays, either as complementary or average ratio views. All regions of gain and loss are determined, with a user-adjustable choice of thresholds for display and exportable tabulation of regions exceeding the threshold. Spot-averaged ratios can also be exported. Examples of profiles generated from hybridisations with IntegraChips of tumor DNA and clinical samples with chromosomal abnormalities will be presented, including detection of mosaicism to about 15%. Agreement is shown between copy number profiles as determined with IntegraChips/GenoCensus or with IntegraGen's Illumina platform and Illumina software using the HapMap arrays of 317,000 oligonucleotides.

P15
POST-GENOMIC TECHNOLOGIESTITLE: DNA QUALITY ASSESSMENT FOR ARRAY CGH BY ISOTHERMAL WHOLE GENOME AMPLIFICATION

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Introduction. Array Comparative Genomic Hybridization (array CGH) is increasingly applied on DNA obtained from formalin-fixed paraffin-embedded (FFPE) tissue, but in a proportion of cases this type of DNA is unsuitable. Due to the high experimental costs of array CGH and unreliable methods for DNA quality testing, better prediction methods are needed. The aim of this study was to accurately determine the quality of FFPE DNA input in order to predict quality of array CGH outcome.
Methods and Materials. DNA quality was assessed by isothermal amplification and compared to array CGH quality on 60 FFPE gastric cancer samples, one FFPE colorectal cancer sample, two FFPE normal uvula samples, one fresh frozen and six FFPE HNSCC samples. Gastric cancer DNA was also quality tested by β-globin PCR.
Results. Accurate prediction of DNA quality using the isothermal amplification was observed in the colorectal carcinoma, HNSCC and uvula samples. In gastric cancer samples, the isothermal amplification was a more accurate method for selecting good quality DNA for array CGH compared to using PCR product lengths. The isothermal amplification product was used for array CGH and compared to the results achieved using non-amplified DNA in four of the samples. DNAs before and after amplification yielded the same patterns of chromosomal copy number changes for both the fresh DNA sample and the FFPE samples.
Conclusions. The efficiency of isothermal DNA amplification is a reliable predictor for array CGH quality. The amplification product itself can be used for array CGH, even starting with FFPE derived DNA samples.

P16
18p DE NOVO DELETION STUDIED BY ARRAY CGH: A NEW APPROACH FOR A PHENOTYPE MAP

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The 18p- is one of the most common autosomal terminal deletion syndromes. Frequent clinical features include moderate to severe mental retardation, postnatal growth retardation and round face with typical facial features. These features have been correlated to different breakpoints along the chromosomal arm. However, a reliable phenotype map has not been established since most of the cases involve the entire short arm of the chromosome or are secondary to unbalanced translocations. Genotype-phenotype correlation studies using FISH and microsatellite analyses have suggested the presence of a critical region for the mental retardation between 18p11.1 and 18p11.21. We present a 22-year-old female studied using 1Mb-resolution array CGH. A 7.85Mb pure terminal deletion 18p (pter?p11.23) was observed. The deletion includes the region suggested to be related to the characteristical face and the postnatal growth retardation but not the critical region for mental retardation. In fact, the patient showed the characteristic rounded face with downturning corners of the mouth and a normal mental development. However, she showed normal stature. Additional features observed were hirsutism and progressive stiffness of hands and feet. Along the 7.85Mb deletion there are some genes that could be implicated in muscle development,

lipodystrophy, holoprosencephaly and chromosomal segregation among others. Moreover, DYT7 and DYT15 loci causing dystonia have been mapped to 18p. Further studies in pure 18p deletion patients using array CGH should be performed in order to establish a reliable phenotype map for the 18p- syndrome. *Dan Diego-Alvarez and Ana Bustamante-Aragones contribute equally to the present work.

P17
GENOTYPING MICROARRAY FOR DIAGNOSIS IN 156 FAMILIES ASSOCIATED WITH ABCA4: SPECIFICITY AND SENSIBILITY

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Introduction. Mutations in the ABCR (ABCA4) gene have been associated with Stargardt disease (STGD) or fundus flavimaculatus (FFM). In addition, a few cases with autosomic recessive cone-rod dystrophy (arCRD) and autosomic recessive retinitis pigmentosa (arRP) have been found to have ABCA4 mutations. Comparative genetic analyses of ABCR variation and diagnostics have been complicated by substantial allelic heterogeneity.
Patients and methods. We analyzed a total of 156 unrelated families. Mutation analysis was performed in 107 STGD families, 32 arCRD families and 31 arRP families. Patients were analyzed with a commercial microarray, ABCR400 (AsperBio). In 9 families we have employed denaturing HPLC (dHPLC) for the detection of he second mutated allele.
Results. Using the microarray, the respective frequencies of mutant alleles for the 50 exons are: 54,7% (117/214) for STGD with 73 mutated families (44 with two mutant allele and 29 with only one mutant allele), 25% (16/64) for CRD with 10 mutated families (6 with two mutant allele and 4 with only one mutant allele) and 8% (5/62) for RP with 5 mutated families (all with only one mutant allele). All the 88 mutated families (138 mutated alleles) were re-sequencing. However in 3 STGD families, the microarray detected a mutant allele which was not confirmed by direct sequencing, giving us a specificity rate of the microarray of 97,8% (135/138). The microarray was able to detect 138 mutant allele, however could not detect 3 other mutations (in 1 CRD family and 2 STGD families) by dHPLC and/or by direct sequencing. Therefore, the microarray sensibility obtain in this study was 97,9% (138/141).
Conclusions. The ABCR400 genotyping microarray is a comprehensive screening tool for genetic variation in patients with ABCR-associated retinal pathology. The present study suggests the combination of microarray and dHPLC as an optimal screening strategy for mutation analysis in this huge gene.

P18
DNA COPY NUMBER PROFILES OF PRIMARY COLORECTAL CANCERS AS PREDICTORS OF RESPONSE TO THERAPY

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Introduction. Colorectal cancer (CRC) is biologically a heterogeneous disease, which gives rise to different clinical behaviors, including risk of metastasis and response to drug therapy. The success of both classical as well as novel targeted drug therapies can be improved by matching the right combination of drugs with different biological classes of CRC. The aim of this study was to correlate genome wide DNA copy number status in advanced colorectal cancer with response to chemotherapy.
Materials and methods. Thirty-two patients with advanced CRC were selected from the patient series of the CAIRO study of the Dutch Colorectal Cancer Group (DCCG), based on either a good (n=17) or a poor response (n=15) to first-line combined irinotecan and capecitabine therapy. DNA copy number profiles were determined by oligonucleotide-based array comparative genomic hybridization (array CGH).
Results. The group of the non-responders had fewer aberrations ($P = 0.04$) than the responders, especially regarding losses ($P = 0.01$). The striking difference in aberrations between the two groups were losses of 1p36 ($P = 0.05$), 18p ($P = 0.02$), and 18q ($P = 0.01$), that were more frequent in the CRC of patients which had a good response to chemotherapy. Hierarchical cluster analysis of the array CGH data revealed two clusters with cluster 1 containing twenty-one tumors and cluster 2 eleven

tumors. Fifteen out of twenty-one tumors of cluster 1 consisted of responders, while in cluster 2 nine out of eleven tumors were non-responders. Cluster membership showed a significant correlation with response status (P = 0.01).
Conclusion. Primary tumors of patients with advanced colorectal cancer with a good or poor response to systemic chemotherapy show different DNA copy number profiles. Tumors of patients with a good response to combined irinotecan and capecitabine treatment had overall more chromosomal aberrations, especially losses of 1p36, 18p and 18q.

P19
TARGETS OF GENOME COPY NUMBER REDUCTION IN PRIMARY BREAST CANCERS IDENTIFIED BY INTEGRATIVE GENOMICS

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Introduction. The identification of specific oncogenes and tumor suppressor genes in regions of recurrent aneuploidy is a major challenge of molecular cancer research.
Methods and Materials. Using both oligonucleotide single-nucleotide polymorphism (SNP) and mRNA expression arrays, we integrated genomic and transcriptional information to identify and prioritize candidate cancer genes in regions of increased and decreased chromosomal copy number in a cohort of primary breast cancers.
Results. Confirming the validity of this approach, several regions of previously-known copy number (CN) alterations in breast cancer could be successfully re-identified. Focusing on regions of decreased CN, we defined a prioritized list of eighteen candidate genes, which included ARPIN, FBN1, and LZTS1, previously shown to be associated with cancers in breast or other tissue types, and novel genes such as P29, MORF4L1, and TBC1D5. One such gene, the RUNX3 transcription factor, was selected for further study. We show that RUNX3 is present at reduced CNs in proportion to the rest of the tumor genome and that RUNX3 CN reductions can also be observed in a breast cancer series from a different center. Using tissue microarrays, we demonstrate in an independent cohort of over 120 breast tissues that RUNX3 protein is expressed in normal breast epithelia but not fat and stromal tissue, and widely downregulated in the majority of breast cancers (>85%). In vitro, RUNX3 overexpression suppressed the invasive potential of MDA-MB-231 breast cancer cells in a matrigel assay.
Conclusions. Our results demonstrate the utility of integrative genomic approaches to identify novel potential cancer-related genes in primary tumors.

P20
A NOVEL GENOMIC DISORDER AT 16P13.11: WHERE IS THE SYNDROME?

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Many genomic disorders are caused by non-allelic homologous recombination between low copy repeats flanking copy number variable regions. Chromosome 16 is especially rich in a chromosome 16 specific low copy repeat, with 20 distinct copies on 16p. However, thus far, no genomic disorder has been associated with rearrangements between these LCR16s. During our screen of 600 patients with multiple congenital anomalies and mental retardation (MCA/MR) at 1 Mb resolution, we detected four patients with a similar microdeletion and two with the reciprocal duplication. Three of the microdeletion patients presented with MR, microcephaly, short stature and epilepsy while a fourth microdeletion carrier presented with holoprocenphaly. The 1.5 Mb region encompassing 25 genes is flanked by multiple LCR16. No copy gains or losses of this region has been reported as benign variants. Fine mapping by 500K SNP array showed that the different deletions and duplications result from NAHR between distinct LCR16s located within a 500kb interval on either side of the rearrangement. For one patient, the microdeletion is inherited from a normal father while one duplication occurred de novo. Within 725 normal individuals three duplications but no deletions were detected. Hence, the microdeletion is only presented in the patient population suggesting that hemizygosity of the 16p13.11 region is a susceptibility factor for MCA/MR (Fisher exact test $p<0.01$) while the duplication may be a benign variant. In addition, "atypical" larger rearrangements were also

identified, some mediated by NAHR between different LCR16s located at longer distances.
In conclusion, this study shows that LCR16 is another LCR mediating chromosomal rearrangements by NAHR. The phenotypic variation in both microdeletion, microduplication patients and normal individuals underscores the difficulties in the clinical interpretation of imbalances detected by high resolution array CGH: (1) a de novo duplication 16p13.11 is probably not causal while (2) an inherited 16p13.11 deletion is a susceptibility factor for the MCA/MR phenotype. To evaluate the involvement of rare imbalances in the etiology of MCA/MR, large scale association studies may be required to pinpoint those imbalances with high penetrance for MCA/MR.

P21
EXTENSIVE PROMOTER METHYLATION IS PRESENT IN BOTH MICROSATELLITE INSTABLE ANDCHROMOSOMAL INSTABLE COLORECTAL CARCINOMAS

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Introduction. Colorectal cancer (CRC) is a complex and heterogeneous disease in which microsatellite instability (MSI), chromosomal instability (CIN) and epigenetic instability, characterised by simultaneous hypermethylation of multiple CpG islands, play an important role. The aim of this study was to investigate the interrelationship of MSI, CIN and extensive promoter methylation (EPM).
Methods and Materials. 71 CRCs were analysed for CIN by comparative genomic hybridization and MSI by the mononucleotide marker BAT-26. Promoter methylation of the tumour suppressor and DNA repair genes hMLH1, O6-MGMT, APC, p14ARF, p16INK4A, RASSF1A, GATA-4, GATA-5 and CHFR was analysed using methylation-specific PCR (MSP).
Results. Unsupervised hierarchical cluster analysis identified a group of CRCs (n= 21.1%) with a high methylation index (MI: 0.82). The majority of the genes showed the highest frequency of promoter methylation in this EPM-H group, except for O6-MGMT and APC which showed an inverse relationship (P value: 2.4 x 10-3) and higher frequencies of promoter methylation in clusters of CRCs with lower MI's. Although the EPM-H group showed a strong association with MSI (P value: 6.6 x 10-5) and an inverse relation to CIN (P value: 3.4 x 10-2), approximately half of the EPM-H group were CRCs with CIN.
Conclusions. Although EPM shows a stronger association with MSI than with CIN, tumours with the highest levels of promoter methylation can be found with equal frequency within these two groups. This suggests that epigenetic changes are important for colorectal cancers developing with MSI or CIN.

P22
IDENTIFICATION OF CHROMOSOMAL ABNORMALITIES IN PROSTATE CANCER BY ARRAY CGH.

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Genetic alterations are the hallmark for various diseases like cancer, mental retardation and birth defects. Identifying these alterations and the genes they contain will provide molecular targets for diagnosis and therapy. Genomic DNA was isolated from LNCaP, a human prostate cancer model cell line. Labeled genomic DNA probes were generated from LNCap cells and normal female genomic DNAs using CyScribe™ Array CGH Genomic Labeling Kit (GE Helathcare) and BioPrime™ Array CGH Genomic Labelling System (Invitrogen). The labeled fragments were hybridized on to a Spectral Chip™ 2600 used for whole genome scanning and Constitutional Chip™ 3.0 containing clones for 48 constitutive diseases. Image analysis was performed by Axxon GenePix® Pro 6.0 software and chromosomal abnormalities were identified using web-based Spectral Ware™. Higher dye labeled DNA yields (>16%) and Cy dye dCTP-nucleotide incorporation (10%) were achieved with Amersham CyScribe Array CGH Genomic Labeling Kit. Analysis of hybridization data showed similar signal intensities, log2 ratios and signal to noise ratios for the two kits. Both kits identified loss in 2p22.3-2p14; 10q23.1-10q24.33; 13q21.1-13q22.1 chromosomal regions and strong gain in Yq11.2 region that were previously detected by conventional CGH.

P23
GENOMIC MARKERS OF SURVIVAL IN SEROUS OVARIAN CANCERS

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Introduction. Ovarian cancer is frequently diagnosed at late stage due to absence of obvious precocious symptoms. In this study, we proposed to explore genomic aberrations in the most commonly diagnosed stages (III-IV) and to correlate them with survival in a series stratified in short and long survivors.
Methods and Materials. Array-CGH for 46 ovarian cancer of stage III-IV and mostly of serous histology was carried out using 4500 BAC chips. The cohort is composed of short survivors with less than 29 month of survival (median = 18 months) and long term survivors with more than 48 months of survival (median = 65 months).
Results. The most commonly gained regions were 8q22.3-q24.3, 3q26.1-q27.2, 5p15.33-p15.2, 1q25.3, 2p14-p12, 11q13.5-q14.1 and losses were 17p13.3-q12, 13q13.3, 16q21-q22.1, 22q13.31-q13.33, Xp21.1, 5q14.3-q15. Some losses occurs more frequently in short term survivors as 17p21 and 22q losses. Statistical analysis is ongoing to determine if regions taken individually or in combination allow to define a signature predictive of outcome. Results will be compared with previous studies using conventional CGH or array-CGH and aimed to identify chromosomal regions associated with survival or chemoresistance.

P24
INCOMPLETE GENOME COVERAGE BY HIGH RESOLUTION SNP ARRAY PLATFORMS

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Introduction. Copy number variations (CNVs) may represent susceptibility loci for common diseases (e.g. diabetes, cardiovascular disease, cancer, dementia), and serve as markers in association studies.
MicroRNA-coding genes may be contained within CNVs. Deletion or insertion of CNVs may induce position effects that affect gene expression patterns without alteration of gene copy numbers. In order to achieve complete identification of CNVs in our study population we analysed human DNA samples with both a high coverage, low resolution BAC-based array-CGH platform and a high resolution 317 K SNP Infinium array platform.
Methods and Materials. Peripheral blood DNA samples were obtained from patients with multiple congenital abnormalities and mental retardation (MCAMR) and their unaffected parents. Comparative genome hybridization on a 3,782 BACs containing array platform (average spacing 0.7 Mb and 19.9% coverage of the human autosomes) was performed as described. For genome-wide SNP genotyping the 317 K SNP Infinium array platform of Illumina (San Diego, CA, USA) was used.
Results. By BAC-based array-CGH we identified 53 CNVs that occurred at frequencies higher than 10% among 48 healthy individuals. Attempting to retrieve these 53 CNVs on the 317 K SNP array platform, we found that 13 (24.5%) were not represented.
Conclusions. Our data indicate that due to the selection criteria used to build the SNP array platform a significant portion of the most frequently occurring CNVs would go undetected. We conclude that caution should be applied when SNP arrays are being used for genome wide studies of CNVs.

P25
GENE EXPRESSION PROFILING OF HEREDITARY BREAST TUMOURS USING A CDNA MICROARRAY

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Introduction. Around 25% of hereditary breast cancer cases are attributable to inherited mutations in the high penetrance BRCA1 and BRCA2 genes, resting a 75% in which the genetic defect responsible for the susceptibility in unknown. The tumoral tissue analysis may give us a large amount of information about the molecular pathways that are altered in each group and about which genes are differentially implicated in the pathobiology of the distinct types of tumors.
Methods and Materials. The main goal of this project is to establish the gene expression profile of breast tumors

developed by BRCA1 and BRCA2 mutation carriers and subclassify the BRCAX group using a cDNA Microarray developed by the Spanish National Cancer Centre (Oncochip v2.0), containing 11.500 clones corresponding to 9.300 genes. For that purpose, we have collected samples of frozen tumoral tissue from 16 BRCA1 mutation carriers, 8 BRCA2 and 14 BRCAX patients, in addition to 22 sporadic tumors. Total RNA from all the samples was extracted using Tri Reagent® solution, amplified using the Eberwine method, labeled with Cytm3 dUTP and Cytm5 dUTP and hybridized onto the Oncochip.

Results. Preliminary results, including part of the BRCA1, BRCA2 and sporadic tumors, showed differences in the expression profiling of the three groups. The whole series, including the BRCAX tumors, has already been hybridized and further analysis is in process.

Conclusions. The main differences were found between BRCA1 and sporadic tumors. Maybe due the low number of BRCA2 samples preliminary analyzed, the differences between this group and the BRCA1 positive or sporadic samples were not significant.

P26
GENETIC STUDY OF ANTI-MYCOBACTERIAL IMMUNE RESPONSES

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Numerous studies have identified a strong genetic component contributing to tuberculosis. However, little is known about how gene variants affect immune responses to Mycobaterium tuberculosis. Identification of these genes is essential to identify critical pathways of disease susceptibility. Here, we report on three immune phenotypes that are being used for a quantitative genome-wide linkage study and for candidate gene-based association analysis. 165 multiplex families, including 522 children, were enrolled from an area in Cape Town with one of the highest reported tuberculosis incidence globally. The enrolment criteria aimed to maximize the number of children from large families and with a high probability of previous mycobacterial exposure. Three types of immune phenotypes were determined on all enrolled children: i) Mantoux skin reactivity, ii) frequency of mycobacteria-specific, IFN? producing CD4+ T cells in peripheral blood, and iii) mycobacteria antigen-induced IFN? and TNFa production by blood lymphocytes. Stimulating antigens used were: live BCG, M. tuberculosis purified protein derivative (PPD), and M. tuberculosis secreted protein ESAT-6. Over 60% of children displayed evidence of mycobacterial exposure using Mantoux skin reactivity as a measure. The distribution of TNFa and IFN? response to each antigen follow essentially a Gaussian distribution of primed individuals. There was high correlation for the same measure at different days. Moreover, considerable correlation of host responses exists across antigens for the majority of individuals, with a small subset differing substantially in their ability to respond. This subset potentially represents individuals with specific genetic defects of immune responsiveness. Previous studies showed that the studied immune phenotypes are under strong genetic control with heritability estimates of 70% for Mantoux reactivity, and 40% and 80% for PPD-triggered IFN? and TNFa production respectively. Given the high heritable estimates, it is likely we will identify at least a subset of the genetic factors underlying host responsiveness to mycobacteria.

P27
MOLECULAR- CYTOGENETIC CHARACTERIZATION OF A 9Q34 DUPLICATION / TRIPLICATION

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Partial trisomy 9 is rare and heterogeneous with respect to the chromosomal region involved in the aberration and the clinical phenotype. We report on a 16-year old female referred to our laboratory with mental retardation. Cytogenetic analysis of G-banded chromosomes demonstrated an abnormality on the end of the long arm of chromosome 9. Additional subtelomeric MLPA analysis confirmed the aberration and showed a duplication of the telomeric end of chromosome 9q. Fine mapping by 250K SNP array (Affymetrix) showed a terminal duplication of ~ 3 Mb: dup(9)(q34.3). Interestingly, the MLPA and SNP array results gave high intensity values suggesting a 9q34 triplication.

Confirmation of this possible triplication is currently under investigation.

P28
IMPLEMENTATION OF ARRAY CGH AND THE DETECTION OF CHROMOSOMAL IMBALANCES IN DANISH PATIENTS WITH MALFORMATIONS AND MENTAL RETARDATION

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Research, diagnostics and treatment of human diseases associated to mental retardation has been a major engagement for the Kennedy Institute in Denmark during almost four decades. Following the sequencing of the human genome, molecular genetics methods have advanced in recent years and full genome scanning for chromosomal imbalances has become routine with the array comparative genomic hybridisation technique on patient samples. We have established the aCGH method based on the Cytochip platform (BlueGnome Ltd., Cambridge, UK). Our setup with Cytochips includes improved pre-blocking as well as optimised washing under formamide-free conditions while maintaining a sensitive and robust detection of chromosomal imbalances. In combination with conventional karyotyping, FISH analysis, and MLPA we detect chromosomal aberrations by multiple methods. For example, we have analysed a child presenting with only slightly dysmorphic, but mental retardation and found a duplication on chromosome 22q11, which is associated with the known 22q11 duplication syndrome. Confirmation by MLPA analysis using primers for the DiGeorge syndrome is pending. In another child presenting with mental retardation we found an 8 Mb duplication of the 22q13 region by aCGH. When we analysed the chromosomes by FISH with a 22q13-bound ARSA probe and a subtelomeric probe we discovered that the 22q13 region was translocated to the 22p telomere. Investigations of parents are pending. A duplication of 22q13 is rare with only about 20 patients reported so far. So far we have performed 40-50 aCGH analyses on patient samples and detected approximately 10% with pathogenic abnormalities. By combining various molecular genetics methods we are able to detect genetic aberrations in patient samples and each method contribute to the characterisation with unique information. Currently, we are setting up a real-time PCR method for the quantification and verification of delicate imbalances in samples and allowing us to perform even more improved diagnosis and genetic counselling of patients and their families.

P29
IDENTIFICATION OF BIOLOGICAL PATHWAYS INVOLVED IN COLON ADENOMA TO CARCINOMA PROGRESSION

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Introduction. Colorectal cancer arises from premalignant adenomas. The aim of this study was to identify which biological pathways are differentially expressed between colon adenomas and carcinomas.
Methods and Materials. Microarray-expression data of 34 adenomas and 30 carcinomas were analyzed by the program Pathway Level Analysis of Gene Expression (PLAGE). PLAGE identifies differentially expressed gene groups from a collection of predefined gene sets by comparing the activity levels of each pathway among two sample groups. PLAGE offers the possibility to include 135 predefined KEGG and 259 Biocarta pathways, as well as custom defined pathways. In addition to the KEGG and Biocarta pathways, several expression signatures published in literature that originated from microarray-expression data regarding different biological processes and entities were also analyzed.
Results. Data analysis by PLAGE using only the predefined KEGG and Biocarta pathways, revealed several pathways that were significantly differentially expressed between colon adenomas and carcinomas. Most of these pathways were involved in amino acid and lipid metabolism. When experimental derived expression signatures were included in the PLAGE-analysis, significant differences were found in five out of ten signatures tested. An expression signature that discriminated colorectal adenomas from carcinomas (Lin et al, 2002), also turned out to be significant in our dataset ($p<0.0001$). Moreover, significant differences were observed for two chromosomal instability signatures (Carter et al, 2006) - CIN25 ($p=0.0001$) and CIN70 ($p<0.0001$), proliferation (Whitfield et al, 2006; $p=0.0001$), as well as metastazation signatures (Li et al, 2006; $p<0.0001$). To our knowledge none of these pathways were previously tested in colorectal tumors.

Compared to the KEGG and Biocarta pathways, these five experimental derived pathways all rank in the top 15 significant pathways.
Conclusions. Colorectal adenoma to carcinoma progression is associated with significant changes in gene expression levels in multiple cancer associated biological processes like proliferation, metastazation and chromosomal instability.

P30
GENETIC DISSECTION USING A NOVEL WHOLE GENOME TILING PATH BAC ARRAY REVEALS DRAMATIC HETEROGENEITY IN TORIELLO-CAREY SYNDROME, INCLUDING A NOVEL MICRODELETION SYNDROME AT 22q12

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Introduction. The use of array-based Comparative Genomic Hybridization (aCGH) has substantially increased the success rate in detecting pathological chromosomal lesions in a variety of disorders.
Methods and Materials. We have used aCGH to study 24 individuals with Toriello-Carey Syndrome (OMIM#217980-TCS), a multiple congenital anomaly syndrome characterized by corpus callosum abnormality, cardiac defects, cleft palate/Robin sequence, hypotonia, mental and postnatal growth retardation, distal limb anomalies and suggestive facial dysmorphology.
Our aCGH analysis was based on a novel high-resolution tiling-path bacterial artificial chromosome (BAC) microarray, designed in collaboration with the Roswell Park Cancer Institute. This microarray was constructed using end-to-end placement of ~19,000 RPCI-11 BACs resulting in over 93% coverage of the euchromatic genome at a resolution of ~160 KB. The lack of significant end overlap of BACs in our design has resulted in an array that uses significantly less clones than others described.
Results. We have found significant heterozygous deletions in 4 cases. In 2 cases, the deletions (~6MB) involve the same region of chromosome 22q12, suggesting a novel microdeletion syndrome. Deletions were also found in one individual at 1q42 (~6MB) and in another at 6q15 (~5MB). Furthermore, we have also studied a patient with a de novo balanced translocation involving 2q33 and are aware of two further cases – one with a complex rearrangement involving chromosome 4, and one associated with a 8:18 translocation.
Conclusions. We have found evidence for 4 additional loci in this condition, bringing the total to at least 6. Further work is required to determine which genes within these heterozygous deletions are significant in the aetiology of the syndrome.

P31
GENE REARRANGEMENTS IN PAPILLARY THYROID CARCINOMAS FROM RET/PTC3 AND TRK-T1 TRANSGENIC MICE

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Rearrangements of genes along the MAP kinase pathway are frequent alterations in papillary thyroid carcinomas. To investigate whether additional gene alterations are present in these tumours we investigated tumours from RET/PTC3 and TRK-T1 transgenic mice for chromosomal imbalances. Therefore, in a first step conventional comparative genomic hybridisation (CGH) has been performed. A subset of tumour samples also has been analysed by array CGH using 1 MB mouse BAC arrays. CGH of 24 tumours (17 TRK-T1 and 7 RET/PTC) revealed 37 gains and 70 losses. The most frequent imbalances became apparent on chromosomes 1, 3, 4 and 14. In three tumours (one RET/PTC3 and two TRK-T1 tumours) a deletion on 14E5 (RET/PTC) and 14E4-E5 (TRK-T1) has been detected. This deletion has been further narrowed down by positional cloning using 26 BAC clones and was confirmed by FISH with BAC clones RP23-154G18 to RP23-378E14 indicating a

deletion of 1.5 Mb. Within this region SLITRK5 has been identified. Preliminary results indicate a down-regulation of SLITRK5 also in human tissue samples. Array CGH has been performed on six cases. In each case a deletion on chromosome 4 (4D1 – E2) (33.6 Mb) has been detected. Minimal alteration analysis and subsequent confirmatory studies by interphase FISH will allow the identification of further candidate genes involved in the tumourigenic process. The findings indicate that additional gene alterations are present in RET/PTC3 and TRK-T1 positive tumours. Thus, it appears that multiple genetic changes are necessary for malignant transformation of epithelia thyroid cells.

P32
DNA COPY NUMBER CHANGES IN HISTOLOGICAL TYPES OF INVASIVE BREAST CANCER

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Introduction. The classification of breast tumors by the World Health Organization (WHO) distinguishes 30 different morphological types of breast cancers. The rare 'special type' breast carcinomas exhibit favorable prognostic features, but are often not recognized and subsequently classified as invasive ductal carcinoma, not otherwise specified (IDC NOS). The genetic make-up and relationship between the histological special type breast tumors are largely unknown. Gene expression profiling - of 113 samples of 11 histological breast cancer types revealed novel relationships between the special types of breast tumors, and identified molecular profiles that distinguished groups of histological subtypes with favorable outcome (Weigelt et al., Cancer Research, submitted). The goal of the study presented here is to identify DNA copy number alterations and the relationship between DNA copy number, gene expression and the histological special type breast tumors.
Methods and Materials. We isolated DNA of 94 tumors of 11 histological breast cancer types including: invasive lobular, tubular, medullary, metaplastic, mucinous type A and B, apocrine, endocrine, micropapillary, adenoid cystic carcinoma as well as invasive ductal carcinoma with osteoclastic giant cells. Comparative genomic hybridization was performed using a 3.2K BAC array. For all samples gene expression-profiling data from 35k oligo microarrays was available. DNA copy number changes are compared with gene expression data for all the samples.
Results. The results of the DNA copy number changes and their comparison with gene expression profiling and validation by immunohistochemistry on tissue microarrays are presented. Special emphasis is placed on the correlation of our data with DNA copy number alterations of invasive ductal carcinomas not otherwise specified.
Conclusions. We expect that our findings will help establish a link between genetic changes and gene expression profiles that determine tumor cell behavior in breast cancer. Our results will improve not only the understanding of the histogenesis of breast cancer subtypes and their interrelationships, but will also enable better prognostic stratification since some special-type breast cancers exhibit a particular favorable prognosis.

P33
ACTIVATION OF SRC KINASES DOWNSTREAM A NOVEL COMPLEX CHROMOSOMAL TRANSLOCATION INVOLVING CHROMOSOMES 2, 3 AND 5 ASSOCIATED WITH CML RESISTANCE TO IM THERAPY

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Introduction. Imatinib (IM) resistance is a highly relevant issue in CML therapy. More evidence emerges of the role of Src family kinases (SFK) in both Bcr-Abl dependent as well as independent mechanisms of resistance and disease progression. Here we present a CML patient with hematological resistance to IM therapy with activated SFK. The patient was diagnosed as Ph-positive CML with additional change involving chromosomes 2, 3 and 5, forming a novel complex translocation.
Methods and Materials. The conventional cytogenetics, FISH with locus specific, painting, armspecific paints and BAC probes, M-FISH and arrayCGH were used to define additional complex translocation. Phosphospecific antibody was used for determination of activated SFK and Q-RT-PCR to follow BCR-ABL transcripts.

Results. Using all mentioned methods the chromosomal aberration was precisely determined as a complex rearrangement created by translocation, insertion, inversion and deletions, which were determined by arrayCGH. Although the BCR-ABL positive clone was not RT-PCR detectable after 13 months of IM therapy, the clinical course of the disease worsened with appearance of the symptoms identical to those before diagnosis of CML and the number of cells with derivative chromosomes 2, 3 and 5 remained almost the same (>80%). Western blot analysis showed activated SFK probably downstream the novel complex translocation and suggesting possible therapeutic target for dual Abl/SRC kinase inhibitors. Our findings of Ph-negative clones with complex translocation at the time of diagnosis as well as the clinical course before CML diagnosis also suggested that the complex translocation has occurred prior to the acquisition of the Ph-chromosome.
Conclusions. Our study brings further evidence of possible multistep model for the development of CML and confirms importance of the complex analysis of patient´s leukemia cells with clinical impact for targeted therapy of the disease.

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P34
INTEGRATED GENE COPY NUMBER AND EXPRESSION ANALYSIS OF 38 PRIMARY GASTRIC TUMORS

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Introduction. Aberrant gene copy number and its reflection on gene expression is a fundamental factor in solid tumorigenesis. In general, gastric cancers have an extremely poor survival rate since most tumors are detected only at an advanced stage. Identifying gene copy number and expression level changes that drive carcinogenesis is essential for improving early stage diagnosis and therefore prognosis of gastric cancers.
Methods and Materials. Our approach was to map chromosomal alterations in 38 primary gastric tumors and to integrate these results with the gene expression profiles of the same tumors. Gene copy number and expression levels were measured with Agilent's 12K cDNA and 44K oligo arrays, respectively. Gene copy numbers were analyzed using CGH Explorer software and a statistical permutation analysis was then performed with the integrated expression data to find genes whose expression is deregulated due to altered copy number. Tissue and lysate arrays will be used to further validate the selected candidate genes at the protein level in nearly 400 gastric tissue samples.
Results. The most common chromosomal aberrations were gains at 17q12-21, 20q, 8q and X and losses at 4q. Integration of copy number and expression data revealed 895 amplified and overexpressed genes as well as 125 deleted and underexpressed genes. These genes included many known gastric cancer related genes such as MYC (alterations in 21% of the tumors), ERBB2 (20%), GRB7 (18%), MUC1 (11%), and VEGF (11%) as well as novel target genes such as GNAS (29%), HOXA9 (13%) and SMURF1 (13%).
Conclusions. In summary, using whole-genome approach for integration of copy number and gene expression microarray data, we have identified several candidate genes potential as oncogenic markers for gastric cancer. The protein level validation will give additional information about the significance of these identified targets in a larger tumor material.

P35
CHARACTERIZATION OF AMPLICON 17P BY ACGH

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Introduction. Amplifications at 17p, with minimal common region in 17p11.2-p12, are commonly found in 13-32% of high grade osteosarcoma (OS), 24-40% of leiomyosarcoma (LMS), and in 16-43% of malignant fibrous histiocytoma (MFH) of soft tissue, but are rare in other malignancies. Targets of the 17p11.2-p12 region in high-grade OS have been reported, whereas no targets of 17p have been defined in LMS and MFH. We aimed to characterize chromosome 17p and targets therein in high-grade OS, high- and low-grade soft tissue LMS, and high-grade MFH of soft tissue.

Methods and Materials. Two low-grade and two high-grade soft tissue LMS, three OS, and two MFH samples showing gains in 17p by chromosomal CGH were chosen. Array-CGH was performed using oligonucleotide-based human genome CGH microarray 44B (Agilent Technologies) according the manufacturer's instructions. Agilent\'s laser confocal scanner was used for scanning and data from the slide images was acquired with Feature Extraction software v8.1 (Agilent Technologies).
Data was analyzed using Agilent's CGH Analytics software v3.2.32.
Results. Before gain/amplification in17p11.2-p12, p-terminal loss was detected in all but one sample. Minimal common region of loss from the p-terminus spanned TP53-C17orf25 and proximal minimal common region of loss covered TP53-GAS7. The copy number profiles of the minimal commonly gained region (17p11.2-p12) spanned FLJ45455-ULK2, containing 63 genes. This region was continuously gained in low-grade LMS and MFH, whereas in high-grade LMS and OS this region was frequently interrupted by gains and amplifications.
Conclusions. The size and copy number status of 17p differed between the studied sarcoma entities. OS and high-grade LMS showed a discontinuous pattern of amplifications and gains, whereas MFH and low-grade LMS showed continuous pattern of gains. Precise boundaries and target genes within the lost or gained regions were also determined.

P36
DUPLICATION AND REARRANGEMENT OF THE MYB ONCOGENE IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction. T-ALL is an aggressive T-cell malignancy that is most common in children and adolescents. Leukemic transformation of thymocytes is caused by a multistep process involving mutations that affect proliferation, survival, differentiation, cell-cycle control, and stem-cell maintenance. Molecular analysis has identified a variety of specific genetic alterations such as deletion of CDKN2A, ectopic expression of transcription factors, episomal amplification of NUP214-ABL1, and mutation of NOTCH1.
Methods and Materials. In order to detect novel genomic imbalances in T-ALL that can not be detected by standard cytogenetics, we have performed array-CGH, using an array with genomic BAC and PAC probes with an overall resolution of 1Mb over the entire genome, and additional probes around known and candidate oncogenes.
Results. An initial screening of 27 T-ALL samples by array-CGH identified an acquired duplication of the MYB oncogene in 2 patients with normal karyotype and in 3 T-ALL cell lines. FISH located the extra copy of MYB at chromosome 6q. Screening of an independent set of 107 T-ALL patients by quantitative PCR showed that the frequency of MYB duplication in T-ALL was ~8.4% (9/107). In addition, we identified a patient with T-ALL and t(6;7)(q23;q35) with a breakpoint in TCRbeta on 7q35, and the MYB locus on 6q23. The mean expression level of MYB was found to be significantly elevated in T-ALL cases with MYB duplication compared to cases without the duplication. MYB duplication was frequently associated with NOTCH1 mutation in cell lines and T-ALL patients, suggesting that MYB cooperates with NOTCH1 in the pathogenesis of T-ALL. We show that siRNA knock-down of MYB expression results in a partial differentiation, indicating that MYB expression is required to maintain a more immature state of the T-cells.
Conclusions. These data identify the duplication of MYB as a recurrent alteration in T-ALL and suggest that MYB may represent a novel target for therapy.

P37
ARRAY-CGH INVESTIGATIONS IN PATIENTS WITH UNCLEAR SYNDROMIC NEPHROPATHIES IDENTIFIES A MICRODELETION IN XQ22.3-Q23 IN A FEMALE PATIENT WITH ALPORT SYNDROME, MENTAL RETARDATION, AND FOCAL EPILEPSY

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Introduction. Molecular cytogenetic techniques such as array-based CGH have been instrumental in the identification of microimbalances associated with syndromic phenotypes. In this study, patients with unclear syndromic nephropathies are being analyzed.
Methods and Materials. So far, we investigated ten patients with nephropathies and additional clinical features, such as mental retardation, heart defects or growth abnormalities. Array-CGH analysis was performed with a whole-genome array with 8000 large insert clones providing an average resolution of <0.5Mb.
Results. In one 16-year old female patient that presented with microhematuria, glomerular proteinuria, mental retardation including severe speech impairment, senso-neuronal hearing loss, and focal epilepsy, we detected a microdeletion in chromosomal bands Xq22.3-q23. This deletion was verified by FISH, found to be uniallelic, 2.2-3.7Mb in size, and not to be inherited from the mother. X-inactivation studies in the patient are ongoing. A kidney biopsy was performed. Electron microscopy showed splitting of the lamina densa and a thin basement membrane, which is diagnostic for Alport syndrome. By cranial magnetic resonance imaging (MRI), subcortical heterotopia was detected. Presently, 3-tesla MRI including white fibre tracking is being performed to further characterize the brain lesion.
Conclusions. In ten patients with unclear syndromic nephropathies, we identified a female with a contiguous gene syndrome at Xq22.3-q23. The microdeletion includes the X-linked Alport syndrome gene COL4A5 and the LISX gene associated with subcortical heterotopia, mental retardation and epilepsy. Thus, the phenotype observed in our female patient combines features of the AMME-complex (Alport syndrome, mental retardation, midface hypoplasia, elliptocytosis) with X-linked lissencephaly.

P38
MOLECULAR GENETICS OF DISEASETITLE: COMBINED HIGH RESOLUTION ARRAY CGH AND EXPRESSION PROFILING OF ETV6/RUNX1-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIAS REVEAL A HIGH INCIDENCE OF CRYPTIC XQ DUPLICATIONS AND IDENTIFY THE TESTIS-SPECIFIC SPANXB AS A PUTATIVE TARGET GENE

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Introduction. In pediatric B-cell precursor acute lymphoblastic leukemia (ALL), the most common structural genetic abnormality is the t(12;21)(p13;q22) which results in the ETV6/RUNX1 fusion gene. As ETV6/RUNX1 by itself is not sufficient for leukemic transformation, it is believed that additional mutations subsequent to the fusion gene are necessary for overt leukemia.
Methods and Materials. Seventeen ETV6/RUNX1-positive pediatric ALLs were investigated by 32K high resolution array-based comparative genomic hybridization (array CGH), 27K gene expression profiling, and fluorescence in situ hybridization (FISH).
Results. Comparing the array CGH and gene expresion patterns revealed that genomic imbalances conferred a great impact on the expression of genes in the affected regions. The array CGH analyses identified a high frequency of cytogenetically cryptic genetic changes, e.g., del(9p) and del(12p). Interestingly, a duplication of Xq material, varying between 30 and 60 Mb in size, was found in 6 of 11 males (55%). Genes on Xq were found to have a high expression level in cases with dup(Xq); a similar overexpression was confirmed in t(12;21)-positive cases in an external gene expression data set. By using gene expression profiling and reverse transcription polymerase chain reaction, the testis-specific gene SPANXB, located in the commonly gained Xq25-28 region, was shown to display a higher expression in ETV6/RUNX1-positive ALLs compared to other B-lineage ALLs. The high expression of this gene also in ETV6/RUNX1-positive ALLs from female patients may be associated with demethylation of both its alleles.
Conclusions. The present data reveal that dup(Xq) is a frequent abnormality in t(12;21)-positive childhood ALL and suggest an important pathogenic role of SPANXB in this malignancy.

P39
GENE COPY NUMBER CHANGES IN MALIGNANT PLEURAL MESOTHELIOMA – AN ARRAY COMPARATIVE GENOMIC HYBRIDIZATION STUDY

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Introduction. Mesothelioma is an aggressive malignancy in the mesothelial cells lining the chest and abdominal cavity, such as the pleura and peritoneum. Mainly linked to previous asbestos exposure, it is a rare disease characterized by multiple chromosomal aberrations. Histological mesothelioma subtypes have been shown to correlate with respective prognoses. Our aim was to find a more specific pattern of chromosomal aberrations in malignant pleural mesothelioma. *Methods and Materials.* The mesothelioma samples comprised the epithelioid and sarcomatoid subtypes, but not the biphasic subtype. DNA from fresh-frozen and paraffin-embedded tissues was extracted, digested, and hybridized to Agilent\'s oligonucleotide 44B arrays with reference DNA, according to the manufacturer's instructions. The data was extracted and analyzed using Feature Extraction (v. 8.1) and CGH Analytics (v. 3.2.32) software (Agilent Technologies). The results were compared with clinical data of the patients.
Results. The array CGH results showed a pattern of multiple chromosomal aberrations in mesothelioma. The number of aberrations in the samples ranged from zero to 46. The most prominent homozygous deletion was in 9p21, including CDKN2A. The most common recurrent losses were found at 1p13.3p21.1, 3p14.2p22.1, 6q24.1q25.3, 13q11q14.3, 14q, and 22q. Previously unreported gains included 9p13.3, 7p22.2p22.3, 12q13.2, 16p13.3, and 17q24.3q25.3. *Conclusions.* These results show a pattern where losses are the most common type of aberrations, and the frequency of high-level amplifications is low. The sarcomatoid subtype correlates with a worse prognosis, and the results implicate some distinct aberrations between the epithelioid and sarcomatoid subtypes.

P40
CHARACTERIZATION OF TWO DISEASE ASSOCIATED, APPARENTLY BALANCED, COMPLEX CHROMOSOME REARRANGEMENTS

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Balanced, de novo, complex chromosome rearrangements are extremely rare, and little is known about the frequency of sub-microscopic imbalances in these patients. The over-all incidence of de novo balanced chromosome rearrangements is approximately 1:2000 newborns. In this group of children, there is an increased risk of congenital abnormalities and mental retardation, which has been estimated to 6%. This may be explained by the disruption or inactivation of specific genes or sub-microscopic rearrangements that cannot be detected by traditional cytogenetic methods. An unexpected complexity in genome imbalance has recently been described in patients with apparently balanced translocations presenting with an abnormal phenotype. We have studied two patients with developmental delay of varying degree and dysmorphic features. Traditional karyotyping with G-banding showed that both patients had apparently balanced, complex rearrangements involving three chromosomes. Patient 1: 46,XY,t(1;5;10)(q32;q31;p12) and Patient 2: 46,XX,t(2;8;15)(q35;q24.1;q22).
FISH-mapping of the breakpoints was performed using BAC clones that were chosen according to their mapped position in Ensembl and ordered from CHORI or the Sanger Institute. High-resolution array-CGH using a 33K tiling resolution BAC array has so far been performed on one patient, but will be performed on both. The detailed results will be presented at the meeting.

P41
DISRUPTION OF A CLUSTER OF CONSERVED NON-CODING ELEMENTS BY A TRANSLOCATION BREAKPOINT DISTAL TO HMX2/HMX3

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Introduction. It was recently discovered that not only can disruption of important developmental genes cause disease, but so can disruption of the regulatory landscapes surrounding these genes, e.g. by translocations that remove cis-regulatory elements. Here we present a balanced t(10,13)(q26;q31)de novo translocation, in a patient with mental retardation, deafness, epilepsy and ataxia and both chromosomes breakpoints within gene deserts. The objective of this

study is to identify regulatory sequences related to the candidate genes associated with the 10q26 breakpoint and contribute to the understanding of the etiology of mental retardation associated with deafness, ataxia and epilepsy.
Methods and Materials. Chromosomes were prepared from peripheral blood lymphocytes cultures and by analysis of GTG-banded chromosomes a t (10;13)(q26;q31) was identified. The translocation breakpoints were mapped by fluorescence in situ hybridisation (FISH) using bacterial artificial chromosomes (BACs) from the RP11 library. The functional assay was performed as previously described.
Results. Both breakpoints were mapped by FISH to gene deserts downstream of the homeobox genes HMX2 and HMX3, on chromosome 10q26, and downstream of SLITKR5 on chromosome 13q31. The breakpoint on chromosome 10q26 disrupts a cluster of conserved non-coding elements (CNEs) associated with HMX2 and HMX3. The disrupted elements downstream of the breakpoint on chromosome 10q26 have been studied in vivo by a functional assay using zebrafish embryos. Four of these elements revealed GFP expression in the zebrafish central nervous system and sensory organs.
Conclusions. CNEs probably function as tissue-specific enhancers important for the diverse spatio-temporal functions of the associated key developmental genes. In the mouse, HMX2 and HMX3 are co-expressed in the developing central nervous system, including neural tube, hypothalamus and inner ear, overlapping the GFP expression associated with 4 of the 6 elements tested. The GFP expression patterns in the zebrafish embryos are compatible with the hypothesis that the mental retardation, epilepsy, ataxia and deafness in the translocation carrier could be caused by long range position effects on HMX2/HMX3.

P42
USEFULNESS OF ARRAY-CGH FOR VERIFICATION AND MAPPING OF CRYPTIC CHROMOSOME IMBALANCES IN 89 PATIENTS WITH PSYCOMOTOR DEVELOPMENTAL DELAY

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Introduction. Delayed psychomotor development occurs in 2% - 3% of the general population, but the etiology remains unexplained in about half of the cases. Improved chromosomal characterization, together with more knowledge of the genotype-phenotype relations, will improve our ability to give these patients an accurate diagnosis. Submicroscopic chromosomal imbalances are a major cause of psychomotor delay and malformations, and many of these imbalances are so small that they are overlooked or undetectable by conventional cytogenetic methods. Application of molecular cytogenetic techniques allowing whole-genome screening at high resolution, have improved the detection of submicroscopic chromosomal imbalances in patients with psychomotor delay. We have previously reported the detection of imbalances in 10% of patients with psychomotor delay having an apparently normal karyotype, by the use of high-resolution comparative genomic hybridization (HR-CGH) [1]. The development of microarray based comparative genomic hybridization (array-CGH), enables us to investigate the genome at a much higher resolution than HR-CGH, indicating an improvement of the detection of chromosomal imbalances. In this study we wanted to compare the detection efficiency of the HR-CGH method with the 1Mb array-CGH method.
Methods and Materials. 554 patients with delayed psychomotor development were included in the study. G-banding, HR-CGH, and 1Mb array-CGH were used in the study.
Results. 554 patients with delayed psychomotor development, all former found normal by G-banding, were screened by HR-CGH analysis. Imbalances were found in 49 patients (9%), but array-CGH was normal in nine of these patients (18%). Thus a positive (and de novo) chromosome imbalance was found in 40 of the 554 patients, giving a HR-CGH detection rate of 7.2%. The detected imbalances included: 29 deletions (average size 7 Mb), 3 duplications, 4 unbalanced translocations and 4 occult trisomy mosaicisms (three of them having mosaic trisomy 9). 1Mb array-CGH was also used for mapping of smaller deletions and duplications suspected in 36 patients after routine G-banding. Finally, in 4 out of 20 patients with normal findings on both routine karyotyping and HR-CGH, a de novo deletion (3 patients) or duplication (1 patient) was detected by array-CGH, giving a detection rate of 20%. In total, chromosomal imbalances were mapped by 1Mb array-CGH in 89 patients.
Conclusions. 1Mb array-CGH has proven to be a very useful tool for verification and mapping of genomic imbalances, and the array-CGH method will in the future

be an important diagnostic tool for characterization of patients with delayed psychomotor delay.

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P43

ASSOCIATION ANALYSIS OF ALCOHOL DEHYDROGENASE ADH1B AND ADH1C GENE POLYMORPHISMS WITH ALCOHOL DEPENDENCE IN RUSSIAN POPULATION OF WEST SIBERIA

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Introduction. Alcohol-related health and social problems are of great public concern in many countries. Ethanol-metabolizing enzymes have been repeatedly associated with protection against alcoholism. ADH1B*47His protective effect allele was demonstrated in many populations, while ADH1C gene polymorphisms association are controversial. The relationship between alcohol dehydrogenase ADH1B exon 3 A/G (Arg47His) and ADH1C intron 2 A/C gene polymorphisms and the individual predisposition to alcoholism was investigated.
Methods and Materials. The genotypes of ADH1B, ADH1C were examined in 174 male aged less than 30 years (92 alcoholic subjects and 82 healthy controls). Genotyping was performed using PCR-RFLP methods. The estimations of difference in allele frequencies, Hardy-Weinberg equilibrium, linkage disequilibrium by Hill (LD) and odds ratio (OR) was carried out with the Pearson chi-square statistics.
Results. The genotype frequencies of ADH1B and ADH1C obeyed the Hardy-Weinberg equilibrium in both groups. The derived allele frequencies ADH1B*G and ADH1C*C were 1.5 and 25.0% in alcoholic, 9.3 and 29.9% in control groups, respectively. A near significant decrease in ADH1B*G allele frequency in the patient group was revealed. In individuals with ADH1B*G allele OR and 95% confidence interval (CI) for alcoholism was 2.19 and 0.84-5.83 (P=0.076). There was no association ADH1C*A/C polymorphism with alcohol dependence (P=0.308; OR=1,28; 95% CI 0.78-2.11). A near significant LD in control group was observed (P=0.058). LD was not revealed in the alcoholic group. The haplotype frequencies in patient and control groups were:
ADH1B*A_ADH1C*A 71.7 and 60.5%;
ADH1B*A_ADH1C*C 23.9 and 30.2%;
ADH1B*G_ADH1C*A 3.3 and 9.3%;
ADH1B*G_ADH1C*C 1.1 and 0.0%, respectively.
Conclusions. The ADH1B*G allele displayed moderate association with alcohol dependence in Russian population of West Siberia. Our results indicate that it is necessary to analyse haplotype structure in ADH gene cluster to uncover the susceptibility to alcoholism.

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P44

ARRAY CGH ANALYSIS REVEALED A DELETION OF 6P25.3-P24.3 MATERIAL AND A DUPLICATION OF 8Q24.22-Q24.3 MATERIAL PREVIOUSLY UNIDENTIFIED IN A PATIENT WITH SEVERE MENTAL RETARDATION AND DYSMORPHIC FEATURES

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Array CGH analysis has proven to be a very valuable tool in clinical genetic research to identify submicroscopic deletions and duplications in patients with previously normal karyotypes by cytogenetic examination. We report on an 8-year old girl presenting with mental retardation, rieger anomaly, cardiac anomalies (atrial septal defect, dysplastic pulmonic valve, periphere pulmonic stenosis), hearing impairment, brachycephalus, hypertelorismus, mid-face hypoplasia, short philtrum, teeth anomalies, short neck, and scoliosis. Cytogenetic examination (GTG banding at 550 band resolution) revealed a normal female karyotype 46,XX. We performed an array CGH analysis using the 35k OpArray V4 70mer oligonucleotide chips (Operon, Köln). Our data analysis pipeline includes ImaGene (BioDiscovery) for spot analysis, arrayCGHbase (Menten et al. 2006) for flagging and normalization

procedure, and the CGH-Miner (Wang et al. 2006) tool for the analysis of copy number changes. The array CGH analysis revealed a deletion representing 9.7 Mb of consensus genomic sequence within chromosomal bands 6p25.3-p24.3, as well as a duplication representing 14 Mb of genomic sequence covering chromosomal band 8q24.2-q24.3. As previously described, a distinct Axenfeld-Rieger phenotype, with hearing impairment and cardiac defects, is correlated with distal 6p deletion including the FOXC1 gene located in 6p25.3 (Maclean et al. 2005, Le Caignec et al. 2005). The FOXC1 gene lies within the deleted chromosomal region 6p25.3-p24.3 detected by the array CGH analysis. Thus, array CGH resolved a chromosomal imbalance, which was not detectable by conventional cytogenetic analysis. FISH experiments will be performed in order to identify the nature of the aberration.

P45
GET A HANDLE ON ALL YOUR ARRAYCGH DATA: EASY, EFFICIENT, AND FREE

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A single arrayCGH experiment can result in tens of thousands of rows of data. A typical genetics lab can produce millions of these data in a single week. By a simple volume-based definition, today's genetics lab has become an information technology center. There are many tools available to handle and store all of this data - some may be purchased, some are free (open-source), and some may be developed in-house by individual labs. Our lab has chosen a combination of the last 2 choices - we're not only using the open-source application ArrayCGH*base* (Menten et al. 2005), we've also begun a collaborative project with the original (and other) developers of the application. The result is a strong, stable, well-documented and supported application that is available to the research community at large.
This paper will discuss details of our collaboration, such as:

- A summary of new and improved features of the application (improved normalization techniques, new reports, inclusion of R-based analysis tools, etc).
- Providing well-written documentation to our users, relating to installation, application usage, backup and recovery strategies, and communication of bugs and feature requests back to the development group.
- Allowing for growth of the collaboration; establishing processes and procedures for additional coders to submit new features, including testing, documentation, and coding requirements.
- Integration of this application with existing analysis "pipelines" that may already exist or be subsequently developed in the lab.
- Using established IT "best practices" (use-case documentation, quality assurance, network-enabled source code management tools for remote users).

Additional aspects of open-source development (as it relates to the academic and research environment) will be explored and addressed in this paper.

P46
STROMA CONTENT OF COLON CARCINOMAS IS AFFECTED BY LOSS OF 9Q34 AND 1P36

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Introduction. In addition to accumulation of somatic mutations in neoplastic cells, tumor development is also influenced by non-neoplastic stromal cells like fibroblasts, macrophages, and endothelial cells, which affect processes like immune surveillance, angiogenesis, and metastasis. The aim of this study is to identify chromosomal aberrations that affect stroma composition of colon carcinomas.
Material and methods. A panel of 23 human colon carcinomas was used to evaluate genome-wide gains and losses of chromosomal segments by BAC-array Comparative Genomic Hybridization (array-CGH). Stroma content of these tumors (% non-tumor cells within carcinoma) was determined by quantitative measurements of hematoxylin-eosin stained sections. *Results*. Stroma content varied from 18% to 64%. Array-CGH analysis indicated a total of 39 chromosomal segments that exhibited gains or losses in more than 20% of colon carcinomas. Gain of 20q was the most frequently observed genomic alteration, in 18 out of 23 carcinomas (78%). Upon statistical evaluation (ANOVA) and correction of P-values for multiple comparisons

(Bonferroni) two regions were identified that affected stroma content significantly, i.e. loss of 9q34 (P=0.002) and loss of 1p36 (P=0.02). Loss of 9q34 was accompanied by a two-fold increase in the total amount of stroma (from 25% to 48%), while loss of 1p36 had the opposite effect (from 44% to 28%). Considering the importance of 20q gain for adenoma-to-carcinoma progression, we repeated this analysis using the subset of colon carcinomas with 20q gain. Loss of 9q34 and 1p36 still affected stroma content significantly (P=0.001 and P=0.01, respectively). In addition, gain of 8q was now also significantly associated with increased stroma content (from 31% to 44%; P=0.04).
Conclusion. Collectively, these data indicate that certain somatic mutations affect tumor stroma composition, implying that they modulate functional characteristics of the tumor microenvironment.

P47
TUMOR NECROSIS FACTOR ALPHA -308 GA/AA GENOTYPES ARE ASSOCIATED WITH HIGH INFLAMMATORY ACTIVITY AND HIGH SERUM TUMOR NECROSIS FACTOR A IN JUVENILE IDIOPATHIC ARTHRITIS PATIENTS

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Purpose. The aetiology of Juvenile Idiopathic Arthritis (JIA) is still unknown. Environmental and genetic factors play an important role in the development of the disease. Considering the relevance of Tumor Necrosis Factor alpha (TNFalpha) in the physiopathology of JIA, it is possible that polymorphisms in the promoter area of this gene may be relevant for the susceptibility and activity pattern of the disease. The -308 TNFalpha position has been shown to correlate with disease activity and response to treatment in Portuguese patients with Rheumatoid Arthritis.
Objectives. To verify if clinical measures of JIA activity and TNFalpha serum levels were influenced by TNF-alpha -308 genotypes.
Material and methods. Patients with the diagnosis of JIA followed in 3 Hospitals in the Lisbon area were consecutively recruited. A protocol was applied including demographic and clinical data (number of joints with active disease and/or limited range of motion, visual analogue scale for disease activity (VAS), patient's functional status, as evaluated by the Portuguese version of childhood health assessment questionnaire (CHAQ)). A blood sample was collected from each patient for the determination of erythrocyte sedimentation rate (ESR) and TNF-alpha serum levels (ELISA). DNA was extracted for analysis of the TNFalpha gene at position -308 by Restriction Fragment Length Polymorphism. Genotyping of the same polymorphism was also performed in a sample of healthy controls.
Results. Eighty patients were evaluated, 52 females and 28 males, with a mean age of 12,6 ± 5,9 years.The mean disease duration was 6,46 ± 0,06 years and the mean follow-up duration was 4,86 ± 4,9 years. Fifty-one patients presented the oligoarticular subtype, 15 the polyarticular subtype, 7 the systemic subtype, 5 had enthesitis-related arthritis and 2 presented psoriatic arthritis. In the study group, sixty-six (82,5%) patients presented the -308 GG genotype and 13 (17,5%) the -308 GA/AA genotypes, and in the control group 48 (75,0%) patients presented the -308 GG genotype and 16 (25%) the -308 GA/AA genotypes. Patients with the polyarticular subtype presented a higher frequency of the -308 GA/AA genotype (40%) as compared to the patients with the oligoarticular subtype (8%). Patients with the -308 GA/AA genotypes had a significantively higher degree of functional impairment as compared to those with the -308 GG genotype (CHAQ= 0,58±0,71 vs 0,17±0,34, $p<0,05$). In addition, in comparison with those with the -308 GG genotype, patients with the -308 GA/AA genotype had a trend for a higher ESR (29,4±27,5 vs 13,7±10,6, $p<0,05$), VAS 21,7±26,8 vs 14,6±21,5) and TNF alpha levels (406,0±598,9 vs 99,6±129,0, $p<0,05$).
Conclusions. These results suggest that the TNFalpha -308 GA/AA genotypes are associated with a lower functional capacity, polyarticular involvement, higher inflammatory activity and higher TNFalpha serum levels. Although, they do not appear to have a relevant role in the susceptibility for JIA.

P48
GENETIC PROFILING OF CHROMOSOME 1 IN BREAST CANCER

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Chromosome 1 is involved in quantitative anomalies in 50-60% of breast tumors and interestingly in tumors presenting low level genomic aberrations. However, the structure of these anomalies and the identity of the affected genes remain to be determined. Our aim was to characterize these anomalies and define their consequences on gene expression in breast cancer cell lines and primary tumors. We studied 30 breast cancer cell lines and 30 primary breast tumors using specialized arrays for array CGH and expression analysis. In order to validate our data and statistical methods, we assessed copy number and relaltive expression of candidate genes using Q-PCR. Array-CGH data showed that 1p was predominantly involved in losses and 1q almost exclusively in gains. Noticeably, high magnitude amplification were infrequent. In an attempt to fine map regions of copy number changes we defined 19 shortest regions of overlap (SRO) for gains (1 at 1p and 18 at 1q) and of 20 SROs for losses (all at 1p). These SROs, whose sizes ranged 170 Kb to 3.2 Mb, represented the smallest genomic intervals possible based on the resolution of our array. To identify genes with modified expression in relation with genomic alterations, we studied expression profiles of 307 genes located at 1q. Genomic and expression data were compared using statistical approaches. We identified 30 candidate genes showing significant overexpression correlated to copy number increase. Q-PCR showed that 11/29 genes were significantly overexpressed in presence of a genomic gain, while 21/28 genes were overexpressed when compared to normal breast. To the exception of (PLU-1/JARID1B) all the genes identified in our study were newly proposed as candidate cancer genes. Genes selected in our study belong to diverse functional groups such as positive regulators of cell proliferation (PIP5K1A, MAPBPIP, RAB25A, PCTK3, RAB4 and MPZL1), transcriptional regulators or chromatin remodeling (USF1, JARID1B, TBX19, CROC4) or cellular trafficking (VPS45A, ARF1, LYST, CCT3). Our study mapped at high resolution regions of loss and gain on the whole length of chromosome 1 and proposed a series of candidate genes affected by copy number changes.

P49
THE CD14 FUNCTIONAL GENE POLYMORPHISM -260 C>T IS NOT INVOLVED IN

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Introduction. C. trachomatis is the most prevalent STD in Europe and the USA. Clear differences in the clinical course of infection have been described and are due to an interaction between environmental, bacterial and host factors. A limited number of studies have recently demonstrated the influence of host genetic factors on the susceptibility to and the severity of CT infection. The promotor region of the CD14 gene contains a SNP affecting the binding of transcription factors. We investigated the role of CD14 -260 C>T the susceptibility to and severity of CT infection.
Methods. The study was performed in 2 cohorts of Dutch cuacasian women either visiting the STD outpatient clinic in Amsterdam or the department of Obstetrics and Gynaecology of the azM. Relevant clinic data was collected as well a blood samples for genotyping.
Results. The CD14 genotype distribution was comparable to the healthy controls. No differences could be observe between women with or without positive Chlamydia serology, or in the severity of infection.
Discussion. We did not find an association between the CD14 -260 C>T polymorphism and the susceptibility to or subsequent severity of sequelae of CT infection. However, these results do not exclude that a still unknown CD14 expression decreasing SNP may influence the course of CT infection. Chlamydia LPS is capable of inducing an inflammatory response through CD14. The absence of an association between CD14 and susceptibility to CT infection might be explained by the compartmentalisation of TLR4. The differential expression of TLR4 has been described in immortalised cell-lines derived from the female urogenital tract and recently demonstrated in cells isolated from patients by Pioli and Fazeli. TLR2 and TLR4 were the only Toll like receptors with a clear differential expression in the female urogenital tract. Low expression in the lower

urogenital tract and high expression in the upper genital tract. It is hypothesized that through this expression pattern TLR4 modulates immunological tolerance in the lower genital tract and induces host defence against ascending infection in the upper genital tract. Further studies on the immunogenetics of C. trachomatis infection will provide more insight in the clear differences in the clinical course that this microorganism induces in individuals and lead to potential vaccine candidates.

P50
CLONE-END SEQUENCE MAPPING FOR IDENTIFICATION OF STRUCTURAL VARIATION IN THE HUMAN GENOME

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Introduction. By mapping sequence reads from clone-ends onto the reference assembly the expected size and orientation of clone-ends can be used to infer insertions, deletions and inversions in the genome. Our lab has adopted this approach using the clone-end libraries created by Celera Genomics for their whole genome shotgun-sequencing project. The average library sizes were 2kb, 10kb and 50kb, and they contained sequences from 5 different individuals. There is one sequence read from each end (a mate pair) of every clone, and the clones are mapped across all parts of the human genome. *Methods and Materials.* Using BLAT and several stringent filtering criteria, we are able to map the mate pairs to their best placement in the reference list assembly. Then we checked for consistent discrepancies in size and orientation in order to infer putative variations. If the distance between sites of a pair-end mapping is smaller than expected, we define the event as a deletion in the donor's genome. If the distance is larger than expected, then we call the discordant event as an insertion. Inversions are identified when the orientation of the mate pair is discordant with the reference genome for each break point. Using the 2kb, 10kb and 50kb libraries, we identified structural variations of length >340bp, >2.6kb, and >18.7kb, respectively. For each donor, we require at least two discordant mate pairs showing similar size, coordinate position and orientation to define a potential variation. *Results and Conclusions.* About 6.25 million of the clone ends demonstrated best placements in the genome, and approximately 0.6% of them were classified as the insert size to be too large or too small. Afterwards, we require at least two discordant mate pairs to show similar size and position to infer mutation events, and with this criterion our preliminary results suggest that there are about 60 deletion and 163 insertion events detected in an individual. We will proceed to characterize inversions, and also we will also perform further analysis to refine the putative breakpoints. This study uses a high-throughput genome-wide approach to identify variation breakpoints. Further experimental and literary confirmations are on-going and will be used to estimate the false-positive rate and fine tune the parameters of the approach needed to elucidate the structure, distribution and role of these variants.

P51
DEVELOPMENT AND VALIDATION OF THE "CHROMOSOME X GENE-SPECIFIC ARRAY" THAT ENABLES IDENTIFICATION OF COPY NUMBER CHANGES IN GENES OF THE X CHROMOSOME

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Introduction. The aim of this study was to design and produce a highly specialized and novel oligonucleotide microarray that enables identification of copy number genetic changes in genes of the X chromosome. Identification of genetic alterations is extremely biologically relevant both for research and clinical purposes. Results may be utilized in diagnosis and genetic counselling for genetic syndromes that they are implicated in or in the identification of new candidate genes. Recent studies have indicated that microdeletions and microduplications occur at a high frequency in the human genome. Analysis by array-CGH approaches, that does possess a much higher resolution, therefore represents a unique opportunity. Recent advances in the production of high density oligonucleotide arrays have paved the way for previously unimaginable coverage and resolution. We took advantage of this new technological advancement and designed an oligonucleotide array covering the exons of the genes on the X chromosome. This ensures a more complete and robust screen of all X

chromosome genes for the identification of genetic changes.
Methods and Materials. The "chromosome X gene-specific array" contains about 22,000 60mer oligonucleotides providing a complete coverage of all X chromosome genes. The array provides coverage of more than 92% of all X chromosome exons and micro RNA (miRNA) regions, as well as regions from other chromosomes serving as controls. Two known abnormal patient control samples with a well characterized known isochromosome X abnormality and with Xp22.2 duplication were interrogated on the array to test its efficiency. The above hybridizations experiments also served a role in identifying probes of the microarray that do not perform efficiently. Following the above testing, modifications were carried out and the final "chromosome X gene-specific array" was developed. The "chromosome X gene-specific array" was then utilized in the screening of 20 XLMR families (kindly provided by the X-Linked Mental Retardation Consortium).
Results. Three out of the twenty families were identified by array-CGH analysis as having copy number changes on X chromosome genes. The genetic changes found in the three families are a 1.78Kb containing all exons of one gene, a 27Kb deletion containing all exons of two genes, 22.4Kb duplication containing one gene. The above first two genetic changes found were confirmed with PCR, while the third one is still under investigation.
Conclusions. We achieve the development and validation of the "chromosome X gene-specific array" that enables identification of copy number changes in genes of the X chromosome. The "chromosome X gene-specific array" can be potentially utilized for research and diagnostic purposes to detect genetic changes in X-linked disorders and other X chromosome abnormalities. The well known "Oxford Gene Technology" (OGT) biotechnology company will manufacture and sell the "chromosome X gene-specific array" within the next few months.

P52
CHARACTERIZATION OF HLA GENETIC POLYMORPHISM IN A PORTUGUESE POPULATION WITH ANKYLOSING SPONDYLITIS

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Introduction. We aim to perform a study regarding the distribution of reported potential gene variants from MHC class I (A,B,Cw) and class II (DRB1, DQB1) in a sample of Portuguese AS patients.
Methods and Materials. Patients with AS diagnosis, according to the New York criteria, followed in our hospital were consecutively recruited. Blood samples were collected from each patient and DNA was isolated for determination of the MHC class I and II polymorphisms by polymerase chain reaction with sequence-specific-primer (PCR-SSP).Genotyping of the same genes was also performed in a sample of 174 healthy controls randomly selected from the Portuguese bone marrow donors registry. Individuals of both populations positive for HLA-B*27 group were further defined at allelic resolution. Genotypes and allele frequencies were calculated from direct counts and HLA multi-locus A/B/Cw/DRB1/DQB1 haplotype frequencies were estimated through an iterative Expectation-Maximization (EM) algorithm (PyPop - http://allele5.biol.berkeley.edu/pypop/). For statistical analyses, Fisher exact test was applied (statistically significant at $p<0.05$).
Results. Fifty patients were evaluated, 21 females and 29 males, with a mean age of 69 ± 13.5 years, mean disease duration: 21.6 ± 14.0 years. Forty-three AS patients (86.0%) were B27-positive compared to 16 (9.2%) of healthy controls confirming these gene high association to
AS susceptibility ($p<0.001$) The distribution of B27 alleles in AS patients was B*2705 (88.4%) and B*2702 (11.6%), and in healthy controls B*2705 (81.3%), B*2702 (6.3%) and B*2707 (12.5%). In this Portuguese population B27 alleles associated with AS appeared to be more frequently carried by the haplotypes A*2 B*27 Cw*2 DRB1*01 DQB1*05 (5.6%), A*24 B*27 Cw*2 DRB1*04 DQB1*03 (4.5%) and A*2 B*27 Cw*7 DRB1*13 DQB1*06 (4.5%).
Conclusion. As in other populations, in Portugal, the presence of HLA-B27 is the highest known genetic factor for susceptibility to AS. The distribution of B*27 allele subtypes is very similar in AS patients and disease free individuals. Others genetic and non-genetic factors must be studied to understand the mechanisms underlying AS susceptibility. This is the first study in which high-resolution B27 genotyping, in the Portuguese population, is performed.

P53
THREE DELETED REGIONS IN A PATIENT WITH A DE NOVO PERICENTRIC INVERSION 6 (P11.5Q15) IDENTIFIED BY A 317 K SNP ARRAY PLATFORM

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Introduction. By GTG banding a patient with characteristic dysmorphic facial features, a striking, non-progressive deficit of motor control, lack of speech development and abstract mental abilities, while well adapted to family life and to a school for mentally retarded persons was found to carry an interstitial deletion 6q14 contained within a pericentric inversion with breakpoints in 6p11.2 and 6q15 as determined by high resolution cytogenetic analysis (E. Passarge, Cytogenet. Cell Genet. 91: 192-198, 2000).
Methods and Materials. A segmental aneuploidy profile of the patient and her parents was obtained with an Infinium 317 K SNP Array (Illumina, San Diego, CA, USA).
Results. We found three separate deletions in 6p12, 6p12.3, and 6q14;q16. The paternal chromosome 6 appeared to be deleted in the patient. The deletion in band 6p12 comprised 1.15 Mb of DNA containing the PKHD1, IL17, MCM3, EFHC1, and TRAM2 genes. The deletion in band 6p12.3 involved a 360 kb stretch of DNA containing the RHAG, CRISP1, 2, and 3, and PGK2 genes. The third deletion of 11.9 Mb in band 6q14;q16, affects 27 known genes, including genes involved in growth regulation and tissue remodeling during development, and pain sensation.
Conclusions. We hypothesize that haploinsufficiency of the genes contained within the deletions may contribute to this patient´s phenotype. This is the first complete mapping of three adjacent, but non-contiguous deletions on a single chromosome by applying the novel 317 K SNP array system.

P54
ARRAYCGH INDETIFIED INTERSTITIAL DEL(14)(q) INVOLVING IGH, A NOVEL RECURRENT ABERRATION IN B-NHL

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Introduction. Chromosomal deletions occurring in human malignancies are regarded as hallmarks for the localization of tumor suppressor genes. To identify the gene(s) targeted by del(14)(q) frequently observed in B-cell malignancies, we initially mapped the deletions using a high-resolution arrayCGH.
Methods and Materials. 23 leukemia/lymphoma cases with del(14) were subjected to chromosome 14-tilling path arrayCGH. This platform covering 14q with a resolution of approximately 42 kb comprises 838 BAC/PAC clones from the Chori 32K set.
Results. ArrayCGH identified 2 categories of del(14)(q): (1) involving the IGH locus at 14q32.33 and (2) not involving IGH. This latter category grouped 9 cases with interstitial deletions ranging in size from 17-70 Mb and distributed along 14q13->ter. 14 cases showed interstitial del(14)(q) involving IGH (loss of 3'IGCH). The proximal breakpoints of these deletions varied in 6 cases and clustered in the one-BAC/14q24.1 region in 8 cases. The latter del(14)(q24.1q32.33) covering approximately 36 Mb was further detected by FISH in 12 additional patients. Further analysis of del(14)(q24.1q32.33) mapped the 14q24.1 breakpoints within and outside of the ZFP36L1 gene and showed clustering of the 14q32.33 breakpoints in the constant region of IGH, proximal to the 5' (Ei) enhancer. These findings suggest that the del(14)(q24.1q32.33), and other analogous IGH-involving del(14)(q), might represent a novel aberration leading to activation of unknown oncogene(s) at 14q by its juxtaposition with IGH enhancer(s). Extensive expression analysis via quantitative PCR and microarray profiling, however, failed to identify a 14q gene uniformly upregulated in cases with del(14) (q24.1q32.33).
Conclusions. IGH-involved interstitial del(14)(q) are novel recurrent aberrations in B-NHL. They likely happened during an illegitimate Ig-class switch recombination and are supposed to operate in a translocation-like manner. The gene targeted by the extensively studied del(14)(q24.1q32.33), however, has remained elusive. Further investigations are needed to unravel the mechanism(s) and role of these deletions in B-cell malignancies.

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P55
CHARACTERIZATION OF THE ROLE OF THE ONCOMIR HSA-MIR-17-92 CLUSTER LOCATED IN 13q DURING COLORECTAL CANCER PROGRESSION

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Recently, a novel class of non-coding RNAs called microRNAs (miRNAs) have shown to regulate central mechanisms of tumorigenesis and contribute to tumour development as tumour suppressors or oncogenes due to altered expression. Chromosomal instability is a major pathogenetic mechanism in colorectal adenoma to carcinoma progression. We have previously documented that in colorectal chromosomal instable tumours, the combination of gain of the chromosomal arms 8q and 13q is one of the major genetic events associated to colorectal cancer (CRC) progression. In CRC, up to one fourth of all annotated human miRNAs are differentially expressed or located on regions of chromosomal instability primary implicated in CRC progression. Functional studies on the hsa-mir 17-92 cluster localized on 13q31 locus have demonstrated that its transcription is activated by the transcription factor c-myc, located on the 8q region and has antiapoptotic, proliferative and angiogenic activities in lymphomas. These results show an important oncogenic function of this miRNA cluster. Therefore, we propose to investigate the contribution of the hsa-mir-17-92 cluster in the tumour biology of colorectal adenoma to carcinoma progression. RNA expression levels of the hsa-mir-17-92 cluster will be determined in 44 carcinomas, 41 adenomas by quantitative RT-PCR. The identification of putative target genes of hsa-mir-17-92 cluster in CRC is being accomplished by integrating the already existing array comparative genomic hybridization (aCGH), mRNA expression data from this same group of tumours, with the computational miRNA target prediction programs TargetScan and Pic Tar-Vert. We have set up quantitative RT-PCR experiments to determine the levels of expression of the hsa-mir-17-92 cluster in colorectal tumours taking in account their levels of c-myc expression and presence of 13q gain in the tumours. The integration of RNA expression and aCGH data has led us to the identification of five down-regulated putative target genes, one of which has known tumour suppressor activity.

P56
ASSOCIATION BETWEEN FUNCTIONAL EGF+61 POLYMORPHISM AND GLIOMA RISK

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Introduction. Epidermal growth factor (EGF) plays a critical role in cancer. A polymorphism in the EGF gene (EGF+61) may influence its expression and contribute to cancer predisposition and aggressiveness. In the present study we aim to elucidate the role of EGF+61 in glioma susceptibility and prognosis.
Methods and Materials. A case-control study involving 197 glioma patients and 570 controls was performed. Univariate and multivariate logistic regression analyses were used to calculate odds ratio (OR) and 95% confidence intervals (95% CI). The luciferase reporter gene assay was used to ascertain the functional consequences of this polymorphism.
Results. Corroborating the univariate analysis, the multivariate model showed that the G allele conferred higher risks for gliomas (OR 1.35, 95% CI 1.07-1.72), glioblastomas (OR 1.48, 95% CI 1.03-2.12) and oligodendrogliomas (OR 1.58, 95% CI 1.10-2.28). The GG genotypes were associated with increased risk for gliomas (OR 1.80, 95% CI 1.12-2.89), glioblastomas (OR 2.07, 95% CI 1.04-4.15), and oligodendrogliomas (OR 2.85, 95% CI 1.23-6.62). In addition, the AG+GG genotypes were associated with higher risk for gliomas (OR 1.56, 95% CI 1.05-2.32) and oligodendrogliomas (OR 2.88, 95% CI 1.39-5.98). No significant association was observed between the EGF+61 polymorphism and glioblastoma or oligodendroglioma patients' overall survival. The luciferase reporter gene assay exhibited a significant increased promoter activity for the G variant compared to the reference A allele.
Conclusions. These findings support the role of the EGF+61 polymorphism as a susceptibility factor for development of gliomas and show its implication on EGF promoter activity.

P57
ANALYSIS OF GENOMIC IMBALANCES AND GEP IN PATIENTS WITH POOR PROGNOSIS DIFFUSE LARGE B-CELL LYMPHOMAS

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Introduction. Diffuse large B-cell lymphomas (DLBCL) are the most common type of aggressive no-Hodgkin lymphoma. Conventional chemotherapy and progenitor stem cell transplantation (SCT) induces complete remissions and cure of more than 60% of these patients. However a group of DLBCL, identified by clinical and biological parameters and classified according to the International Prognostic Index (IPI), did not respond to these therapy. DLBCL have been analysed by means of both genomic and expression arrays as a homogeneous group but little information of the poor prognosis DLBCL group is available. Aim: To study patients diagnosed as poor prognosis DLBCL by CGH arrays and GEP and to identify genetic factors as markers of aggressive disease.
Patients and methods. Array-CGH and expression microarrays analysis were performed in 41 DLBCL with IPI score>3 tretated with MEGACHOP polychemotherapy followed by SCT. The DNA of 22 specimens was isolated from the paraffin-embedded tumor material ("QIAamp DNA Mini Kit", QIAgen) and the rest of samples used in this study were obtained of fresh frozen. A total of 3500 genomic targets DNAs, Bacterial Artificial Chromosome (BAC) and P1-derived artificial chromosome (PAC) clones were compounded from RP-11 libraries (Sanger Institute,Cambridge, UK) which were spaced approximately 1Mb The PCR products after purification were arrayed in triplicate onto CMT-GAPS-coated glass slides (Corning) using a MicroGrid II arrayer (BioRobot). DNA was labelled by using a Bioprime array CGH Labeling Kit (Invitrogen). Denaturation, preanealing and hybridization were carried out for 48h at 42ºC by using a GENETAC hybridization chamber (Genomic Solutions). Arrays were scanned by means of an Axon 4000B scanner (Axon Instruments). Images were analysed by using GenePix Pro 4.0 software (Axon Instruments). Spots were defined using the automatic grid feature of the software and manually adjusted where necessary. Fluorescence intensities of all spots were then calculated after the subtraction of local background.
Results. Most of DLBCL of cases showed DNA copy changes. The most frequent aberrations were gains of 7p22.1 (58.5%), 11q23.3 (248.8%), 3p21.1 (48.8%), 6p21.1 (43.9%) and 11q12.2-q13.1 (43.9%). Losses of chromosomes regions were frequently observed on 13q13.3 (68.3%), p53 (39%) and 5q21.1(31.7%). In 46% (19/41) of patients we observed association between the gains in chromosomes 11q23 and 7p22 ($p=0.001$). In this group, the changes more frequent showed were gains on 11q12.2, 12q14.1 (79%), 7q22.1 (47%) and 20q (42%), while losses were on 3p21 (89%), 13q13 (79%) and 8q23.1 (53%). DNA copy number changes correlated with clinical features. Thus gains on 3p21, 6q21, 12q12, 12q24 were associated with age <60 years ($p<.05$), whereas the presence of B-symptoms were significantly associated with losses on 2p12, 2q36, and 11q14 ($p<0.05$). Gains on 1p36, 1q21, 3p21, 6p21, 20q and loss on 5q21 were associated with Ann Arbor stage III-IV ($p<0.05$). Cases with Bulky mass (>10 cm) showed gains on 11q23 and 12q13-14 ($p<0.05$). By contrast, bone marrow involvement was associated with gains on 12q14. Increased lactate dehydrogenase (LDH) level was observed in cases with gains or losses on (p53). The losses on 10q23.1 and 17p13.2 were significantly associated with shorter overall survival ($p=0.018$ and $p=0.021$). Overall a good correlation between GEP and genetic changes was observed.
Conclusion. Arrays-CGH showed that the most frequent changes in DLBCL with aggressive clinical behaviour are gains on 7p22.1, 11q23.3, 3p21, 6p21.1 and losses on 13q13.3, 17p13.2 and 5q21.1. In 46% of cases an association of gains on 7p22 and 11q23 was observed, and this could represent a different genomic subtype of DLBCL. Therefore, arrays-CGH identified relatively small genomic regions associated with clinical behaviour and outcome.

P58
A COMBINED GENE EXPRESSION PROFILE AND CGH ARRAY STUDY DEFINE NEW GENES INVOLVED IN THE DIFFERENT GENETIC TYPES OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction. B-cell lymphoproliferative disorders (BCLPD) are a group of neoplasias characterised by the clonal expansion of neoplastic cells derived from one single transformed cell leading to the proliferation and accumulation of mature B-cells in different tissues. B-cell chronic lymphocytic leukemia (B-CLL) is the most common leukemia in adults. Microarray-based methodology provides genomic information of the whole genome in a single experiment. Thus both CGHarrays and expression arrays provide a full scope of the cancer genome. The aims of this project were to get a better knowledge of both chromosomal imbalances and gene expression profile of clonal lymphocytes in B-CLL patients and to study the relationship between the data obtained and the genetic characteristics of the disease.
Patients and methods. Peripheral blood from 72 patients with CLL was analysed by combining GEP (Human Genome U133 Plus GeneChip, Affymetrix) and 1Mb-CGHarray (Sanger Institute). In all cases clinical data, FISH, IGH somatic mutations and inmunophenotype were also available. *Results.* GEP distinguished cases with IGH mutated and unmutated. Mutated B-CLL cases showed deregulation of ZAP70 (protein kinase activity), CASP3 (apoptosis related) and MDM2 and SMAD4 (signal transduction). Regarding cases with loss of 13q a total of 270 genes were deregulated, 200 of them were down regulated in the group showing loss of 13q, including HDAC4, BCL2, IL15 and protein-tyrosine kinase activity genes (SRC and STK11). Overall array CGH results correlated with FISH analysis. At present time, correlation between CGHarrays and GEP is in progress.
Conclusion. The B-CLL genomic groups showed different genomic abnormalities both in CGH arrays and in GEP.

P59
HIGH-RESOLUTION PROFILING OF GENE COPY NUMBER ALTERATIONS PERFORMED ON SMALL AMOUNTS OF TUMOR DNA EXTRACTED FROM FRESH-FROZEN OR FORMALIN-FIXED AND PARAFFIN-EMBEDDED TISSUE

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Introduction. The application of high-resolution techniques, in particular array-CGH, to the study of cancer genetics has made important contributions to the identification of genes and pathways associated with tumor development and/or progression. However, limiting size of human biological material often yield insufficient amount of genomic DNA. Another concern is that archival pathology specimens are often fixed in formalin and embedded in paraffin (FFPE), and the quality of DNA obtained from these samples is often insufficient for array-CGH studies. These facts have driven the development of efficient whole genome amplification (WGA) methods that produce quantitatively unbiased DNA.
Materials and methods. We have produced an array comprised of 32,396 BAC clones (32K array) covering 99% of the human genome. The clone set which provides a resolution of 60 kb was acquired from BACPAC Resources Center (http://bacpac.chori.org/), amplified by degenerate oligonucleotide-primed-PCR (DOP-PCR) and printed on CodeLink-HD slides. The presence of gene copy number alterations is computationally evaluated using a newly developed approach which uses a hidden Markov model to assign the most probable copy number class for each clone (Andersson R. et al., submitted).
Results. We evaluated the ability of DOP-PCR and GenomePlex WGA kit to amplify DNA for 32K array-CGH experiments using DNA derived from a well characterized patient affected with neurofibromatosis type-2. GenomePlex produced best results with highly reproducible and representative data. Only 10 ng of DNA were required as starting material. The method was also optimized to amplify DNA extracted from tumor tissues. WGA from both fresh-frozen and FFPE tissue derived from a glioblastoma multiforme and a bladder tumor sample (T1) produced highly reproducible profiles and identified the same copy number aberrations detected in unamplified DNA. These regions included candidate tumor suppressor and oncogenes.
Conclusions. We show that isolation of genomic-DNA from either frozen or FFPE tissue samples in combination with a subsequent WGA produces reliable results upon hybridization on a whole genome clone based array. This approach will allow us to profile extensive series of archival pathology samples. Results

from such studies would be of enormous benefit for translational research.

P60
TP53 MUTATIONS ARE ASSOCIATED WITH A PARTICULAR PATTERN OF GENOMIC IMBALANCES IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Introduction. It has been established that p53 maintains the genomic stability of cells through cell cycle arrest, DNA repair, and apoptosis and that the mutation of TP53 (the gene encoding p53) is one of the most frequent genetic alterations reported in solid tumors. A TP53 mutation is present in more than 60% of the head and neck squamous cell carcinomas (HNSCC) and is considered one of the earliest genetic alterations in HNSCC carcinogenesis. Within the remaining 40% of the tumors containing wild type TP53, a subgroup of approximately 10% is present for which the human papillomavirus (HPV) is considered the etiological factor for cancer development by blocking p53 by viral oncogene E6. In the remaining 30% the role of p53 and the pathway it acts in is still unknown.
Purpose. Aim of the study is to identify specific genetic changes associated with mutant or wild type TP53 in HNSCC of non HPV-etiology. Methods: We analyzed by means of microarray comparative genomic hybridization (maCGH) 39 tumors containing either mutated (n=19) or wild type TP53 (n= 20). Results: HNSCC containing wild type TP53 have significantly less genomic alterations compared to TP53 mutant HNSCC. Chromosomal regions, differentially and significantly altered in the TP53 mutant tumors, are gains at 7q21, 11q13, and 14q21 and loss at 13q31. The TP53 wild type tumors did not show any differentially altered region compared to TP53 mutant tumors. Gain at 8q23-qter and loss at 11q22-qter were detected in both tumor types. *Conclusions.* Two routes to HNSCC can be discriminated: one with the involvement of a mutated TP53 gene, and one with wild type TP53. A mutation in the TP53 gene during head and neck carcinogenesis is associated with a relatively high level of genomic instability.

P61
GENOMIC AND EXPRESSION PROFILING DEFINES THE MOLECULAR FEATURES OF INTRACHROMOSOMAL AMPLIFICATION OF CHROMOSOME 21 IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction. Intrachromosomal amplification of chromosome 21 (iAMP21) is arguably the most important molecular prognostic marker for patients with B-lineage acute lymphoblastic leukemia (ALL) identified in recent years. Patients have a significantly inferior event free survival at 5 years compared with other patients: 29% (95% CI 13%-48%) versus 78% (76%-80%). As a result of this three-fold increase in relapse risk, it is vital that this aberration is fully characterized to facilitate accurate diagnosis. Previously published data (Strefford et al 2006, PNAS 103, 8167-8172) showed common regions of copy number alteration (CNA) and a distinctive expression profile.
Methods and Materials. To further dissect the genome of these patients, we have performed a comparative genomic hybridization (aCGH) study with 244K genome-wide (n=23) and 190K chromosome 21 tiling (n=16) arrays. Gene expression was profiled using the Affymetrix U133A and exon ST 1.0 array (n=8), with confirmatory quantitative PCR.
Results. The location and degree of CNA involving chromosome 21 were heterogeneous. Centromeric CNA were highly variable, suggesting chromosomal instability. All patients shared a common region of amplification (CRA) of 3.5Mb in size, positioned between 32.8 and 36.3Mb. Nineteen patients showed 21q deletions, 13 of which shared a common region of deletion (CRD) of 0.25Mb close to the telomere. The CRA and CRD have been significantly refined compared to our previous data. Molecular copy number counting (MCC) has confirmed the position and extent of these alterations and facilitated structural PCR analysis, while FISH has provided accurate quantification, revealing up to a five-fold copy number gain. Metaphase FISH and mBANDing showed complex patterns of intrachromosomal rearrangement, including inversions and duplications, implicating a series of breakage-fusion-bridge cycles in the formation of this abnormality. The

expression of 32 and 5 well-characterized genes in the CRA and CRD respectively, correlated with the copy number changes in these regions.
Conclusions. This study provides detailed characterization of this clinically relevant chromosomal abnormality, with refinement of genomic profiles and precise mapping. This has identified a small subset of genes in these patients from which important biological mechanisms are likely to emerge.

P62
COMBINATION OF DIFFERENT GENOMIC APPROACHES TO DEFINE A CANDIDATE DELETED GENE IN AML

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Acute myeloid leukemia patients with complex karyotype are characterized by presenting more than three cytogenetic aberrations and an adverse outcome. Recently, array-CGH studies over this population showed discrete losses at chromosome 17, including TP53 and NF1 genes.
Among a series of 120 de novo AML, on which we have conducted DNA profiling analysis using a high density oligonucleotide-based arrayCGH, we detected 7 cases of AML that showed DNA copy number aberrations involving chromosome 17. Aberrations in chromosome 17 were cryptic deletions affecting the regions 17p13.1 (including TP53 gene) or 17q11.2 (including NF1 gene). We observed that all our seven samples displayed losses involving NF1. Only two of them showed concomitant loss of TP53. FISH confirmed arrayCGH data.
Two more approaches were used to better characterize the genomic and genetic features of the rearranged chromosome 17. The presence of loss of heterozygosity (LOH) was studied with the Illumina Infinum 300K SNP platform. Only one case showed LOH affecting the TP53 genomic region. Finally, TP53 mutation analysis was performed by complete sequencing of exons 4 to 9 in all cases. Two harboured mutations, one case had lost the wild type allele of TP53 and the other was heterozygous for the mutation.
In conclusion, we confirmed the existence of recurrent cryptic deletions in the area of 17q11.2, targeting the gene NF1. The minimum deleted region in 17q11 covered 1.5 Mb. Only one case with deletion or UPiD in TP53 showed mutations in the gene, suggesting that TP53 may not be the principal target in de novo AML. Therefore, we think our genetic data support the proposed role of NF1 as the main target in rearrangements of chromosome 17 in de novo AML, based up on previous reports, which set NF1 as a leukemogenic agent when mutated or haploinsufficient.

P63
SIGNIFICANCE OF MOLECULAR CHROMOSOME ANALYSES IN THE CLINICAL INTERPRETATION OF APPARENTLY BALANCED TRANSLOCATIONS

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Introduction. By definition, balanced translocations are associated with normal phenotype and detected due to multiple miscarriages or severely affected offspring as a consequence of inherited imbalance. Several mechanisms have been proposed to explain how a small percentage of apparently balanced translocation is associated with abnormal phenotype.
Methods and Materials. Although molecular cytogenetic techniques are nowadays available to reveal small imbalances or additional chromosomal aberrations, the interpretation of an apparently balanced translocation or cryptic chromosomal rearrangements is even now a challenge for the clinical geneticist. We report on three cases with translocation where molecular chromosomal analysis (arrayCGH, FISH, subtelomeric FISH) helped to explain the associated abnormal phenotype.
Results. A de novo apparently balanced translocation in a boy with mental retardation and dysmorphic features proved to be imbalanced after performing arrayCGH. According to genotype-phenotype correlation analysis the small deletions around the breakpoints may be responsible for the clinical features. A balanced translocation inherited from the healthy father was associated with a small de novo duplication on a third chromosome in a mentally retarded girl with VSD and epilepsy. The partial duplication suspected even by G banding was confirmed by FISH analysis. The association between the duplication and the translocation may be a coincidence; however, a possible instability of the cell division due to the translocation couldn't be

excluded. A cryptic unbalanced translocation was detected by arrayCGH in sibs with severe mental retardation and minor anomalies. The results were verified by chromosome-specific telomere FISH which also revealed a balanced translocation in the mother involving the ends of the chromosomes. *Conclusions.* Our cases demonstrate that molecular cytogenetic techniques as a part of a well-reasoned approach significantly increase the diagnostic efficiency of a clinical genetic unit; namely, abnormalities remaining cryptic by conventional methods may become detectable.

P64
MOLECULAR CHARACTERIZATION OF DOUBLE MINUTES/HOMOGENEOUSLY STAINING REGIONS IN SELECTED TUMORS

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Double minutes (dmin) and homogeneously staining regions (hsr) are the cytogenetic hallmarks of genomic amplifications in tumors. Little is known on their genomic structure, gene expression pattern and possible mechanisms for their genesis. Previous results obtained on 34 patients affected by acute myeloid leukemia (AML) with MYC (8q24)-containing dmin provided evidence in favor of the episome model for the formation of dmin (Storlazzi et al., 2006). Following the same rationale as in leukemia patients, we have performed Array-CGH, FISH and PCR analyses in order to map the breakpoints of dmin/hsr in six and eleven tumor cell lines with MYC and MYCN amplifications, respectively. Preliminary results regarding the definition of the sequence at the amplicon junction indicate that both the episome model and the breakage-fusion-bridge (BFB) cycles could be important mechanism behind dmin/hsr formation in solid tumor cell lines, differently from leukemia patients.
We found that the sequence junctions between amplicons arrayed in a "head to tail" fashion were generated by non homologous end joining (NHEJ); amplicons arrayed in a "head to head" fashion displayed the presence of interstitial cryptic sequences, surprisingly mapping outside the amplicon region, at their junctions. Sequence analysis revealed that one of the junctions between these sequences and amplicons was the result of homologous recombination (HR) triggered by Alu elements with a ~80% sequence similarity. Further characterization of amplicon arrangements and junctions in the remaining cell lines are needed to unveil the driving force towards the episome model or the BFB cycles mechanism in the genesis of dmin/hsr.
Moreover, preliminary results showed that an unexpected gene [EYA1(8q13.3) in GLC3] was co-amplified with MYC. We plan to test the expression of this gene, and other ones that would be eventually found to be amplified, in order to show if they could be the targets of the amplification.

P65
HIGH FREQUENCY OF SUBMICROSCOPIC DNA COPY NUMBER CHANGES IN PATIENTS WITH CONGENITAL HEART DISEASE

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Introduction. Congenital heart disease (CHD) is the most frequent birth defect and affects nearly 1% of newborns. Chromosomal aberrations have been shown to cause syndromes that involve malformations of the heart, however, hardly anything is known about the frequency of chromosomal rearrangements in non-syndromic forms of CHD.
Methods and Materials. Here we present a submegabase resolution BAC array CGH screen of 105 patients with CHD as the sole abnormality at the time of diagnosis.
Results. We have detected 18 chromosomal changes that do not coincide with frequent DNA copy number variants: 2 de novo deletions, 4 de novo duplications, 5 familial duplications and one familial deletion. In one further deletion and five duplications inheritance could not be verified due to lack of parental DNA.
Conclusions. The striking underrepresentation of deletions suggests that such rearrangements are less tolerated, leading either to complex phenotypes or death. Aberrations inherited from healthy parents may predispose to disease, but may be in need of a modifier or a "second hit" to express the phenotype. For genetic counselling these changes represent an increasing challenge.

P66
IDENTIFICATION OF CANDIDATE GENES PLAYING A ROLE IN THE INDUCTION OF RADIATION-TRANSFORMATION OF THE FEMALE BREAST

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Breast carcinomas are the most frequent cancers in women. We are interested to uncover the process of radiation-induced transformation of breast cells. Therefore, we have investigated the initial steps of tumor formation by characterizing immortalized radiation-transformed epithelial breast cell lines (B42) by applying molecular cytogenetic and molecular biological approaches like spectral karyotyping (SKY), array CGH, array painting, RACE-PCR, sequencing, Taqman expression analysis and immunohistochemistry. Here we present the results of the investigation of one cell line (B42-16) with a tumorigenic phenotype. SKY, array CGH and array painting allowed us to identify the karyotype of the cell line, to define chromosomal gains and losses with a resolution of 1 Mb and the identification of nearly all chromosomal breakpoints with a tiling resolution which is just limited by the size of the used large-insert clones (BACs and PACs). Thus, candidate genes could be identified which may be altered in structure or gene dosage and will therefore contribute to the tumorigenic phenotype. Array painting using flow-sorted marker chromosomes of the cell line allowed us to uncover the genes GRID1 (10q23.2) and HAS2 (8q24.13). The breakpoints within the genes GRID1 and HAS2 have been cloned by RACE-(rapid amplification of cDNA ends) PCR and their over-expression was shown on RNA (Taqman quantitative realtime-RT-PCR) and protein level (immunohistochemistry). Further more three chromosomal breakpoints including one micro-deletion on chromosome 12 as well as two breakpoints on chromosome 10 became apparent. Within these breakpoints the candidate genes MDM2, WNT1, CPM are located which are likely to be altered. The micro-deletion covers a small region which encodes for the genes TBX5 and TBX3 (deleted in Holt-Oram and ulnar-mammary syndromes). The results indicate the involvement of new candidate genes which may play a role in radiation-induced breast carcinogenesis.

P67
THE USE OF LEBER CONGENITAL AMAUROSIS GENOTYPING MICROARRAY IN 299 SPANISH FAMILIES AFFECTED WITH RETINITIS PIGMENTOSA

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Introduction. Leber Congenital Amaurosis (LCA) is the most severe inherited retinopathy with the earliest age of onset. Individuals affected with LCA are diagnosed at birth or in the first few months of life with severely impaired vision or blindness, nystagmus and abnormal or extinguished ERG. Non syndromic LCA has been associated with mutations in ten genes: ten genes: AIPL1 (17p13.1), CEP290 (12q21.3), CRB1 (1q31-q32.2), CRX (19q13.3), GUCY2D (17p13.3), IMPDH1 (7q31.3-q32), RPE65 (1p31), RPGRIP1 (14q11), RDH12 (14q23.3-q24.1) and TULP1 (6q21.3). These genes are involved in different physiologic pathways in the retina.
Material and methods. We have screened a group of 299 families divided in three sub-groups: 42 LCA, 107 early-onset ARRP and 150 non early-onset ARRP. We have studied 6 of these genes (AIPL1, CRB1, CRX, GUCY2D, RPE65 and RPGRIP1) and in addition, two early-onset RP genes (LRAT and MERTK) using a genotyping microarray (www.asperbio.com)
Results. The respective frequencies of mutant alleles were: 22.6% (19/84) for LCA with 13 mutated families, 6,1% (13/214) for early-onset (ARRP) with 12 mutated families and 5% (13/300) with 12 mutated families with non early-onset ARRP. CRB1 was the most mutated gene in Spanish affected families. We have studied 5 families with possible digenism or triallelism; digenism was discarded in all cases but triallelism was not.
Conclusion. Using LCA microarray should be the first step in the molecular diagnosis when the clinical diagnosis indicates that is an LCA or early-onset ARRP case. The relative percentage of mutations found with the microarray suggests that more LCA-associated genes remain to be discovered.

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BRCA1 DIAGNOSTIC SCREENING BY ARRAY-CGH

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Introduction. Only 25% of familial breast cancer can be attributed to known breast cancer genes including BRCA1, BRCA2, CHEK2, ATM, TP53, and PALB2. The rest could be either missed in current screening, carry unclassified variants (UVs), contain germ-line mutations in yet unknown breast cancer genes or be primarily non-genetic. In the Netherlands alone, 312 BRCA1-UVs are reported. Here we aimed use aCGH to re-examine BRCA1/2 negative cases fulfilling the criteria of hereditary breast and ovarian cancer syndrome (HBOC), as well as unclassified variants (UVs)
Methods and Materials. We profiled paraffin embedded tumors by 1Mb BAC array-CGH to identify a BRCA1 profile. A nearest shrunken centroid classifier [1] was trained on 32 control tumors and 18 proven BRCA1-tumors and subsequently validated on independent tumors (17 controls and 10 BRCA1).
Results. This classifier included features on chromosome 3q and 5cen similar to the metaphase-CGH classifier [2]. We classified 27/28 BRCA1 and 2/49 control tumors as BRCA1-like. In fact, one of these BRCA1-like controls had been independently proven to carry a BRCA1 mutation. We also classified 3/38 BRCA1/2 screen-negative HBOC cases as BRCA1. Interestingly, one had a BRCA1 truncating splice-mutation and one had BRCA1 promoter-methylation. Seven of 20 BRCA1-UVs classified as BRCA1, mostly in agreement with biochemical and genetic evidence.
Conclusions. Array-CGH can identify BRCA1-carriers among hereditary breast cancers and may facilitate interpretation of UVs. The fact that only tree BRCA1-profiles were found in 38 HBOC tumors suggests that there must be other breast cancer causes in these families.

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CGHCALL: CALLING ABERRATIONS FOR ARRAY CGH TUMOR PROFILES

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Introduction. Array CGH log-ratios resulting from feature extraction are usually converted back to discrete states representing loss (< 2 copies), normal (2 copies), gain (3-4 copies), and, possibly, amplification (> 4 copies). This process is referred to as `calling'. Segmentation algorithms detect breakpoints and levels in single profiles, but do not result in explicit calls. New algorithms were developed to produce calls based on confidence measures as p-values or False Discovery Rates (FDR). When using a testing principle, the null-state, `normal', has a different status than the `loss' and `gain' states: controlling the FDR or the p-value should avoid calling too many aberrations. However, we experienced that biologists prefer to use a more `state-neutral' approach to obtain the most likely classification of the data into discrete states.CGHcall uses a mixture model approach to enable such a classification.
Methods. CGHcall uses the segmentation results of DNAcopy, which was shown to be one of the strongest segmentation algorithms. Since one cannot expect loss, normal and gain levels to be uniform over all data, we allow fluctuations by using random effects. We combine the segmentation results with a mixture model to obtain the most likely classification per segment rather than per individual clone. In our model, the number of states is strongly biologically motivated. Six states rather than the conventionalthree states are used. Moreover, estimation of the mixture proportions per chromosome arm is introduced.
Results. Using FISH validated array CGH data, we illustrate that when multi-copy gains cover rather large regions in someof the samples inclusion of the extra states improves detection of single copy gains. For both simulated and real data CGHcall outperforms several existing calling methods. Finally, incorporation of the chromosome arm information is shown to be promising for a large oral squamous cell carcinoma data set.
Conclusion. CGHcall is an easy-to-use R-package achieving high calling accuracy.

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LARGE SURVEY OF SNPS IN BREAST CANCER DEVELOPMENT, THE EXAMPLE OF MDM2 SNP309 AND TP53 R72P

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Introduction. Association studies in large series of breast cancer patients can be used to identify single-nucleotide polymorphisms (SNPs) contributing to breast cancer susceptibility. Among the candidate genes are those that have a function in DNA damage response such as TP53 and its key regulator MDM2. Studies examining the effects of two variants in TP53 (R72P) and MDM2 (SNP309) have concluded that their individual effects, if any, are small. However, data from epidemiological studies suggest a functional interaction between these genes.
Methods. We have investigated the interaction of these variants on breast cancer risk and the age at onset of breast cancer, in a large pooled series of cases and controls from the Breast Cancer Association Consortium (BCAC). A total of 3834 healthy controls and 5191 breast cancer cases were genotyped for both MDM2 SNP309 and TP53 R72P using Taqman assays.
Results. Breast cancer risk was not found to be associated with the combined variant alleles (OR 1.00 (0.81-1.23)). Individual odds ratios were ratios were 1.01 (95%CI 0.93-1.09) for MDM2 SNP309 and 0.98 (0.91-1.04) for TP53 R72P. Though the German group had initially found evidence for a 4 year earlier age at onset for carriers of both variant alleles, this effect was no longer apparent in the pooled data set, after excluding the hypothesis-generating study.
Conclusion. In a large collaborative study we did not find an indication for an association of MDM2 SNP309 and TP53 R72P, separately or in interaction, with breast cancer, suggesting that any effect of these two variants, would be very small and confined to subgroups that were not separately assessed in our present study. Currently we are analyzing other SNPs in the Dutch cohort (n=1000) included in the BCAC, for which results will be available at the time of the congress.

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HIGH RESOLUTION GENOMIC PROFILING OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA REVEALS NOVEL COPY NUMBER ABERRATIONS RELEVANT FOR CELLULAR GROWTH AND DIFFERENTIATION

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It is well-established now that genomic aberrations can serve as important diagnostic, prognostic, and therapeutic parameters in childhood acute lymphoblastic leukemia (ALL). Currently, cytogenetically visible chromosomal aneuploidies as well as recurrent balanced translocations, such as t(9;22)(q34;q11) translocations involving BCR-ABL fusions and 11q23-associated translocations resulting in MLL rearrangements, are used as highly valuable prognostic and therapeutic markers. However, more subtle (i.e. submicroscopic) anomalies with clinical implications, which could potentially further improve targted therapy of ALL, have thus far not been identified. To this end, we have performed high resolution genomic profiling using microarray-based comparative genome hybridizations (array CGH, using 250k single nucleotide polymorphism (SNP)-based genotyping arrays from Affymetrix) on a panel of 40 childhood ALLs. Using this approach, we were able to detect multiple de novo genetic lesions in most leukemic samples, varying from chromosomal aneuploidies and segmental chromosome gains and losses to sub-microscopic copy number aberrations affecting single genes. On average, we detected 3.9 lesions (2.8 losses and 1.1 gains) per sample. Many of these lesions involved recurrent (partially) overlapping deletions and duplications, including various established leukemia-associated genes, including CDKN2A, ETV6 (TEL), RUNX1 (AML1), and MLL. In 36% of the B-lineage ALLs, microdeletions of the B-lineage transcription factors PAX5 (n=7), EBF (n=2), and Ikaros (n=1), or lesions affecting genes with established roles in B-cell development, i.e., RAG1 and RAG2, FYN, PBEF1, or CBP/PAG were detected. Since we frequently encountered additional lesions affecting genes involved in cell cycle regulation, cell survival, proliferation and apoptosis, we conclude that multiple oncogenic pathways act in conjunction during childhood ALL development.

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LITERATURE MINING FOR CONSTITUTIONAL CYTOGENETICS

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Introduction. We present a text mining application that uses publicly available literature to statistically link phenotype descriptions to deletions and duplications in the genome, within the context of constitutional cytogenetics.
Methods and Materials. Cytogeneticists are familiar with banding patterns of chromosomes at various levels of cytogenetic resolution. Hence, reports in the literature frequently mention bands to delineate a genomic region. A specific nomenclature allows unambiguous detection of cytobands. Based on this premise we constructed a database that links PUBMED abstracts to cytogenetic bands they reference. This highly specific map is then used to characterise individual cytobands based on the content of linked abstracts. To this end 20 different domain specific vocabularies were constructed with which the user indicates from which perspective to annotate the genome. These vocabularies provide dysmorphology, anatomy-specific, gene- or protein-centered, gene ontology, and disease-related perspectives on the literature.
Results. The tool functions in two directions. On the one hand, users enter or click a cytoband on the genome view. the tool will characterize that band with statistically overrepresented vocabulary concepts found in the literature. For example, when aBandApart is queried with '4p16.3' and a disease vocabulary, the most significant concepts are achondroplasia, Wolf Hirschhorn syndrome, Huntington Disease, multiple myeloma, cherubism, dwarfism and hypochondroplasia, all of which are confirmed to be associated to that region. On the other hand, users can start from a concept and query the database for statistically overrepresented chromosomal regions. Overrepresented bands are listed and light up in blue on a genome chart. Links to relevant literature are provided with the cytoband profile.
Conclusions. aBandApart aims at linking phenotype information to genomic aberrations at the level of cytogenetic bands. The generation of a phenotypic genome map based on text mining could enable the identification of genes involved in disease processes and could delineate novel clinically recognizable entities. aBandApart is available at http://www.esat.kuleuven.be/Abandapart.

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GENOMIC STABILITY OF GASTRIC CANCERS ASSESSED BY MICROSATELLITE ANALYSIS AND ARRAY COMPARATIVE GENOMIC HYBRIDIZATION

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Introduction. Gastric carcinomas (GCAs) can be classified into two main types, i.e., intestinal (IGCA) and diffuse (DGCA), which differ in their molecular pathology. Even though these two types are considered as different disease entities, their specific molecular signatures and underlying carcinogenic mechanisms are unclear. The aim of the study was to assess the genomic stability of GCAs by microsatellite analysis and array comparative genomic hybridization (aCGH).
Methods and Materials. Fifteen fresh-frozen GCA samples (12 IGCA and 3 DGCA) previously characterized for their genomic stability with 15 microsatellites were used in the study. The samples were analyzed on a custom-made aCGH platform with 16.000 cDNA targets.
Results. Analysis by both microsatellites and aCGH showed that the genomes of DGCA tumors were more stable than those of IGCA. The samples with loss of heterozygosity at multiple loci by microsatellite analysis showed multiple gains and losses on aCGH and could thus be interpreted as genomically unstable. However, the occurrence of gene amplifications was not restricted to genetically unstable samples. In addition, we observed the otherwise genetically stable DGCA tumors to share a previously unreported amplicon.
Conclusions. The results suggest that genomic instability might represent a major tumorigenic mechanism for IGCA, but not for DGCA.

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DNA COPY NUMBER GAINS AT LOCI OF GROWTH FACTORS AND THEIR RECEPTORS IN SALIVARY GLAND ADENOID CYSTIC CARCINOMA

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Introduction. Adenoid cystic carcinoma (ACC) is a relatively common malignant salivary gland characterized by a prolonged clinical course. However, the disease-specific mortality rate of this neoplasm is high due to distant metastases occurring in 40-60% of the patients after more than 10 years.
Methods and Materials. In order to unravel common genetic abnormalities associated with initiation and progression of ACC, microarray-based comparative genomic hybridization was performed to a series of 18 paraffin-embedded primary ACCs using a genome-wide scanning BAC array. Additionally, we applied categorical and survival analyses to analyze whether chromosomal changes related to clinicopathological characteristics and patient survival.
Results. A total of 238 chromosomal aberrations were detected, representing more gains than losses (205 vs. 33 respectively). The most frequent DNA copy number changes (>60%) included gains of 9q33.3-q34.3, 11q13.3, 11q23.3, 19p13.3-p13.11, 19q12-q13.43, 21q22.3, and 22q13.33. These loci harbored numerous growth factors (FGFs and PDGF), together with their receptors (FGFR3 and PDGFRß). We also compared the chromosomal regions displaying significant differences between ACCs that developed a recurrence/metastasis during the time of follow-up (n=9) and to those that did not (n=9). ACCs with a recurrence and/or a metastasis more frequently displayed a gain on chromosomes 4p16.3 (p=0.03, FDR=0.15), 16p13.3 (p=0.05, FDR=0.2), 16q24.1-q24.2 (p=0.03, FDR=0.15) and 17p13.1 (p=0.03, FDR=0.15). Interestingly, FGFs and FGF receptors were located in some of these genomic areas. Furthermore, patients with seventeen or more chromosomal aberrations had a significantly less favorable outcome than patients with fewer chromosomal aberrations (log rank=5.19, p=0.02).
Conclusions. In conclusion, frequent DNA copy number gains at loci of growth factors and their receptors suggest their involvement in ACC initiation and progression. The presence of FGF and PDGF together with their receptors in increased chromosomal regions suggests a possible role for autocrine stimulation in ACC tumorigenesis.

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ARRAY COMPARATIVE GENOMIC HYBRIDIZATION AND EXPRESSION PROFILING OF WILMS TUMOUR IDENTIFY MOLECULAR CORRELATES OF HISTOLOGY AND RELAPSE

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Introduction. Wilms tumour is the most common renal neoplasm of childhood. Although 85% of affected children achieve long-term survival, relapse remains a significant clinical problem. Patients with anaplastic, rather than favourable tumour histology (FH), have a particularly high (50%) risk of recurrence and a relatively poor prognosis. Anaplastic tumours frequently harbour TP53 mutations, but their genetic basis is otherwise poorly understood. We aimed to identify further molecular changes associated with anaplasia and relapse.
Methods and Materials. cDNA microarray expression profiling with over 17,000 probes, and array comparative genomic hybridization (aCGH) on 1Mb-spaced or full genomic tiling path BAC platforms, were used to analyse panels of >50 anaplastic and >70 FH tumours.
Results. In addition to 17p (the location of TP53), both platforms identified novel loci on 14q and 4q as regions of particular interest, with significant copy number loss and underexpression in the anaplastic samples. Pathway analysis of the cDNA data revealed concerted differential expression of gene interaction networks between the tumour subtypes, including one large network centred on ERBB2 (significantly underexpressed in the anaplastic group). A similar analysis of the FH data detected overexpression of IGF1R in tumours that subsequently relapsed, and highlighted differential expression between relapse classes of several important binding or regulatory interaction partners of this protein, including AREG and CDKN1A. Both the expression and cDNA data could be used to classify tumour samples into histological subtypes using a Support Vector Machine algorithm with

>80% accuracy in cross-validation, a finding of potential diagnostic and prognostic relevance.
Conclusions. Anaplastic and FH tumours have distinct genomic and expression profiles. Further investigation of the roles of specific genes at the loci associated with anaplasia should provide us with valuable molecular and clinical insights into the factors that control tumour behaviour.

P76
GENOMIC PROFILING OF ETV6-RUNX1 POSITIVE ACUTE LYMPHOBLASTIC LEUKAEMIA REVEALS SURPRISING COMPLEXITY AND NOVEL SECONDARY ABNORMALITIES

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Introduction. The translocation t(12;21)(p13;q22) accounts for 25% of children with B-lineage acute lymphoblastic leukaemia (ALL). It results in fusion of 5' ETV6, to almost the entire RUNX1 gene. The long latency between its prenatal formation and disease presentation, the presence of functional ETV6-RUNX1 fusions in normal newborns, and the inability of the fusion protein alone to initiate overt leukaemia, suggests that chromosomal changes in addition to the fusion are required for leukaemogenesis.
Methods and Materials. In light of these observations, the aim of this study was to use CGH (aCGH, Agilent 244K) (n=35) and SNP (Affymetrix 10K) arrays (n=20) to analyze a series of ETV6-RUNX1 positive patients to identify recurrent copy number alterations (CNA) and loss of heterozygosity (LOH) that may contribute to the leukaemic phenotype. Abnormal regions were quantified by fluorescence in situ hybridization.
Results. All patients exhibited abnormal profiles by array-CGH, which correlated well with diagnostic G-banding, FISH and SNP array data. On several occasions (n=10), the genomic profiles were surprisingly complex, exhibiting more than 15 distinct CNA. Large aberrations involved deletions of 6q (n=5), 9p (n=5), 11q (n=4), 12p (n=12), and gains of 21q (n=6) and Xq (n=7). Sub-microscopic alterations targeted 3q (n=3), 6p (n=3), 8q(n=3) and 9p (n=4). CNA breakpoints were accurately mapped to involve the ETV6 and RUNX1 genes in patients with additional copies of the der(21)t(12;21) showing the accuracy and resolution of the aCGH technique. A number of 12p deletions did not involve ETV6 as expected, but genes positioned proximally. In addition to targeting CDKN2A, 9p deletions specifically targeted PAX5 (n=4). A strong correlation between copy number assessment by aCGH and SNP analysis was demonstrated, while additional regions of copy-number-neutral LOH were observed.
Conclusions. Here we describe, for the first time, high-resolution genomic imbalances in ETV6-RUNX1 positive patients. In addition to driving disease-specific leukaemogenesis, genes within these novel regions may represent prognostic markers or therapeutic targets.

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CENTROMERIC DYSFUNCTION/GENOTOXIC EFFECTS IN ETHANOL AND LEAD CO-EXPOSED CULTURED HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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Introduction. Interaction of lead (Pb), known toxicant, with genetic material is well documented, especially in occupationally exposed population. However, the cascade of events involved and mechanisms of genotoxicity are poorly understood. It has also been reported that exposure of environmental pollutants together with other habitual factors like consumption of ethanol and tobacco are key contributors to various diseases including cancer. In the present study, the possible genotoxic threat of ethanol on lead was investigated by analyzing the chromosomal aberrations (CA) and centromeric dysfunction in human peripheral blood lymphocytes (HPBL) as cytogenetic biomarkers.
Methods and Materials. For the analysis of CA and centromeric dysfunction, human peripheral blood lymphocytes cultures were set up and exposed to either of ethanol (50, 100, 200, 250 and 300mM) or lead (10-6M) and combination of both lead-ethanol. Cultures were incubated at 37 °C for 72 h. At 70th h colchicine was added. Cells were harvested, staining was performed by giemsa and analyzed CA by automated software guided karyotyping. For centromeric dysfunction, metaphases were denatured using 70% formamide. The probes were denatured and hybridize with metaphase at 37°C for 24h.

For detection anti dig FITC was added. Counter stained by DAPI.
Results. Individual ethanol and lead showed no genetic damage in HPBL. Interestingly, co exposure of lead and ethanol (200mM, 250mM) was caused a dose dependent induction in CA significantly (<0.01) against the control. Furthermore, centromeric FISH in metaphase revealed the centromeric dysfunction following the co-exposure, where as acentric fragment where reported.
Conclusions. The results suggest that exposure of lead in the presence of ethanol makes genetic system more vulnerable under experimental conditions.

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AN EFFICIENT, VERSATILE AND SCALABLE PATTERN GROWTH APPROACH TO MINE FREQUENT PATTERNS IN UNALIGNED PROTEIN SEQUENCES

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Introduction. Pattern discovery in protein sequences is often based on multiple sequence alignments (MSA). The procedure can be computationally intensive and often requires manual adjustment, which may be particularly difficult for a set of deviating sequences. In contrast, two algorithms, PRATT2 (http//www.ebi.ac.uk/pratt/) and TEIRESIAS (http:// cbcsrv.watson.ibm.com/) are used to directly identify frequent patterns from unaligned biological sequences without an attempt to align them.
Methods and Materials. Here we propose a new algorithm with more efficiency and more functionality than both PRATT2 and TEIRESIAS, and discuss some of its applications to G protein-coupled receptors, a protein family of important drug targets.
Results. In this study, we designed and implemented six algorithms to mine three different pattern types from either one or two datasets using a pattern growth approach. We compared our approach to PRATT2 and TEIRESIAS in efficiency, completeness and the diversity of pattern types. Compared to PRATT2, our approach is faster, capable of processing large datasets and able to identify so-called type III patterns. Our approach is comparable to TEIRESIAS in the discovery of so-called type I patterns but has additional functionality such as mining so-called type II and type III patterns and finding discriminating patterns between two datasets.

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QUANTITATIVE DETECTION OF CYTOKERATIN 20 mRNA EXPRESSION IN BLADDER CARCINOMA BY REAL-TIME REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION

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Introduction. To evaluate the quantification of Cytokeratin 20 (CK20) expression between tumor and normal urothelial cells in early detection of bladder carcinoma.
Methods. Total RNA was isolated from voided urine samples of 6 pre-diagnosed and 34 diagnosed patients and 10 healthy volunteers as control group, cDNA and the quantification and amplification of CK20 mRNA was performed according to the manufacturer's instructions.
Results. In 34 bladder carcinoma cases, the average of relative ratio of CK20 mRNA expression was found as; 11.91±50.01 (median=0.50, minimum=0.005 and maximum=305.41). In 6 pre-diagnosed cases and 10 healthy volunteers, it was determined as 0.06±0.12 and 0.07±0.12 respectively. The positive and negative results of cases that were determined according to the "borderline", the specificity, sensitivity, positive diagnosis value, negative diagnosis value and total diagnosis value of the method were calculated as; 69.56%, 67.65%, 85.19%, 59.26% and 78.00% respectively. There was no correlation between relative ratio of CK20 and tumor grade. The ROC curve was created from sensitivity and specificity values. In receiver operating characteristic (ROC) analysis, the areas under curve (AUC) were calculated as 0,917 (p=0.000, CI95% = 0.841 to 0.994). Cut-off value of CK20 expression as 0.1224 maximised sensitivity (%82.4) and the specificity (% 81.2) on the ROC curve.
Conclusions. The level of CK20 mRNA expression is an available method for the diagnosis and follow up of bladder cancer cases. In addition; we believe that this method can contribute to prolong the cystoscopy follow-up period range that is still being used as a standard reference for monitoring of bladder cancer.

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IDENTIFICATION OF NOVEL GENES RELATED TO PRIMARY NODAL DIFFUSE LARGE B-CELL LYMPHOMAS WITH POOR OUTCOME BY ARRAY CGH AND EXPRESSION INTEGRATION ANALYSIS

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Introduction. Diffuse large B-cell lymphoma (DLBCL) is highly heterogeneous with regard to clinical outcome. A series of 42 diffuse large B-cell lymphomas (DLBCLs) is presented providing compelling evidence for the synergy of arrayCGH combined with expression arrays in identifying candidate oncogenes.
Methods. Array CGH (6K BAC) and expression profiling was performed on 42 DLBCLs. Regions of copy number alterations (CNAs) were determined using CGHcall [4] and CGH regions [5]. The chromosomal regions were clustered using a dedicated cluster algorithm called WECCA (http://www.few.vu.nl/~wvanwie/WECCA/) The array CGH data was subsequently integrated with the expression array results using ACE-it [6].
Results and Discussion. Expression analysis previously identified three different DLBCL subtypes with different clinical outcome [3]. Array CGH analysis of the same samples confirmed previously identified CNAs and could again separate the DLBCLs in 3 distinct clusters. These CNA clusters significantly associated with the expression profile subtypes. Combination of the data of RNA expression and CNA regions identified a total of 316 genes. TP53 apoptosis effector PERP/PIGPC1, was identified as a candidate tumor suppressor at 6q21, which region is frequently deleted only in patients with good prognosis. A 3Mb recurrent deletion at 15q1, contains the cyclin D binding-protein (CCNDBP1/GCIP) [2], recently identified as a tumor suppressor gene in hepatocarcinogenesis, and beta-2-microglobulin (B2M) [1], previously recognized as an important gene in DLBCL. Noteworthy, at the most frequently deleted region, 1p, no genes with recurrent down-regulation were significant after Benjamini-Hochberg's multiplicity correction. For gains with recurrent over-expression 1q, 3q, 7, 12p12, 18q and 21q were identified. Most frequently gained and overexpresses is a sharp peak of 0.3Mb at 1q32.1 MDMX/MDM4, a critical regulator of p53.

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