11th Annual Meeting
Friday, March 4, 2011
Aula Magna, Louvain-la-Neuve

Dynamic DNA
The BeSHG

The BeSHG was launched in March 2000.

The aim of the Society is to allow all scientists involved in the field of human genetics, working in all Belgian universities, and all independent research institutes or genetic centers in Belgium to have an official representative Society in Belgium.

The BeSHG is aimed at promoting Human Genetics in its wider sense, e.a. by supporting genetic research, improving exchanges between Belgium and foreign countries, organizing scientific meetings, and enhance collaborations between Belgian labs. The BeSHG represents the community of Belgian geneticists towards the other national and international Societies of Genetics, and promotes discussion on scientific, professional, social and ethical issues linked to the practice of human genetics.

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Hélène Antoine-Poirel, Nicole Revencu, Yves Sznajer, Miikka Vikkula

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Dear colleagues,

On behalf of the board of the BeSHG, as well as of the entire UCL Local Organizing Committee, I am pleased to welcome you to Louvain-la-Neuve for the 11th Annual Meeting of the BeSHG. “Dynamic DNA” will be the thread of the day, reflecting the fascinating observations that the human genome is much more vivid and modulated than previously thought. The DNA sequence and structure is continuously altered and recombined, somatic alterations are not infrequent, the transcriptional activity of DNA can be modulated in multiple ways, and RNA-level regulation adds an additional level of dynamism. Evolution is ongoing. We, as clinical geneticists, scientists and as members of the society, still have a long way to go to fully understand the human genome, and its use for the benefit of us all.

Renowned experts will lead us to the two extremes of life, from the potential role of spermatogonial paternal age-effect mutations in the origin of common complex diseases, and the mechanisms of meiotic recombination in genome diversity, to the limits of genome plasticity associated with senescence. We will explore the epigenetic changes induced by carcinogenic bacterial infections thanks to genome-wide analyses. We will discover the novel therapeutic strategies targeting genetic defects in cancer. Finally, the wealth of information provided by the new technologies requires that we question how this information can be integrated into patient care in a harmonious and beneficial way. Therefore, we will discuss what are the legal rights of the patients, in order to adapt genetic counseling to the context of genome-wide, dynamic data.

I hope that this diversified program will boost our daily work in genetics.

Welcome and enjoy the meeting!

Hélène Antoine-Poirel
Dynamic DNA

08.00 – 08.45  Registration (Coffee and croissants)

08.45 – 09.00  Welcome address: Hélène Antoine-Poirel, president BeSHG
Miikka Vikkula (for the local organizing committee)

09.00 – 10.30  Invited Presentations I
Chair: Paul Coucke – Nicole Revencu

09.00  Paternal age-effect mutations and selfish testes: causes and consequences in human disease
Anne Goriely (Weatherall Institute of Molecular Medicine University of Oxford, UK)

09.30  Plasticity of genome replication analyzed by deep sequencing and DNA combing
Jean-Marc Lemaître (Institut de Génomique Fonctionnelle, INSERM, Montpellier, France)

10.00  What determines the genetic map?
Bernard de Massy (Institut de Génétique Humaine, CNRS, Montpellier, France)

10.30 – 11.00  Coffee break, Poster session and Exhibition

11.00 – 12.00  Selected Oral Presentations by Young Investigators I
Chair: Sonia Van Dooren – Yves Sznajer

Annelien Massart
Functional analysis of CCDC54, a protein involved in spermatogenesis

Hannah Verdin
Identity-by-descent mapping reveals a new locus for primary congenital glaucoma, GLC3E, on chromosome 19p13.2

Kristien Peeters
A novel dominant mutation in filamin C causing distal myopathy

Sofie Metsu
A novel dynamic mutation in AFF3 associated with developmental delay

Laurence Desmyter
FAF1, the First Gene Associated with Cleft Palate

12.00 – 12.45  General Assembly for all BeSHG members

12.45 – 14.00  Lunch, Poster session, Exhibition & Satellite meetings

13h30 - 13h40  satellite meeting Caliper

13h40 - 13h50  satellite meeting Agilent
### 14.00 – 15.00  Selected Oral Presentations by Young Investigators II
**Chair:** Catheline Vilain – Nisha Limaye

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#### 15.00 – 15.30  Coffee break, Poster session and Exhibition

#### 15.30 – 17.00  Invited Presentations II
**Chair:** Thomy de Ravel – Miikka Vikkula

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#### 17.00 – 17.15  Conclusions and Prizes

#### 17.15 – 18.00  Reception
The BeSHG thanks the sponsors of this meeting:

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Invited Speakers
Paternal age-effect mutations and selfish testes: causes and consequences in human disease

Anne Goriely

Weatherall Institute of Molecular Medicine, University of Oxford, UK

The majority of spontaneous germline point mutations in humans are paternal in origin. It is now widely accepted that this paternal bias originates from gender-based differences in the biology of gametogenesis, but it is less well known that a small class of spontaneous mutations, which we term paternal age-effect (PAE) mutations, present in a distinctive fashion. The shared properties of PAE mutations include an almost exclusive paternal origin, a high apparent germline mutation rate (up to 500-800 fold above background), and a narrow spectrum of mutations encoding dominantly-acting proteins. The best documented examples of PAE mutations are in the FGFR2, FGFR3, HRAS, PTPN11 and RET genes, heterozygous substitutions of which cause a variety of congenital skeletal disorders (such as for example Apert, Costello, Noonan syndromes, achondroplasia and multiple endocrine type2a/b), some of which are additionally associated with cancer predisposition.

Recent evidence, based on quantification of mutation levels in sperm, indicate that spermatogonial cells carrying PAE mutations are positively selected and expand clonally in normal testes through a process akin to oncogenesis. This clonal growth, which is likely to take place in the testes of all men, leads to the relative enrichment of mutant sperm over time - explaining the distinctive PAE of this group of mutations - and in the most extreme cases, to the formation of testicular tumours. To date, all examples of these “selfish” spermatogonial mutations locate within a single signalling pathway, the growth factor receptor-RAS pathway, which is a key determinant of spermatogonial stem cell proliferation and renewal.

Regulation of cell turnover is important in many disease contexts, for example neurogenesis and neoplasia, so the consequences of mutations that hijack this process within germ cells are potentially far reaching. Depending on the spectrum of average PAE mutations levels, they may contribute significantly to the ‘dark matter’ in human heritability, currently speculated to be explained by uncommon alleles of moderate effect. Hence this mechanism is likely to be important in the origins of common complex diseases such as certain cancers and psychiatric disorders, as well as in congenital malformations.
Plasticity of genome replication analyzed by deep sequencing and DNA combing

Emilie Besnard, Amélie Babled, Laure Lapasset, Olivier Milhavet and Jean-Marc Lemaitre

AVENIR INSERM Group « Genome Plasticity and Ageing »
Institute of Functional Genomics, 141 rue de la Cardonille, 34094 Montpellier, France

Eukaryotic chromosomes are replicated from multiple replication origins, which are activated according to a specific spatio-temporal program. Although DNA replication initiates at specific sites, changes in origin selection have been described in embryo development, thought to be influenced by chromatin structure and gene expression, and this distribution can be reset from differentiated nuclei in nuclear transfer experiments to restore an embryonic program.

Currently, we explore the limits of this plasticity in the replication program, associated to the cellular aging phenotype.

In order to analyze modifications in replication origin distribution in details, we first mapped the location of replication origins at the human genome scale in different cell lines, by coupling the purification of short nascent strands elongated at the origins (SNS), to the highly sensitive next-generation sequencing. We discovered an unexpected high number of potential origin positions, heterogeneously distributed, and preferentially associated with specific structural elements of the genome.

Then, we extended our analysis to cellular senescence, which is characterized by a progressive decline of the competence to replicate, and associated to specific modifications of chromatin structure. Using DNA combing of single replicating molecules, we observed in pre-senescent fibroblasts, a global decrease in the mean replication fork velocity and of the mean inter-origin distances, when compared to young proliferative fibroblasts, which is consistent with the activation of stress-induced additional “dormant” origins. Paradoxally, using deep sequencing of SNS, we were able to detect only 40% of potential origins in pre-senescent fibroblasts, compared to proliferative ones, suggesting a decreased choice for origin activation during this process.

A model will be proposed to explain this specific regulation in origin selection associated to senescence entry.
What determines the genetic map?

Bernard de Massy

Department of Genome Dynamics,
Institut de Génétique Humaine, CNRS, Montpellier, France

One major question in the field of genetics, meiosis and genome evolution is to understand the distribution of meiotic recombination events, which are essential for chromosome segregation and which contribute to genome diversity. We have recently discovered that PRDM9, a protein that has a histone methyl-transferase activity and a zinc finger DNA binding domain plays a major role in specifying the sites of meiotic recombination in humans and mice. Our current knowledge about this unexpected and fascinating control, and its implications will be presented.
Dynamic aspects of epigenome - induction of aberrant DNA methylation by carcinogenic bacterial infection

Toshikazu Ushijima

Division of Epigenomics, National Cancer Center Research Institute, Tokyo, Japan

Epigenomes have both dynamic and stable aspects. They undergo dynamic and coordinated changes during development, differentiation, and reprogramming while are very stable during somatic cell divisions. At the same time, exposure to specific environmental stimuli can induce aberrant methylation of CpG islands that eventually leads to disease development. We showed that gastric mucosae of individuals with infection by carcinogenic bacteria, *Helicobacter pylori*, have higher methylation levels of multiple CpG islands than those without, and that the methylation levels were associated with risk of gastric cancers [Maekita, Clin Cancer Res, 2006]. Analysis of 48 promoter CpG islands revealed that methylation was induced in specific promoter CpG islands by *Helicobacter pylori* infection [Nakajima, Int J Cancer, 2009]. Genome-wide analyses of methylation-susceptible genes in cancer cells and of epigenetic modifications in normal cells revealed that susceptibility to DNA methylation was determined by the presence of trimethylation of H3K27 and the lack of stalled RNA polymerase in normal cells, in addition to gene transcription levels [Takeshima, Genome Res, 2010]. Using an animal model, an essential role of inflammation triggered by *Helicobacter pylori* infection in methylation induction was demonstrated [Niwa, Cancer Res, 2010]. In contrast with *Helicobacter pylori*-triggered inflammation, persistent inflammation induced by ethanol or high concentration of NaCl was not capable of inducing methylation [Hur, Carcinogenesis, 2011]. These lines of evidence demonstrated that exposure to specific environmental stimuli, such as *Helicobacter pylori* infection, dynamically induces methylation of specific CpG islands, and forms a field for disease development.
Tumour cells frequently exhibit deficiencies in the signaling or repair of DNA damage. These deficiencies probably contribute to pathogenesis of the disease, but rapid advances in cancer biology have recognized the promise of exploiting these intrinsic DNA damage deficiencies characteristic of tumour cells in the development of novel therapeutic strategies. The potential of therapy based on targeting the underlying genetic defects, is that it may cause highly selective killing of tumour cells while sparing normal cells, resulting in both increased efficacy and reduced toxicity. The identification of synthetic lethal interactions represents an attractive approach for targeting these deficiencies. Two genes are said to be synthetically lethal if a mutation in either gene alone is compatible with viability but mutation of both causes cell death. This approach has been successfully exploited in the clinic using inhibitors of the DNA repair protein, PARP, in the treatment of patients with germline mutations in the tumour suppressor genes BRCA1 and 2. When the genes that mediate the DNA mismatch repair (MMR) pathway, such as MLH1, MSH2 and MSH6 are mutated or epigenetically silenced, the predisposition to cancer is vastly increased. Therefore based on a similar approach to BRCA/PARP, we have shown that silencing of the DNA polymerases, POLB or POLG, is synthetically lethal with MSH2 or MLH1 deficiency, respectively, via an accumulation of oxidative DNA damage. Recently, we have also shown that silencing of the PTEN-induced putative kinase 1, (PINK1), is synthetically lethal in MMR deficient cell lines originating from either MSH2, MLH1 or MSH6 dysfunction. Inhibition of PINK1 in a MMR deficient background results in an elevation of reactive oxygen species (ROS) and the accumulation of both nuclear and mitochondrial oxidative DNA lesions. These synthetic lethal approaches highlight how an understanding of DNA repair processes can be used in the development of novel cancer treatments.
Patients' Rights within the Context of the Technological Revolution in Genetics (Belgium)

Geneviève Schamps (genevieve.schamps@uclouvain.be)

Faculty of Law and Faculty of Medicine, Université catholique de Louvain
Director of the Centre for Medical and Biomedical Law
President of the Federal Commission on Patients' Rights
Member of the Belgian Consultative Committee of Bioethics
Louvain-la-Neuve, Belgium

This presentation highlights several legal provisions that regulate the existing relationship between doctors and patients within the context of genetic testing.

In Belgium, there are no specific regulations governing patients' rights in this field. The latter come under the act of August 22nd, 2002, which is applicable to health care in general.

Several aspects of patients' rights with regard to specific clinical situations will be considered: patient representation in cases where patients are unable (or no longer able) to make decisions in relation to their patients' rights; the autonomy of minors who are considered able to reasonably appreciate their interests; the information that must be communicated to patients in order to obtain their free and informed consent to genetic testing; the information that must subsequently be provided to patients when the results of such medical tests are obtained; the person who must communicate such information. The existence of the patient's right not to know and the possibility for the doctor to invoke the therapeutic exception as a reason for not disclosing information to patients will also be touched on.

The issue of communicating genetic test results to family members and a patient's potential refusal of this will also be considered, as will the issue of access to patient records and the viewing of records by a patient's close relations after his or her death.
Selected Oral Presentations by Young Investigators
O1: Functional analysis of CCDC54, a protein involved in spermatogenesis

Annelien Massart¹, Karine Breckpot², Herman Tournaye³, Willy Lissens¹ & Katrien Stouffs¹

¹ Center for Medical Genetics, UZ Brussel - Department of Embryology and Genetics, VUB
² Laboratory of Molecular and Cellular Therapy, Department of Physiology-Immunology, VUB
³ Center for Reproductive Medicine, UZ Brussel - Department of Embryology and Genetics, VUB

In search for proteins involved in the complex process of spermatogenesis, coiled-coil domain containing 54 protein (CCDC54), a human testis-expressed protein with unknown function, was studied. Expression of this gene is predominantly in testis and in silico analysis of the CCDC54 gene suggested it is a conserved gene. A yeast two hybrid screening was performed, which identified Testis specific Zinc Finger Protein (TZFP), a transcriptional repressor, as a binding partner of CCDC54. To further investigate the effect of the binding of CCDC54 on the activity of TZFP, a luciferase reporter assay was performed. This suggested that CCDC54 is a negative co-regulator of TZFP. Immunohistochemical staining of human testis tissue shows an overall expression of the CCDC54 protein in the seminiferous tubules. At the DNA level, conservation of the CCDC54 gene was verified and mutation analysis was performed. To detect the reported c.316G>T or p.Glu106X polymorphism, a restriction digestion with BccI was performed on a control group of 150 European normozoospermic men, but the polymorphism was not found. The CCDC54 gene of 16 European men with a maturation arrest of spermatogenesis and 32 European men with Sertoli cell-only syndrome (SCOS) was sequenced and only one missense mutation was found in 2 SCOS patients. These preliminary results suggest that CCDC54 could be an important protein for spermatogenesis.
O2: Identity-by-descent mapping reveals a new locus for primary congenital glaucoma, GLC3E, on chromosome 19p13.2

Hannah Verdin¹, Barbara D’haene¹, Frauke Coppieters¹, Steve Lefever¹, Philippe Kestelyn², Bart P. Leroy² & Elfride De Baere¹

¹ Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium
² Dept of Ophthalmology, Ghent University Hospital, Ghent, Belgium

Primary congenital glaucoma (PCG) is caused by developmental anomalies of the trabecular meshwork and the anterior chamber angle, resulting in an increased ocular pressure (IOP) and optic nerve damage from birth or early infancy. The prevalence of PCG is estimated to be 1:10,000 in Western populations with higher prevalences in inbred populations. In general PCG displays an autosomal recessive inheritance and is genetically heterogeneous. To date, four PCG loci are known (GLC3A-D), in which two genes have been identified, CYP1B1 and LTBP2. Here, we aimed to map the disease gene in a large, four-generation consanguineous family with PCG, originating from Jordany.

Mutations in and linkage to the known PCG genes CYP1B1 and LTBP2 were excluded respectively. Homozygosity or identity-by-descent (IBD) mapping was performed in six affected members using genomewide SNP genotyping with 250K arrays (Affymetrix), followed by data analysis with a homemade Perl script.

The identified IBD regions did not overlap with any known PCG loci. Filtering on both size of the region and number of consecutive homozygous SNPs revealed a new candidate region on 19p13.2, named GLC3E. This region measures 1.8 Mb and contains 57 genes. Targeted resequencing of this region is ongoing.

We identified a new PCG locus, named GLC3E, confirming the genetic heterogeneity of PCG, and representing a unique opportunity to identify the third PCG gene.
O3: A novel dominant mutation in filamin C causing distal myopathy


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2 Neurogenetics Group, VIB Department of Molecular Genetics, University of Antwerp, Belgium
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4 Laboratory of Ultrastructural Neuropathology, Institute Born-Bunge, University of Antwerp, Belgium
5 Peripheral Neuropathies Group, VIB Department of Molecular Genetics, University of Antwerp, Belgium
6 Tokuda Hospital, Sofia, Bulgaria
7 University and University Hospital Antwerp, Belgium
8 National Genetics Laboratory, Molecular Medicine Center, Medical University-Sofia, Bulgaria
9 Department of Medical Chemistry & Biochemistry, Molecular Medicine Center, University-Sofia, Bulgaria

Distal myopathies make up a group of clinically and genetically heterogeneous disorders. In this study we describe three distantly related Bulgarian families affected by a dominantly transmitted late onset distal myopathy with unknown genetic defect. Using genome-wide parametric linkage analysis we delineated a 9.76 Mb region on chr7q22.1-q35 containing filamin C, a gene previously associated with myofibrillar myopathy. Filamin C mutation analysis shows a novel frameshift mutation resulting in a premature stop codon that segregates with the disease phenotype. Subsequent mRNA and protein expression studies in patient muscle tissue and lymphoblasts show nonsense mediated decay of the mutant filamin C transcript and a partial loss of filamin C protein expression. These findings suggest that filamin C is dosage sensitive and that mutations leading to haplo-insufficiency can cause myopathy in humans. Our results are in contrast with the previously reported filamin C mutations that cause myofibrillar myopathy through a mechanism of dominant gain of function. This novel filamin C mutation enlarges the clinical spectrum of filaminopathies for it is distinct both in terms of the associated phenotype and the underlying disease mechanism.
O4: A novel dynamic mutation in AFF3 associated with developmental delay

Sofie Metsu¹, Liesbeth Rooms¹, Jozef Gecz², David R. FitzPatrick³ & R. F. Kooy¹

¹ Department of Medical Genetics, University of Antwerp, Belgium
² Women’s and Children’s Hospital, Neurogenetics, Genetics and molecular Pathology, Adelaide, Australia
³ Medical Genetics Section, MRC Human Genetics Unit, Edinburgh, UK

Though specific types of autosomal fragile sites occur more frequently in cohorts of patients with cognitive disorders, few of these fragile sites have been characterized. We studied three families with FRA2A-expression and developmental delay and we identified an elongated polymorphic CGG repeat as the molecular basis of this fragile site. The repeat is located in the 5’ untranslated region of AFF3/LAF4 gene, a paralog of the nuclear transcription factors AF4, AF5q31 and FMR2 involved in regulation of gene expression, cell expansion and embryonic development. Interestingly, loss of expression of the FMR2 gene through dynamic repeat expansion of a CGG-repeat in the 5’ untranslated end of the gene, results in non-syndromic FRAXE mental retardation.

All probands showed cytogenetic FRA2A expression in 21 to 40% of the cells and were further characterized by slow early motor and language development and learning disability. In silico analysis demonstrated a polymorphic CGG repeat in the 5’ end of the AFF3 mRNA. Expansion of the AFF3 associated repeat was shown in the probands and in five relatives with Southern blot analysis. Furthermore a methylated AFF3-allele was present in the probands and in at least three relatives. By cSNP/RT-PCR we could confirm that the methylation of the promotor region silences the transcription of the AFF3 gene. With these results we have collected compelling evidence that transcriptional silencing of the AFF3 gene could play a role in the cognitive impairment present in all patients. After the cloning of FRAXA, resulting in fragile X syndrome, FRAXE, FRA11B in Jacobson syndrome and FRA12A, this is the 5th rare folate sensitive fragile site associated with cognitive disorders, emphasising the role of dynamic mutations in neurocognitive diseases.
O5: FAF1, the First Gene Associated with Cleft Palate

Michella Ghassibé¹, Laurence Desmyter¹, Tobias Langenberg², Karlien Hermans², Bénédicte Bayet³, Philippe Pélerin⁴, Filip Claes², Liesbeth Backx⁵, Pascal Brouillard⁶, Mieke Dewerchin², Nicole Revençu¹, Jacqueline Hecht⁷, Elisabeth Mangold⁸, Jeffrey Murray⁹, Michele Rubini¹⁰, Joris R. Vermeesch⁵, Hélène A. Poirel¹¹, Peter Carmeliet², Miikka Vikkula¹ & CLP group¹²

¹ Laboratory of Human Molecular Genetics, de Duve Institute, UCL, Brussels, Belgium
² Vesalius Research Center, K.U.Leuven, Leuven, Belgium
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¹¹ Center for Human Genetics, Cliniques Saint-Luc, UCL, Brussels, Belgium
¹² other collaborators

Cranial neural crest (CNC) is a multipotent migratory cell population that gives rise to most of the craniofacial bones. An intricate regulatory network mediates CNC formation, epithelial-mesenchymal transition, migration along distinct paths and differentiation. Errors in these processes lead to craniofacial abnormalities, including cleft lip and palate.

The breakpoint of a 46,XY,t(1;2)(p34;q33) balanced translocation present in a family with hereditary Pierre Robin sequence (PRS), was characterized. Expression of the disrupted gene was evaluated. Its implication in non-syndromic clefts was tested by association and relative risk calculations on 7597 individuals. Gene sequencing and SNP arrays were conducted in 368 individuals. In situ hybridizations on murine embryos investigated its role during development. Expression studies of chondrogenic markers, knockdown and mRNA mediated rescue were conducted on zebrafish larvae to unravel the dysregulated pathway.

Fas-Associated Factor-1 (FAF1) is disrupted and its expression is decreased in a PRS family with an inherited translocation. The locus is strongly associated with CPO with an increased relative risk. Faf1 is highly expressed in mouse and zebrafish cartilages during embryogenesis. Knockdown of zebrafish faf1 leads to pharyngeal cartilage defects, due to a failure of CNC to differentiate into and express cartilage specific markers, such as sox9 and col2a1. This phenotype is rescued by administration of faf1 mRNA.

FAF1 is a newly recognized regulator of CNC differentiation. It is necessary for lower jaw development and its disruption results in craniofacial anomalies across species. It is the first gene shown to predispose to cleft palate in man.
O6: Leber Congenital Amaurosis: development of a comprehensive molecular genetic test panel using next-generation sequencing

Frauke Coppieters¹, Bram De Wilde¹, Ellen De Meester¹, Steve Lefever¹, Filip Pattyn¹, Nina De Rocker¹, Bart P. Leroy², Jo Vandesompele¹ & Elfride De Baere¹

¹ Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium
² Center for Medical Genetics and Dept of Ophthalmology, Ghent University Hospital, Ghent, Belgium

Purpose: Leber Congenital Amaurosis (LCA) is caused by mutations in 16 known genes, which together account for approximately 70% of cases. Most often genetic testing for LCA is limited to screening of a selected number of mutations and/or Sanger sequencing of a subset of genes.

The goal of this study was to design an accurate, fast and affordable molecular test for all known LCA genes using next-generation sequencing (NGS).

Methods: We developed a novel protocol consisting of quantitative PCR (qPCR) amplification followed by ligation and fragmentation, sequencing on a Genome Analyzer IIx run, data analysis by NextGENe software. Ten LCA patients were included, five of which had a known molecular defect through prior screening.

Results: Our proof-of-concept study consisted of resequencing of all exons of 16 LCA genes (RD3, RPE65, CRB1, MERTK, IQCB1, LRAT, LCA5, TULP1, IMPDH1, CEP290, RPGRIP1, RDH12, SPATA7, AIPL1, GUCY2D and CRX) in 10 LCA patients. Using in-house developed primer design, 375 primer pairs were designed to cover 236 exons and their intron-exon boundaries. Following amplification, ligation and shearing, all amplicon pools were indexed and together sequenced in a single lane of a Genome Analyzer Ilx run (1x100bp). This yielded sufficient coverage for 93-95% of exons. In total, 104 out of 107 previously identified variants were detected using the NextGENe software, including the known mutations in the positive control group. In addition, mutations were found in two out of five mutation-negative patients.

Conclusions: We developed a novel, cost-efficient workflow for enrichment and parallel sequencing of all currently known LCA genes. The protocol combines reliable and efficient high-throughput qPCR amplification with reproducible ligation and shearing steps, thereby enabling sequencing of regions of interest with variable length, such as exons, on a short-read sequencer. Moreover, the flexibility of the workflow allows easy expansion of the panel with other genes, providing an excellent basis for molecular testing of other retinal dystrophies. Finally, our protocol will allow an early molecular diagnosis in LCA patients, which is essential with respect to reproductive issues, prognosis and eligibility to gene therapy, given recent breakthroughs in gene therapy for RPE65-related LCA.
O7: Genome-wide Haplotyping and Detection of Meiotic Homologous Recombination Sites in Single Cells

Masoud Zamani Esteki, Evelyne Vanneste, Peter Konings, Yves Moreau, Thomas D’Hooghe, Joris R. Vermeesch & Thierry Voet

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Haplotyping is invaluable not only to identify genetic variants underlying a disease or trait, but also to study evolution and population history as well as meiotic and mitotic recombination processes. Current genome-wide haplotyping methods rely on genomic DNA that is extracted from a large number of cells. Thus far random allele drop out and preferential amplification artifacts of single-cell whole genome amplifications as well as algorithmic shortcomings have precluded genome-wide haplotyping of one cell. By developing an innovative data extraction and analysis pipeline of genome-wide SNP-data, the haplotype was reconstructed from single EBV-transformed lymphoblastoid cells as well as human blastomeres derived from in vitro fertilized (IVF) embryos. The methodology applies an optimized single-cell genotype computation and a mixture of novel algorithmic approaches that compute and interpret single-cell haplotypes. When compared to the reference haplotypes which were determined from DNA-samples extracted from many cells of the corresponding EBV cell lines, this computational pipeline improved genome-wide SNP-haplotype discordance rates from ~25% to less than 5% for single cells, thus enabling the detection of homologous recombination sites in individual cells. Furthermore, when applied to single human blastomeres of an embryo carrying a disease allele identified by conventional preimplantation genetic diagnosis the method was able to confirm the diagnosis. This generic haplotyping method will revolutionize reproductive genetic options as it not only enables to select pre-implantation embryos for a single trait, but also broadens the selection spectrum to multiple Mendelian traits as well as to qualitative and quantitative traits present in (animal) embryos. In addition, we envision that the method will increase our insights in the mechanisms of meiotic and mitotic recombination processes and enable the analysis of specimen with limited or degraded DNA.
O8: A cooperative microRNA – tumor suppressor gene network in acute T-cell lymphoblastic leukemia (T-ALL)

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Background: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy of thymocytes. In order to comprehensively assess the action of microRNAs (miRNAs) in T-ALL, we compared miRNA expression patterns in 50 human T-ALL samples and 5 subsets of normal T-cell progenitors with an unbiased miRNA library screen followed by computational target identification and functional assessment of the most relevant candidate miRNAs in a murine T-ALL model.

Methods: Using high-throughput quantitative stem-loop RT-PCR, 430 miRNAs were profiled in a T-ALL patient cohort including 12 HOXA, 15 TAL/LMO, 10 TLX3 and 5 TLX1 rearranged patient samples as well as in 5 different subsets of sorted T-cell populations. An unbiased miRNA library screen was performed in c-MYC transduced MEFs, based upon rescue for c-MYC-induced apoptosis, followed by validation for individual miRNAs in FL5-12 lymphocytes and an in vivo T-ALL mouse model.

Results: In addition to specific sets of differentially expressed miRNAs in the genetic T-ALL subgroups, we identified ten miRNAs which were highly expressed in the entire cohort of T-ALLs, i.e. miR-223, miR-19b, miR-20a, miR-92, miR-142-3p, miR-150, miR-93, miR-26a, miR-16 and miR-342. High expression of the latter subset of 10 miRNAs was confirmed in a series of 18 T-ALL cell lines. A comparison with purified normal T-cell populations revealed leukemia-specific increases in miR-223, and less so for miR-376 and miR-662. Subsequent cross-comparison with the results of a parallel unbiased miRNA library screen, allowed us to identify five miRNAs (miR-19b, miR-20a, miR-26a, miR-92, miR-223) capable of promoting T-ALL development in a NOTCH sensitized murine model. These miRNAs produce overlapping and cooperative effects on validated target genes with known tumor suppressor function in T-ALL, including IKAROS (IKZF1), PTEN, BIM/BCL2L11, PHF6, NF1 and FBXW7.

Conclusion: A comprehensive and unbiased analysis of miRNA action in T-ALL reveals for the first time a cooperative role for a small subset of miRNAs in suppression of key T-ALL suppressor genes and opens the way to new miRNA therapeutic approaches for this disease.
O9: PRDM16 (1p36) translocations define a distinct entity of myeloid malignancies with poor prognosis but may also occur in lymphoid malignancies

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The PRDM16 gene on chromosome 1p36 is reported to be rearranged in sparse cases of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) with t(1;3)(p36;q21). We report the largest series to date of 39 cases of hematological malignancies with PRDM16 alterations out of a series of 120 cases with 1p36 rearrangements screened by fluorescence in situ hybridization (FISH). PRDM16 was found to be rearranged with the RPN1 locus (3q21) in 30 cases and with other loci in 9 cases. The PRDM16 rearrangements are not restricted to myeloid malignancies, as we characterized two cases of lymphoid proliferation with translocations involving PRDM16. We identified novel translocation partners of PRDM16, including transcription factors ETV6 and IKZF1. This is the first report of a translocation involving IKZF1 in a myeloid malignancy. Translocations involving PRDM16 are original in that they lead to its over-expression through 2 different mechanisms (transcriptional upregulation by promoter switch or formation of a chimeric gene). Survival data interestingly suggest that patients with AML/MDS and PRDM16 translocations have a poor prognosis whatever the partner gene. We confirm that AML/MDS with PRDM16 translocations share numerous characteristics with AML/MDS associated with translocations involving EVI1 (3q26), another transcription factor with a PR domain. We propose the addition of a “PRDM16”-entity in the WHO classification of AML/MDS, which could later be broadened to a "PR domain gene rearrangements" subgroup to encompass the AML with EVI1 translocations.
010: Profiling intra tumour heterogeneity with SNP-CGH array based on mono-allelic deletions

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Diagnosis in cancer molecular cytogenetics relies on the ability of Comparative Genomic Hybridisation (CGH) analysis to detect chromosomal alterations that frequently occur in tumours. However, these tumours are often heterogeneous and most current CGH algorithms do not take this heterogeneity into account, leading to frequent failures in detection. Besides, many CGH data algorithms have numerous tunable parameters urging for the development of a simple analysis tool with a good visualisation display of candidate alterations. This tool should also evaluate the intra-tumour heterogeneity (ITH) in non paired samples.

We developed a new analysis method to estimate the ITH with SNP-CGH array based on mono-allelic deletions (CN1). Basically, the commonest alteration is hypothesized to represent the earliest stable genetic alteration in the tumour and has therefore affected the majority of the altered cells in the sample. Conversely, the latest stable genetic alteration has affected the minority of the altered cells in the sample. These early and late events represent therefore different percentage of altered cells.

Our method transforms each CN1 alteration event into its percentage of altered cells. Combining all these percentages, a heterogeneity profile is drawn to reveal the different sub-populations of altered cells constituting the tumour.

We applied this ITH method on freely available GEO datasets, one is composed with chronic lymphocytic leukemia samples and the other is composed with non-small cell lung cancer samples.

Our method obtained a good correlation with the results originally published in these studies using cell counting methods such as FISH, flow cytometry or light microscopy.

Based on our method, the identification of cell sub-clones in heterogeneous tumours was greatly facilitated. Moreover, our method is based on the algorithm "CHROMOWAVE" used to denoise mRNA expression levels and therefore our method makes possible the direct correlation of SNP-CGH array data with gene expression levels. The current method does not provide ITH estimation based on bi-allelic deletions and allelic gains and further work in this direction is needed. However, our method is an important step for future profiling of ITH and for drawing a phylogenetic tree revealing the tumour development.
Poster Presentations
P1: Characterization of deletions in Multiple Osteochondromas with arrayCGH

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Introduction:
Hereditary multiple osteochondromas (MO) is an autosomal dominant skeletal disorder characterized by the formation of multiple osteochondromas. Gross EXT1/EXT2 deletions are responsible for up to 8% of cases. We determined the breakpoints of large EXT1/EXT2 deletions in 16 unrelated MO patients using an MO specific tiling path array and PCR/sequencing analysis.

Methods:
The heterozygous deletions of 16 MO patients were investigated, including with a custom-made Agilent oligonucleotide based chip with a tiling coverage for EXT1/EXT2. For 9 deletions characterization of the deletion breakpoints was possible after fine mapping by performing an allele specific PCR followed by sequencing analysis. The remaining 7 deletions that outlined the limits of the tiling path array were fine mapped on an Illumina HumanCytoSNP-12 v2.0 chip. Additionally, 6 patients with EXT1/EXT2 deletion were added to test for recurrent breakpoints.

Results:
ArrayCGH allowed fine mapping of 9 deletion breakpoints (EXT1 exon 2-11, 2-3, 6-7, 8, 11 and EXT2 exons 2, 8). In 2 patients breakpoints were located in long (> 30 bp) homologous regions. For 2 patients, large homologies in junction regions were identified, but in both cases one of the breakpoints was located outside the homology. Five deletion junction regions did not show any big homologies (> 30 bp), but short identical sequences (2-5 bp) were identified at all breakpoint junctions except for one. Only one of 6 additional samples could be amplified with allele specific PCR, showing the same EXT2 exon 8 deletion as an initial patient. The 7 samples outlining the array limits were additionally analysed on an Illumina chip for fine mapping (EXT1 complete, EXT1 exons 1, 2-11). Two deletions outlined the array boundaries on both the proximal and the distal end. Both patients were shown to have contiguous gene syndromes, respectively Langer-Giedion (chromosome 8) and WAGR-syndrome (chromosome 11).

Discussion & conclusion:
Out of 9 characterised deletions, 7 are supposed to be caused by non-homologous end-joining. Remarkably, 2 of these showed large homologous regions but in both cases one of the both breakpoints was located elsewhere. The two remaining deletions are supposed to be caused by homologous end-joining and one of these was found to be recurrent in a second patient. Additional patients with a deletion of the same exon(s) however had different deletion boundaries, suggesting the absence of recurrent deletions causing MO. It can be concluded that the custom-made Agilent oligonucleotide based chip is a powerful tool for the precise characterization of larger deletions involving EXT1 and EXT2.
P2: PCR-RFLP, sequencing and quantification in molecular diagnosis of spinal muscular atrophy: limits and advantages

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Spinal muscular atrophy (SMA) is a severe neuromuscular disease. It is a common cause of infant mortality. Its incidence is estimated at 1 in 10,000 (1). Clinically, age of onset and the symptoms can distinguish four types of SMA (2). Type 1 was the most severe form of SMA (Werdnig-Hoffman Syndrome). It appears before six months, and life expectancy does not exceed 7 years (3). Type 2 onsets before 18 months and patients are able to sit, but always walk with support. Patients with SMA type 3 or Kugelberg-Welander disease can walk without aid. For Type 3, patients can walk unaided. However, SMA type 4 or Adult form appears after 30 years (4). The gene SMN (survival motor neuron) was located on chromosome 5q13. There are two nearly identical copies (SMN1 / telomeric and SMN2 / Centromeric) (5). The sequence homology between genes SMN1 and SMN2 is 99% with only five nucleotide differences (6). SMA is caused by a homozygous deletion of exon 7 (+ / - exon 8) of SMN1 gene. Thus, the number of SMN2 copies determines the severity of the SMA (7). It has been shown that mild forms of SMA (3 and 4) are associated with more than two copies of SMN2 (8).

The presence of SMNc, the sequence homology with SMNt as well as the correlation between the phenotype and number of copies of SMN2 make molecular diagnosis difficult. Thus, many methods have been used for molecular diagnosis of SMA (RFLP-PCR, sequencing, quantification, ARMS-PCR ...)

The objective of this study is to make available to clinicians a reliable and reproducible test for the molecular diagnosis of SMA. We evaluate the benefits and limitations of three tests used in our laboratory (RFLP-PCR, sequencing and Q-PCR)
P3: Factor V Leiden, prothrombin and MTHFR gene mutations in Moroccan patients with stroke

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The association between vascular and hemostatic factors and risk of stroke has been studied and reported in many studies. Blood coagulation system is thought to be involved in the pathogenesis, and inherited prothrombotic risk factors are supposed to be predisposing to stroke events. The pathogenesis of stroke is complex with the interaction of genetic and environmental factors. Single point mutation in the factor of Leiden gene, prothrombin gene and tetrahydrofolate reductase (MTHFR) gene are confirmed to be associated with stroke. Our work aimed to evaluate the prevalence of factor V Leiden, prothrombin and methylene tetrahydrofolate reductase (MTHFR) gene mutations in 346 stroke Moroccan patients and to compare with that found in healthy controls. Heterozygote factor V Leiden mutation was found in 6.6% stroke patients versus 0% in control subjects. Heterozygote and homozygote MTHFR mutation was determined in 44.5% and 7.3% respectively in patients versus 41.7% and 6% respectively in controls. Heterozygote prothrombin G20210A mutation was found in 28.8% patients and 5.4% in control subjects. FV-Leiden and prothrombin G20210A, but not MTHFR C677T, was associated with stroke in the population studied. Our results are in harmony with some previous reports studying the association of the markers stroke and also in agreement with studies about Moroccan population.
P4: Array-CGH analysis in patients with maturation arrest of spermatogenesis

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Introduction. The past decades, many studies have been performed aiming to identify genetic causes of maturation arrest of spermatogenesis (MA). However, these studies were rather disappointing. In the present study, we looked for the presence of copy number variations (CNVs) in patients with MA and controls with normal sperm parameters

Material and methods. CNVs were identified using 244K arrays in 9 patients. For regions of possible interest, more patients and especially larger groups of control men were investigated by qPCR.

Results. In the patient and control groups, on average 25±4 and 24±7 CNVs were detected, respectively. After elimination of regions that are not containing any known genes and regions that were also detected as CNVs in controls, 32 regions remained. A further reduction to nine regions was done by eliminating deletions that are completely intronic (fi in PRKG), regions that were recently amplified in evolution (f.i. BAGE and PNMA genes) and regions with a well-known function not involved in spermatogenesis. The remaining nine regions are being investigated by qPCR in order to investigate large numbers of controls (>100) and to conclude whether the observed CNVs are common or related to the fertility problems. Preliminary results show that at least some of the regions are exclusively detected in the patients.

Conclusions. In this study, a small group of patients with MA have been thoroughly investigated using array-CGH. No common region was detected in the patient group that was absent in control samples. Six promising regions will be further investigated.
P5: Molecular analysis in Belgian patients with late-onset glycogen storage disease type II

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Glycogen storage disease type II (GSDII or Pompe disease) is an autosomal recessive disorder caused by a deficiency of the lysosomal alfa-glucosidase (GAA). Clinically, the disease represents a continuous spectrum of phenotypes from rapid progressive early-onset subtypes to late-onset milder subtypes. We present here the results of molecular analysis of all coding exons and parts of the flanking introns of the GAA gene in 16 unrelated patients, aged between 25 and 59 years, with late-onset GSDII on clinical and biochemical bases. All 16 patients were carriers of the leaky splice site mutation c.-32-13T>G (previously described as IVS1-13T>G) that is known in literature to occur at a high frequency in adult-onset patients of Caucasian origin. The mutations on the other alleles comprised 9 small deletions/insertions/duplications with c.258dupC present in 3 patients, 4 missense (3 not previously described) and one nonsense mutation, one donor splice site mutation and one deletion from I17 to I18. The independent segregation of the mutations was proven in all families.

Our results confirm the high incidence of the c.-32-13T>G mutation in Caucasian patients with late onset GSDII. Therefore, mutation analysis in late-onset GSDII patients could be started by sequencing for the presence of this mutation in our population. The other mutations are scattered all-over the GAA gene; many of these mutations are insertions or deletions of a few bases.

We would like to thank the Neurologists who have sent samples from their patients for molecular analysis.
P6: No genetic association between Wnt10b polymorphisms and obesity in a Belgian case-control population

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Introduction: The Wnt pathway has been shown to play an important role in maintenance of stem cells and cell fate decisions in embryonic and adult stem cell populations. Activation of the Wnt pathway in mesenchymal stem cells and 3T3-L1 cells inhibits adipogenesis and can lead to osteoblastogenesis. To evaluate the role of the Wnt pathway in adipogenesis (and obesity) further, we analyzed the genetic association between polymorphisms in Wnt10b, an activator of the Wnt pathway, and various obesity parameters in a Belgian population.

Methods: Four tagging SNPs that captured variation of eleven SNPs (MAF>5%) in a 15,2 kb region spanning the Wnt10b gene and its 3’ and 5’ UTRs were genotyped using Taqman® SNP Genotyping Assays. Our population consisted of 1013 obese patients (BMI≥30 kg/m²) and 307 lean healthy individuals (18,5 kg/m² ≤ BMI ≤ 24,9 kg/m²).

Results: In our Belgian case-control population, no significant OR was found for common SNPs in and around Wnt10b. Wnt10b variants were not associated with BMI or with seven additional obesity parameters (waist circumference, waist-to-hip ratio, fat mass, fat mass percentage and total/visceral/subcutaneous fat as measured by a CT scan).

Conclusion: Whereas common variation in Wnt10b was previously shown to be associated with total abdominal and subcutaneous fat in a female Korean population (Kim et al., 2010, doi:10.1016/j.jnutbio.2010.02.002), we were not able to replicate these results or prove any other obesity-related association with Wnt10b in our Belgian population.

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P7: Association between polymorphisms of the Nesfatin gene, NUCB2, and obesity in men

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Introduction: Nesfatin-1, which originates from its precursor protein nucleobindin-2 (NUCB2), is a novel appetite-regulating molecule that might be associated with the melanocortin signalling pathway in the hypothalamus. The secreted protein appears to play an important role in metabolic control through its anorexigenic and anti-hyperglycemic effects. Therefore, we hypothesized that polymorphisms in the NUCB2 gene might influence the susceptibility for the development of obesity.

Methods: In this study, we investigated the association of NUCB2 polymorphisms with the development of obesity in an extensive Caucasian population comprising 1049 obese subjects and 315 normal weight control individuals. We selected and genotyped 8 tagSNPs, which after additional analysis of 6 multi-marker tests, cover most information on common genetic variation in the selected region of 74.78kb surrounding NUCB2 including 10kb upstream and downstream of the gene.

Results: When performing logistic regression analysis, we found association with obesity for 3 SNPs (rs1330, rs214101 and rs757081) and 3 multi-marker tests, only when analyzing the male population separately (p-values ranging from 0.007 to 0.034). We subsequently performed linear regression analysis, again in the male population only, and found that several SNPs were associated with BMI, weight and fat free mass (p-values ranging from 0.002 to 0.036).

Conclusion: These data indicate that polymorphisms in the NUCB2 gene could play an important role in the protection against the development of obesity in male subjects and might have an influence on energy homeostasis. Nevertheless, further research including replication of our results and elucidation of the molecular mechanism remains necessary.
P8: Replication of the SH2B1 rs7498665 association with obesity in a Belgian cohort

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Objective
SH2B1 has been identified as an interesting candidate gene for complex obesity through genome-wide association studies. Therefore, we set out to replicate the reported association with rs7498665 in our Belgian cohort and to extend our study with an additional tagSNP in order to cover most genetic variance in the SH2B1 gene region.

Method: We genotyped both rs7498665 and rs7201929 in a population of 1045 obese adults and 317 healthy lean individuals. Statistical analyses were performed to evaluate the role of these polymorphisms in the development of obesity.

Results
We found that the rs7498665 minor allele increases obesity risk by 26% (ORage-sex adj = 1.26, 95% CI 1.04-1.52, nominal p = 0.016). Logistic regression showed that the rs7201929 minor allele decreases obesity risk by 24% in the population investigated (ORage-sex adj = 0.76, 95% CI 0.61-0.94, nominal p = 0.011). Conditional analyses showed that both associations represent the same association signal (rs7498665 ORadjusted for rs7201929 = 1.17, 95% CI 0.95-1.45, nominal P = 0.14; rs7201929 ORadjusted for rs7498665 = 0.82, 95% CI 0.65-1.04, nominal p = 0.10).

Conclusion
With the current study we were able to replicate and confirm that the SH2B1 gene locus is significantly associated with complex obesity in a Caucasian population.
P9: Towards a Venous Malformation Mouse Model

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Objectives
Germline substitutions in the endothelial cell tyrosine kinase receptor TIE2/TEK cause a rare inherited form of venous anomalies, Mucocutaneous Venous Malformation (VMCM). Moreover, at least 50\% of common sporadic venous malformations (VM) are caused by somatic mutations in TIE2. The identification of the etiopathogenic cause forms the basis for the generation of in vivo mouse models of the disease.

Methods
Conditional “knock-in” lines carrying the most frequent somatic Tie2 mutation, L914F, have been generated. The targeting construct replaces wild-type exon 17 with cDNA starting at exon 17, followed by the remaining 3’ coding region flanked by loxP sites, and then the mutant exon. Upon Cre-expression, the cDNA containing the wild-type exon is floxed out, leaving only the mutant exon and endogenous 3’ exons to be expressed. After electroporation, homologously recombined murine ES cells were identified using PCR and Southern blot, and selected clones were injected into blastocysts to generate targeted mice.

Results
Eight chimeras have been obtained, and germline transmission of the targeted locus was confirmed. Ubiquitous Cre-expression, causing wild-type Tie2 excision, is being induced by crossing with PGK-Cre mice. Preliminary results indicate embryonic lethality before E10.5. Tamoxifen-inducible, endothelial cell-specific replacement will be obtained by crossing with Cd\textsuperscript{h}5-CreERT2 transgenics.

Conclusion
The knock-in strategy replaces wild-type Tie2 with VM-causative mutations, hopefully generating an in vivo model of the disease. This will allow us to study the effects of the mutations and better understand their etiopathogenic mechanisms, as well as test novel therapeutic measures.
P10: Genetic variation in secreted Frizzled related protein 4 (sFRP4) is associated with femoral neck BMD and hip geometry parameters in Danish men – Results from the Odense Androgen Stud

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Osteoporosis is a common multi-factorial disease characterized by low bone mineral density (BMD) and increased fracture risk. Heritability studies demonstrated that genetic factors may account for up to 80% of the variability in BMD. BMD is depending on the peak bone mineral density (pBMD) reached around 25 years and on age related bone loss. The senescence accelerated mouse P6 (SAMP6) is a spontaneous developed mouse characterized by a lower pBMD and has several features which are similar to age-related bone loss in human. Nakanishi et al showed in 2006 that the osteoporotic phenotype in these mice is due to an increased expression of sfrp4, a modulator of the Wnt signalling pathway. By binding to Wnt ligands, sFRP4 is able to suppress the osteoblast proliferation in vitro. Based on those results we examined the effect of variation in and around sFRP4 on BMD and hip geometry parameters in men.

Using HapMap we selected 11 tagSNP’s and 3 multimarker tests which tag the common genetic variation (MAF>5%) in and around sFRP4. All SNP’s are genotyped in 780 Danish men aged 20 to 29 years which are included in the Odense Androgen Study (OAS). The effect of the genotyped SNP’s on the selected phenotypes was tested using linear regression analysis. Statistical analysis resulted in four associated SNP’s (p=0.009 - 0.04) with femoral neck (FN) BMD. Two of them are also associated with at least one hip geometry parameter (p=0.021-0.038). Based on those results, we have selected the four associated SNP’s and replicated those in a population of 590 Danish men aged 60 to 74 years which are also included in the OAS. Since the second population is a smaller population we had less power to detect small effects. Therefore we were only able to replicate the results for only one SNP and some hip geometry parameters. However combined analysis of both population showed smaller p-values for the SNP’s associated with FN BMD (p= 0.007–0.012) and hip geometry parameters (p= 0.006-0.027).

In conclusion, genetic variation in sFRP4 is associated with FN BMD and some hip geometry parameters in a population Danish men aged 20-29 years. We were not able to replicate all these results in older population with same ethnical background. However in a meta-analysis of both populations we found smaller p-values compared to the results we found in the first population.
Hyperostosis cranialis interna (HCI) is a rare autosomal dominant disorder characterised by intracranial hyperostosis and osteosclerosis which is confined to the skull, especially the calvarium and the skull base. The rest of the skeleton is not affected. CT scans show excessive growth of the temporal bone and the narrowing of the internal auditory canals, optic canals and orbital fissures. Clinical symptoms are recurrent facial nerve palsy, disturbance of the sense of smell and touch, hearing and sight impairments and disturbance of balance due to vestibular areflexia. The treatment of this disease is symptomatic.

Histomorphological investigations showed an increased bone formation with a normal tissue structure. Until today the disease is only described in three related Dutch families with common progenitors and consist of 31 individuals over five generations. HCI was observed in twelve family members over 4 generations. Patients are mildly to severely affected. Clinical symptoms appear in the second part of childhood and involve different degrees of function loss of the cranial nerves I, II, VII and VIII. Besides HCI, several bone dysplasias with hyperostosis and sclerosis of the craniofacial bones are known. Examples are Van Buchem disease, sclerosteosis, craniometaphyseal dysplasia and Camurati-Engelman disease. However, in these cases the long bones are affected as well. So far, HCI is the only disorder affecting the skull alone.

A genomic search was performed on this family. More than 300 polymorphic markers spread across the genome were analysed. With these results, LOD-scores were calculated for each chromosome. Based on the results of the LOD-score calculations, genes already known to cause bone dysplasias with involvement of the skull such as SOST, LRP5, LRP4, ANKH and TGFB1 can be excluded as disease causing genes in this family because of LOD-scores below -2.

These results indicate that a gene not previously involved in a sclerosing bone dysplasia is responsible for the abnormal bone growth in the skull of these patients.
P12: Inactivation of murine glomulin results in early embryonic lethality

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Glomuvenous malformations (GVM) are characterized by aberrantly differentiated vSMC-like “glomus cells” around distended vein-like channels, and result from loss-of-function mutations in Glomulin. We have shown that the lesions result from complete lack of Glomulin due to somatic second-hit mutations. To generate a model to study the etiopathogenesis of GVM, in which to test potential therapies, and to understand the developmental role of glomulin, we inactivated the murine gene by inserting the LacZ reporter gene at the start codon. Glmn-heterozygotes were healthy for more than one-year-old, and showed wide vascular expression of LacZ. In contrast, no live knockouts were obtained, embryonic lethality occurring around E8.5, with severe mesodermal defects. To enable studies beyond the lethality time point, we created transgenics with conditionally inducible expression of two glomulin-specific RNAi. When down-regulation of glomulin was induced ubiquitously, embryonic lethality ensued. However, knockdown embryos developed further than the knockouts, and showed vascular abnormalities such as absent yolk-sac vasculature and hemorrhages. Thus, the early lethality in absence of glomulin suggests a crucial role in embryogenesis, even before vascular development. Spatio-temporally controlled induction is thus needed to elucidate the role(s) of glomulin during development, and to mimic the double-hit mechanism underlying GVM, so as to obtain a model to study novel GVM therapies.
P13: Analyses of rare variants in the mitochondrial genome contributing to Age-Related Hearing Impairment

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ARHI (Age-Related Hearing Impairment) is the most common sensory disorder and the most important cause of hearing loss in the elderly. ARHI negatively impacts quality of life. Despite a heritability of about 50%, only weak evidence has been found for the involvement of common genetic variants in ARHI. Here we test whether rare variants in the mitochondrial genome increase susceptibility for ARHI. Mitochondria are the intracellular organelles responsible for energy production by oxidative phosphorylation. They have their own replicating genome, which is highly susceptible to mutations. Many studies highlight the important role of mitochondrial DNA (mtDNA) mutations in age-related complex disorders. Past strategies for screening the whole mitochondrial genome for common and rare variants were limited by the lack of a high throughput platform for mutation detection. We sequenced for 200 ARHI case subjects and 200 ARHI control subjects the entire mitochondrial genome on the Genechip Human Mitochondrial Resequencing Array 2.0 (Affymetrix, Santa Clara, CA, USA).

The common disease rare variant (CDRV) hypothesis states that multiple rare DNA sequence variants with mildly to moderately deleterious effects can be the major contributors to complex diseases. However, classical statistical association tests have low power to identify rare variants. To test the role of rare variants in ARHI, we first looked for a global effect of allelic variation on the phenotype, examining the mutation load of each individual. For each locus showing allelic variation, we subtracted the number of minor alleles in cases by the number of minor alleles in controls. Second, we compared the average mutation load of rare variants between cases and controls. We repeated this test weighting each rare variant with a conservation score obtained by MegAlign (DNastar, Wisconsin, USA). Lastly, to test whether the presence of rare alleles in one of more particular genes was associated with the phenotype, we used the collapsing method of Li and Leal for rare variants (Li and Leal, 2008). For each of the genes separately, we subdivided the individuals into two groups according to their mutational status: individuals carrying no minor allele were scored as wildtype, whereas individuals with at least one mutation were scored as mutant. Association between mutational status and case control status was tested using the Fischer's exact test.

P14: A new locus for otosclerosis, OTSC10, maps to chromosome 1q41-44

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Otosclerosis is a common form of hearing loss characterized by a disordered bone remodeling in the otic capsule. The abnormal bone remodeling can result in conductive hearing loss due to fixation of the stapes footplate, but sensorineural hearing loss can also be present. Although its etiology remains unknown, otosclerosis can be considered a complex disease with rare monogenic forms. Linkage analysis in large families segregating autosomal dominant otosclerosis has led to the identification of 7 loci (OTSC1-5, 7-8). However, none of the corresponding genes has been cloned. In the OTSC2 region however, indications have been found that TCRB is the causative gene in the region.

In this study a new large Dutch otosclerosis family with autosomal dominant inheritance is investigated. After exclusion of the known loci, a genome scan was performed using the HumanCytoSNP-12 BeadChips (Illumina Inc, San Diego, USA). Linkage analysis using Simwalk version 2.91 localized the gene on chr1q41-44 with a maximum LOD score of 3.3. This locus, named OTSC10, measures 26.1Mb and contains 306 genes/gene predictions. This new gene localization confirms the strong genetic heterogeneity of otosclerosis, as almost every new large family still maps to a different locus. As no mutation for monogenic otosclerosis has been identified yet, this represents another opportunity to identify the first one.
P15: Involvement of LTBP4 and FBLN5 mutations in patients with autosomal recessive cutis laxa: clinical and molecular considerations

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Hereditary cutis laxa delineates a heterogeneous group of conditions characterized by abnormalities of the elastic fibers and presenting with loose, sagging and inelastic skin and variable systemic manifestations. Mutations in the fibulin-5 gene (FBLN5) cause an autosomal recessive form of cutis laxa (ARCL) characterized by severe skin laxity, pulmonary emphysema and peripheral pulmonary artery stenosis. Very few FBLN5 mutations however, have been identified so far and the genetic defect remains unknown in a significant proportion of patients. Recently the gene encoding the latent transforming growth factor-beta binding protein 4 (LTBP4) was shown to be implicated in families with an ARCL phenotype. In the current study, we examined a cohort of 16 patients with ARCL. Direct sequencing of FBLN5 in all patients identified 1 known and 1 novel mutation (p.C217R and p.E391X) in 2 probands, whereas molecular analysis of LTBP4 in the 14 remaining probands identified 9 novel loss-of-function mutations (p.R448X, p.C617X, p.S803X, p.Q1221X, p.Q1296X, p.R1377X, c.1263delC, c.4114dupC and c.780+2T>G) and 1 known mutation (c.4127insC) in a total of 8 patients. These results show that LTBP4 mutations are more prevalent than FBLN5 mutations in ARCL. Phenotypic comparison between LTBP4 and FBLN5 mutation positive patients shows overlapping but also distinguishing clinical features, i.e. LTBP4 mutation positive patients have more severe gastro-intestinal and urinary tract involvement. Our results also suggest LTBP4 as the first gene to test in the molecular work up of patients with ARCL.
**P16: Deleterious mutations in RRM2B causing severe mitochondrial DNA depletion in infancy**

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Mitochondrial DNA (mtDNA) copy number reduction, known as the mitochondrial DNA depletion syndrome (MDS), is a common cause of severe mitochondrial disorders of infancy and early childhood. MDS results from defects in nuclear encoded factors involved in mtDNA maintenance and within the past years mutations in the POLG1, DGUOK, MPV17, PEO1, SUCLG1, TK2, SUCLA2 and TYMP genes have proven to be implicated in the pathogenesis of this disorder. The clinical phenotypes associated with the different gene alterations vary considerately but present either as a hepatocerebral or a myopathic syndrome.

We have identified a homozygous p.E85 deletion in exon 3 of the RRM2B gene in a neonate. The patient, born to first-cousin Caucasian parents, presented with lactic acidosis, severe hypotonia, deafness, blindness and hyperammonemia. Muscle biopsy showed RRF, a combined respiratory chain defects and massive subcomplexes of ATP synthase both with traditional spectrophotometry and BN-PAGE. Western blotting using antibodies against selective OXPHOS subunits indicated the preservation of nuclear encoded complex II. She died at 2 months of age. Mutations of the RRM2B gene, encoding the cytosolic R2 subunit of a p53 controlled ribonucleotide reductase (p53R2), have been reported to cause severe depletion of muscle mtDNA by Bourdon et al. 2007. Indeed, < 5% residual amount of mtDNA was measured in muscle tissue of our patient. Aberrations in these 9 genes count only for a minority of all MDS cases. It is expected that other genes involved will be identified soon.
Familial Progressive Hyperpigmentation and Hypopigmentation (FPHH) is an autosomal dominant disorder with reduced penetrance. Clinical signs consist of progressive diffuse, partly blotchy hyperpigmented lesions, multiple café-au-lait spots, intermingled with scattered hypopigmented-appearing maculae, and lentigines. FPHH is distinct from Familial Progressive Hyperpigmentation (FPH), in which no hypopigmented features are present, and which is phenotypically and histologically closer to Dyschromatosis Universalis Hereditaria 2 (DUH2). It also differs from the Legius syndrome, characterized by familial café-au-lait spots and skin fold freckling, caused by mutations in SPRED1.

We performed a genome-wide linkage analysis in seven families with FPHH, and identified linkage on 12q21.12-q22, which overlaps with the DUH2 locus. We investigated whether KITLG in the locus is mutated in FPHH. We discovered three different mutations in four families. A reported FPH-substitution was observed in two FPHH families, and two novel substitutions, p.Val33Ala and p.Thr34Pro, cosegregated with FPHH in two separate families. All three mutations were located in a highly conserved β-strand in KITLG, suggesting this motif to have an important role in the activation of the KITLG receptor c-Kit. In aggregate, mutations in a single gene cause various pigmentation disorders: FPH, FPHH and likely DUH2. Therefore KITLG is an important modulator of skin pigmentation.
P18: Genetic causes of primary lymphedema

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Lymphedema is caused by a dysfunction of the lymphatic vessels, leading to disabling swelling occurring mostly on the extremities. There exist two major categories: primary (idiopathic) and secondary (acquired) lymphedema. Familial lymphedema usually segregates in an autosomal dominant or recessive manner, but it can also occur in association with other clinical problems.

In our lab, the screening of about 200 primary lymphedema samples led to the discovery of different mutations in three major lymphangiogenic genes: VEGFR3, FOXC2 and SOX18. VEGFR3, a class III receptor tyrosine kinase, is predominantly expressed in the lymphatic endothelial cells in adults. Mutations in the gene cause primary congenital lymphedema or Nonne-Milroy disease.

Mutations in the transcription factor genes FOXC2 and SOX18 have been reported in lymphedema-distichiasis syndrome and hypotrichosis-lymphedema-telangiectasia syndrome, respectively.

Recently, we screened two new genes: CCBE1 and PTPN14. CCBE1 (collagen and calcium binding EGF domain 1) gene was screened in 164 lymphedema cases. Mutations were identified only in patients with Hennekam syndrome. The PTPN14 gene, encoding a non-receptor tyrosine phosphatase, was reported to encompass an exon 7 deletion in one consanguineous family, in which affected individuals also present choanal atresia. This deletion leads to a loss-of-function of the protein. We have screened 188 samples by high resolution melting and sequencing for mutations. Only one mutation was found.

We conclude that mutations in the two new genes are rare causes of primary lymphedema, likely limited to specific syndromic forms. Yet, the identification of the genetic causes of primary lymphedema allows more precise diagnoses, follow-up and treatment, and enables the generation of disease models for future developments of novel therapeutic approaches.
P19: A locus for Keloids with Hypertrophic Scarring

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Keloids consist of pathologic fibrosis, which occurs in the skin after trauma and which grows beyond the boundaries of the injury. These cutaneous lesions are formed by excessive deposition of extracellular matrix, mainly collagen. Keloids occur in people of all racial backgrounds; however, individuals of African descent are more susceptible. Linkage of two African-American and Japanese descent families with inherited predisposition to keloids has been reported to loci on 2q23 and 7p11 (Marneros et al., 2004). We have performed linkage analysis in a Belgian family to test if it is also linked to one of these loci. The affected individuals of this family showed formation of small keloids and hypertrophic scars. Linkage analysis was performed using dense microsatellite maps inside the linked regions. The LINKAGE and GENEHUNTER packages were used for parametric (LOD) and non-parametric (NPL) analysis. They excluded both 2q23 and 7p11. Genome scan using the GeneChip Human Mapping 10K SNP Array shows linkage to a locus of 13 Mb with maximum Lod score 2.5.

This finding provides further evidence for locus heterogeneity with genes that regulate formation of keloids with hypertrophic scars.

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P20: A novel splice variant in the N-propeptide of COL5A1 causes an EDS phenotype with severe kyphoscoliosis and eye involvement

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Classic Ehlers-Danlos Syndrome (EDS) is a rare dominantly inherited connective tissue disorder, characterized by hyperextensible skin, poor wound healing and joint hypermobility. Mutations in the type V collagen genes (COL5A1 and COL5A2) are found in about half of the classic EDS patients. While most mutations generate a non-functional COL5A1 allele, only a minority affect the structure of the central triple helix domain. We report a novel splice acceptor mutation (IVS6-2A>G) in the multidomain amino(N-)propeptide of the α1(V) collagen, a highly conserved region in which few mutations have been identified so far, in a classic EDS patient presenting with mild skin involvement, severe progressive scoliosis and muscular hypotonia. cDNA cloning showed that two in-frame mRNA transcripts are present in equal amounts: in one product only exon 7 is skipped while in the other product both exon 7 and the upstream exon 6 are deleted. Both mutant mRNA transcripts affect the N-propeptide variable domain, a domain highly important in regulating the type I/V collagen fibril diameter. Western blot analysis proved that both mutant transcripts are translated and secreted to the extracellular matrix. These truncated α1(V) collagen chains can be incorporated in type V collagen molecules and perturb collagen fibrillogenesis as illustrated by the presence of composite collagen fibrils in the patients dermis. We determined the order of intron removal and showed that two pathways of splicing exist: one in which intron 5 is removed prior to splicing of intron 6, resulting in skipping of exon 7, the second in which intron 5 is removed slowly and results in skipping of exons 6 and 7. Possibly, the poor strength of the exon 6 splice sites contributes to the double exon skip.

In conclusion, these findings add further insights to the order of splicing of COL5A1 and show that a single acceptor splice site mutation can generate multiple mutant transcripts thereby affecting the upstream exon.
P21: Mutations in TMEM165 cause a new Congenital Disorder of Glycosylation type II and affect pH homeostasis

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Protein N-glycosylation is one of the major biosynthetic functions of the endoplasmic reticulum (ER) and Golgi compartments. The correct oligosaccharide structures observed on secreted and/or membrane glycoproteins depend on the activities of glycosyltransferases and remodeling glycosidases but also on other factors such as a correct localization of Golgi enzymes and/or an adequate Golgi environment. Genetic defects affecting the glycosylation pathway cause a range of diseases known as Congenital Disorders of Glycosylation (CDG).

We identified one family with a peculiar phenotype of hypotonia, muscle hypotrophy, osteoporosis, very short stature and joint laxity. The patients were diagnosed as CDG-II, they presented sialylation and galactosylation deficiencies.

Using different markers for Golgi sub-compartments, a dilated Golgi morphology associated with a fragmentation of the trans Golgi network (TGN) in fibroblasts from affected individuals was observed. A slight delay in the retrograde translocation of Golgi membranes to the ER was observed in fibroblasts after treatment with brefeldin A. In order to identify the responsible gene, homozygosity mapping was combined with a genome wide transcriptomic analysis to look for genes whose expression would be down regulated. A deep intronic mutation was identified in a novel gene, TMEM165 (TPARL), coding for a protein whose cellular functions are unknown. After screening unrelated CDG-II patients, we identified missense mutations in the same gene in 2 additional cases.

To study its subcellular localization, a fusion protein with a fluorescent tag was generated. Co-localization of the tagged protein with relevant markers revealed a late endosomal/lysosomal localization. Because of its peculiar localization, we hypothesize that the function of this new protein is that of a proton transporter. To investigate this possibility, pH measurements in late endocytic structures were done using the Lysotracker and Lysosensor dyes, in patient and control fibroblasts. Compared to control cells, a much stronger staining was observed in patient cells, indicating a more pronounced acidity in the acidic compartments. This result was confirmed in Hela cells by using an siRNA strategy to knock down TMEM165.

All together, the discovery of mutations in this unknown gene defined a new type of CDG-II and revealed a completely novel connection between lysosomal pH, glycosylation and intracellular trafficking.
P22: Origin of constitutional mosaicism for segmental aneusomy

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Constitutional chromosomal mosaicism comprising a de novo aberration and a normal cell line is rare. Post-zygotic errors have often been proposed as the causal mechanism for this type of rearrangements. Evidence from preimplantation embryos, where the mitotic error rate is high and mosaicism of 75 to 90% has been detected, support this hypothesis. However, previous molecular analyses have shown that rearrangements of meiotic origin also occur. We analysed a series of six patients with these rare mosaic structural aberrations to determine their origin and to look at the prevalence of the different mechanisms of origin. Patients were ascertained during prenatal diagnosis or referred for cytogenetic/molecular investigations after clinical evaluation for dysmorphic features. Karyotyping and array CGH results showed 4 segmental duplications and 2 unbalanced translocations. Parental karyotypes were all normal indicating the de novo origin of the rearrangements. Molecular analyses of sequence-tagged-site (STS) markers were consistent with a post-zygotic origin for 4 patients and a meiotic origin for the other 2 patients. We therefore provide evidence that cases with 46,normal/46,aberrant mosaicism can be caused by both postzygotic and meiotic mechanisms; and show that investigation of the origin of such aberrations can lead to a better understanding of chromosomal instability during early development.
P23: A 1 bp deletion in the dual reading frame deafness gene LRTOMT causes a frameshift from the first into the second reading frame

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Autosomal recessive nonsyndromic hearing impairment is genetically highly heterogeneous, with a growing list of 37 responsible genes. LRTOMT is one of the recently discovered genes, with only six known mutations. We analyzed an Iranian consanguineous family, segregating ARNSHI. We found a single base pair deletion, c.104delC, in the LRTOMT region cosegregating with the HI. This gene has two major protein products, LRTOMT1 and LRTOMT2 whose coding regions overlap. The mutation causes a frameshift that eliminates the predicted catechol-O-methyltransferase domain of LRTOMT2, which is most likely disease causing. For LRTOMT1 the frameshift leads to a unique predicted chimeric protein containing parts of both proteins, due to a shift from the first into the second reading frame.
P24: Venous malformation causative mutations in TIE2 cause an aberrant endothelial cell phenotype

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Purpose
Venous malformations (VMs) are the most frequent vascular malformations referred to specialized vascular anomaly centers, as they are often clinically problematic. A rare (1-2%) familial form, termed cutaneomucosal venous malformation (VMCM), is caused by gain-of-function mutations in TIE2, the most common of which is R849W. We showed that at least 50% of common sporadic VMs, characterized by the presence of large unifocal lesions, are caused by somatic mutations in TIE2. These include a frequent L914F change and a series of double-mutations in cis, all of which cause ligand-independent hyperphosphorylation of the receptor in vitro. In addition, the two common mutations affect the sub-cellular localization of the receptor, and its translocation in response to the angiopoietin-1 (ANGPT1) ligand.

Methods
To test for transcriptional differences between TIE2-mutant and wild type (WT) cell-lines, we performed expression profiling using GeneChip Exon 1.0 ST Array on retrovirally infected HUVECs expressing R849W, L914F, WT or uninfected controls. Changes of interest were confirmed by Real-Time quantitative PCR (RT qPCR). We now need to confirm that these changes hold true in vivo. To do this, we will carry out in situ hybridization/immunohistochemistry on patient tissues, and RT qPCR on VM-derived endothelial cells (ECs).

Results
Transcriptional profiling of HUVECs showed that almost all commonly used EC markers assessed are dysregulated by TIE2 mutations. These results were confirmed by RT qPCR. Downregulated transcripts include those for several growth factors, such as the VEGF receptors and the EGF receptor. This may explain our observation that VM derived ECs fail to proliferate robustly in standard-to-growth factor supplemented medium.

Conclusion
Chronic hyperphosphorylation and aberrant localization of the TIE2 receptor seem to result in a profoundly abnormal EC phenotype in vitro. The presence of these changes in vivo, and their potential implication for VM-derived EC-function, remain to be explored.
In vivo quantification of cerebral GABA(A) receptors in fragile X patients using [11C] flumazenil Positron Emission Tomography

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In the brain of fragile X animal models, we found a decreased mRNA expression of several subunits of the GABA(A) receptor. GABA(A) receptors are the major inhibitory neurotransmitter receptors in the mammalian brain. This ionotropic receptor plays a role in learning and memory, hyperactivity, insomnia, epilepsy, anxiety and depression; processes that are disturbed in fragile X patients. This indicates that a dysfunction of the GABA(A) receptor is involved in the behavioural problems observed in the fragile X syndrome. The GABA(A) receptor is therefore a target for rational treatment of the syndrome.

In a set of experiments we already demonstrated that GABAergic drugs are able to correct some of the symptoms in fragile X mice. However, before therapy on patients can be initiated, it is required to validate GABAergic abnormalities in human. As post-mortem studies are not feasible, we set up a study to image and quantify the GABA(A) receptor distribution in vivo. Using positron emission tomography (PET) with [11C]Flumazenil, a known GABA(A) receptor antagonist, as a radioligand we determined the amount of GABA(A) receptors in the brain. We found an overall significant decrease (10-20%) in GABA(A) receptor availability in fragile X patients. The decrease was most prominent in specific brain regions, including cortical regions. This confirms that the GABAergic system is involved in the pathophysiology of the fragile X syndrome and suggests that GABAergic drugs might ameliorate several symptoms of fragile X patients, improving their quality of life.
P26: Musculocontractural Ehlers-Danlos Syndrome (former EDS type VIB) and adducted thumb clubfoot syndrome (ATCS) represent a single clinical entity caused by mutations in the CHST14 gene

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We present clinical and molecular findings of three patients with an EDS VIB phenotype from two consanguineous families. The clinical findings of EDS kyphoscoliotic type (EDS type VI A and B) comprise kyphoscoliosis, muscular hypotonia, hyperextensible, thin and bruisable skin, atrophic scarring, joint hypermobility and variable ocular involvement. Distinct craniofacial abnormalities, joint contractures, wrinkled palms, and normal urinary pyridinoline ratios distinguish EDS VIB from EDS VIA. A genome-wide SNP scan and sequence analyses identified a homozygous frameshift mutation (NM_130468.2:c.145delG, NP_569735.1:p.V49*) in CHST14, encoding dermatan-4-sulfotransferase 1 (D4ST-1), in two Turkish siblings. Subsequent sequence analysis of CHST14 identified a homozygous 20-bp duplication (NM_130468.2:c.981_1000dup, NP_569735.1:p.Glu334Glyfs*107) in an Indian patient. Loss-of-function mutations in CHST14 were recently reported in adducted thumb-clubfoot syndrome (ATCS). Patients with ATCS present similar craniofacial and musculoskeletal features as the EDS VIB patients reported here, but lack the severe skin manifestations. By identifying an identical mutation in patients with EDS VIB and ATCS, we show that both conditions form a phenotypic continuum. Our findings confirm that the EDS-variant associated with CHST14 mutations forms a clinical spectrum, which we propose to coin as “musculocontractural EDS” and which results from a defect in dermatan sulfate biosynthesis, perturbing collagen assembly.
P27: Orofacial phenotype of van der Woude and Poplyteal Pterygium Syndrome patients mutated for IRF6

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Introduction: The most common cause of syndromic orofacial clefts is the van der Woude syndrome (VWS), a dominantly inherited developmental disorder characterized by pits and/or conical elevations of the lower lip, cleft lip and/or palate, and hypodontia (VWS, OMIM #119300). The popliteal pterygium syndrome (PPS, OMIM #119500) shares the clinical features of VWS with the addition of other signs, such as popliteal webs (pterygia), synechiae connecting the upper and lower jaws, ankyloblepharon, syndactyly and genital anomalies.

In 2002, Kondo et al. demonstrated that mutations in the interferon regulatory factor 6 gene (IRF6) were responsible for these two allelic syndromes. Because of incomplete penetrance, only some of the clinical symptoms may be present. Since many signs occur in the orofacial region, it is of great interest to better characterize VWS/PPS patients and to see if a phenotype / genotype correlation exists.

Materials and Methods: Nineteen families (17 VWS + 2 PPS) from Trousseau hospital (Paris) were seen by a multidisciplinary staff, focusing on the orofacial phenotype. Panoramic imaging were used to check for dental agenesis and teleradiography of the profile to perform the Delaire analysis.

The coding exons of the IRF6 gene, including intron-exon boundaries, were sequenced. When no mutation was found, Multiplex Ligation-dependent Probe Amplification was performed to test for intragenic deletion or amplification.

Results: We identified a mutation in IRF6 in 82% (14/17) of VWS and 100% (2/2) of PPS families. Affected individuals had cleft (79%), lower lip pits (78%), conical elevations (36%), dental agenesis (72%), and abnormal dental morphology (60%). The upper lateral incisors and the second upper premolar were the most affected. In one VWS family, one asymptomatic carrier was detected.

Conclusion: No genotype / orofacial phenotype correlation was found. However, the study refines the orofacial symptoms present in VWS/PPS patients, especially the dental features. For any individual with abnormal structure or agenesis of a upper lateral incisor, and/or a 2nd upper deciduous molar and/or a 2nd premolar, especially if any of his/her relatives has a cleft, the dentist should carefully look for a lower lip anomaly and other possible signs of VWS/PPS. If presents, patients should be addressed towards appropriate clinical and genetic diagnosis.
P28: Nasal speech and hypothyroidism are common hallmarks of 12q15 microdeletions

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Array CGH has led to the identification of several microdeletion syndromes by screening individuals with shared phenotypical characteristics. By combining array CGH data from large cohorts of patients, the screening of overlapping microdeletions has become possible, which in turn can lead to the identification of shared phenotypic characteristics. Here we present the clinical and molecular data of three previously unreported patients with submicroscopic overlapping deletions distal to the 12q14 microdeletion syndrome at chromosome bands 12q15q21.1. The deletions presented in this study vary from 2.5 Mb to 2.57 Mb in size with a 1.29 Mb common deleted region containing six RefSeq genes. To our knowledge, only seven patients have been reported with deletions in this region (Meinecke et al., 1987; Watson et al., 1989; Perez Sanchez et al., 2004; James et al., 2005; Tocyap et al., 2006; Yamanishi et al., 2008; Schluth et al., 2008). The deletion breakpoints in the literature vary from 12q13 to 12q23 and are associated with growth retardation, developmental delay and dysmorphic features. The previously reported cases were detected with standard cytogenetic techniques, except for the patient of Tocyap et al. (2006) where localization of microsatellite markers was investigated and the patient from Schluth et al. (2008) which was fine mapped using a 1 Mb resolution array. Although a variable clinical phenotype is present in all patients, the three patients reported here all present with developmental delay or learning disability, nasal speech and hypothyroidism.
P29: Comparison of 3 molecular methods to detect aneuploidy in prenatal diagnostics

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Background
Detection of aneuploidy is currently performed by FISH, caryotyping or array CGH. These techniques are long lasting, costly, and require a considerable amount of expertise. The purpose of this work is to evaluate the performance of three molecular assays (MLPA from MRC-Holland, MAQ from Multiplicon and BoBs from Perkin Elmer) in the detection of aneuploidy as well as their sensitivities for the detection of various types of mosaics.

Patients and methods:
Between April and September 2010, a total of 30 amniotic fluid and 17 chorionic villi samples were prospectively collected. These were firstly analyzed by FISH; then, DNA was purified using home brew phenol chloroform extraction and analyzed using the three molecular techniques. Analysis was performed in blind. Artificial mosaics for t21, t18, XXY, XYY, XO and XXX were obtained by dilutions of DNA from positive samples in a negative DNA pool; mosaics with 50%, 40%, 30%, 20% and 10% of abnormal cells were made available.

Results
Analysis with MLPA and MAQ produced 6.4% and 10.6% of uninterpretable results, respectively. Analysis with BoBs gave no uninterpretable result and concordance with FISH was 100%, including one Klinefelter syndrome, one Turner syndrome, five t21, three t18 and 37 normal results. The best results regarding mosaics detection were achieved with BoBs, with sensitivities of 20% of abnormal cells for t21 and 30% for t18, XXY, XYY, XO and XXX mosaics. Sensitivity for mosaicism detection was mediocre with MLPA and ranged from 50% (t21, t18, XXX) to 30% (XXY and XYY) with MAQ. Five microdeletion DNA samples were obtained from our biobank in order to evaluate the capability of BoBs to detect microdeletion syndromes: all five microdeletion syndromes (Di George, Williams-Beuren, Prader-Willi, Angelman, Smith-Magenis) were correctly identified.

Conclusions
The best results regarding analytical performance as well as the best sensitivities for mosaics detection were obtained using BoBs technique, with a total concordance with FISH. Moreover, this molecular method allowed the accurate detection of 5 microdeletion syndromes in our study.
The Marfan (MFS) and Loeys-Dietz (LDS) syndromes are caused by a wide variety of mutations in the Fibrillin 1 (FBN1) and Transforming Growth Factor Receptor 1/2 genes (TGFBR1/2), respectively. With conventional mutation screening technologies, analysis of this set of genes is time-consuming and expensive.

We have optimized a cost-effective and reliable mutation discovery strategy using Next Generation Sequencing technology. In a first phase, five MFS or LDS patient samples with previously identified mutations and/or polymorphisms in FBN1 and TGFBR1/2 were amplified using 17 multiplex PCR reactions combining 120 amplicons into panels of 4 to 11 amplicons. Subsequently, the PCR products were equimolarly pooled and sequenced on a Genome Sequencer FLX (Roche) using the GS-LR70 long-read sequencing kit (Roche). The standard flow files were analyzed with the in-house developed Variant Interpretation Pipeline (VIP, version 1.3) software package. All expected mutations could be identified. In a second phase, we validated the technique on 87 samples from MFS patients fulfilling the Ghent criteria using two GS-LR70 runs and one Amplicon Titanium XLR70 run (Roche). To differentiate the samples within a single run, we have used up to 30 different Multiplex Identifiers (MIDs). This resulted in the identification of 65 different FBN1 mutations in a total of 73 patients. Multiplex Ligation-dependent Probe Amplification (MLPA) analysis of the remaining 14 negative samples identified one duplication and two deletions in FBN1. For the remaining 11 negative samples, Sanger sequencing of the coding sequences which were investigated with NGS, did not reveal additional variants.

Our data support that multiplex PCR of all coding exons of FBN1 and TGFBR1/2 followed by Next Generation Sequencing analysis and complemented with MLPA, is a clear-cut strategy for a time- and cost-effective identification of mutations.
P31: Concomitant inhibition of MDM2 and Aurora kinases induces synergistic antitumor effects in neuroblastoma cells

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Objectives
Neuroblastoma is the most common pediatric cancer, and it accounts for 15% of cancer deaths in children. Alterations in p53 pathway are important for the pathogenesis in a high proportion of cancers; however, less than 2% of neuroblastoma tumors harbor a mutation in p53 at diagnosis. This makes the release of p53 from its antagonist MDM2 an attractive therapeutic strategy especially after the discovery of nutlin-3 that can specifically target MDM2. Parallel to this, aurora kinase inhibitors such as VX-680 show strong inhibitory effects on the proliferation of cancer cells. We investigated the synergistic effects of combined treatment of both compounds on neuroblastoma cells.

Method
Neuroblastoma cell lines were treated with nutlin-3 alone, VX-680 alone, or with a constant ratio combination of the two drugs. Cell viability was measured using a system that monitors cellular growth in real time without incorporation of labels (xCELLigence). mRNA was isolated to evaluate the expression of genes in the p53 pathway by RT-qPCR.

Results
The combined treatment of nutlin-3 and VX-680 synergistically reduced the viability of p53 wild type neuroblastoma cells. p53 mutant cell lines respond to the treatment with VX-680 but not to nutlin-3. As a consequence, the synergistic effects were observed only in p53 wild type cells.

Conclusion
Simultaneous inhibition of MDM2 and Aurora kinases synergistically induced antitumor effects in neuroblastoma cells with wild-type p53. Research is still ongoing to determine the key players that mediate these synergistic effects. We believe that such a strategy in drug combinations could have therapeutic benefits by decreasing the dosage and yet maintain the same efficacy with less side effects or toxicity.
P32: Functional analysis of the P53 pathway in Neuroblastoma cells using small-molecule MDM2 antagonists Nutlin-3

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BACKGROUND
Inactivation of the p53 pathway is essential for tumor cells to survive and thrive, and improved understanding of the mechanisms behind p53 inactivation may guide the development of targeted therapeutic strategies. We set out to examine the nature of p53 pathway defects in a large panel of neuroblastoma cell lines using the selective MDM2 antagonist nutlin-3 as a tool to directly activate p53.

METHODS
The entire coding region of the p53 gene from 34 human neuroblastoma cell lines was analyzed by direct sequencing. Functional integrity of the p53 pathway was probed by measuring the reduction in cell viability after treatment with nutlin-3. Activation of the p53 pathway in experiments aimed at identification of modulators of the response to nutlin-3 was assessed by real-time quantitative RT-PCR analysis of p53 target genes and by cell viability and caspase assays.

RESULTS
We identified 9 cell lines (26.5%) with a mutation in the p53 gene, including 6 missense mutations, 1 nonsense mutation, 1 in-frame deletion, and 1 homozygous deletion of the 3' end of the p53 gene. Sensitivity to nutlin-3 was highly predictive of absence of p53 mutation. Cell lines with wild-type p53 were subject to marked nutlin-3–induced cytotoxicity in 23 out of 25 cases, indicating that p53 downstream signaling pathways are functionally intact in the vast majority of neuroblastoma cell lines. The presence of a homozygous CDKN2A (p16INK4a/p14ARF) deletion in one of both nutlin-3–refractory cell lines with wild-type p53 (i.e., in SHEP cells) prompted us to investigate the role of p14ARF and p16INK4a in the response to nutlin-3. The nutlin-3–resistant phenotype of SHEP cells could not be reversed by reintroduction of p14ARF or p16INK4a, but knockdown and overexpression experiments in several other neuroblastoma cell lines pointed to a stimulatory effect of p14ARF on the response to nutlin-3.

CONCLUSIONS
Mutational inactivation of p53 is not uncommon in neuroblastoma cell lines, whereas defects in effector pathways downstream of p53 are rare. Expression levels of p14ARF may modulate the response to nutlin-3, dependent on the cellular context.
P33: A girl with blepharophimosis and strabismus and a de novo interstitial microdeletion proximal to the 14q32.2q32.2 imprinted gene cluster

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Most of the reported cases with a distal deletion on 14q are terminal deletions and have been identified through conventional karyotyping. Here we report a female patient with a de novo interstitial microdeletion in the distal part of chromosome 14q and compare this observation with previous reported cases.

The proband was born at term after an uneventful pregnancy. Her birth weight, length and head circumference were on the 3th centile. She was referred to us at 7 months of age because of a high arched palate and strabismus. Beside facial dysmorphism, mild feeding problems and a mildly delayed motor development were noted. Ophthalmologic examination showed blepharophimosis, convergent strabismus and hypermetropia. Array CGH analysis revealed a de novo 2,7-3 Mb interstitial deletion 14q32.2q32.2. Seven genes are located within the deleted region, but their function can not be directly linked to the phenotype of our patient.

This is the first report of an interstitial microdeletion within 14q32.2, proximal to the 14q32.2 imprinted gene cluster. Eight previous reported cases with a linear interstitial or terminal 14q deletion (identified by conventional karyotyping) overlap with the deleted region in our patient. Phenotypic findings in patients with this type of deletion are confined to hypotonia, developmental delay and facial dysmorphism (high and prominent forehead, blepharophimosis, strabismus, epicanthal folds, flat nasal bridge, short bulbous nose, thin upper lip, low-set ears and micrognathia), whereas major congenital malformations seem uncommon.
P34: A girl with truncus arteriosus, ectopic kidney, facial dysmorphism and 14q24.1q24.2 microdeletion

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Seven cases with partial monosomy of the 14q23q31 region have been previously reported. All of them were identified through conventional karyotyping with limited definition of breakpoints. Here we report a female patient with a de novo 14q24.1q24.2 microdeletion and compare the phenotypic findings with previous reported cases.

The proband was born at term after an uneventful pregnancy. An echocardiography was performed because of a heart murmer, revealing a truncus arteriosus type II. Clinical examination showed a relatively tall stature (P97) and distinct facial dysmorphism. Psychomotor evaluation revealed a generalised mild developmental delay and a pronounced speech delay. Renal ultrasound showed an ectopic right kidney (but renal function was normal). Radiographs of the vertebral column only showed a mild lumbar scoliosis. Bone age was found to be advanced: 5y9m – 6y10m at the age of 3 years 7 months. Array CGH analysis showed a de novo 2,6-3,4 Mb deletion on 14q24.1q24.2. Based on their function, none of the 22 RefSeq genes within the deleted interval are good candidates for the congenital anomalies observed in our patient.

This is the first report of a patient with a deletion on 14q24.1q24.2 identified by array-CGH. Seven previously reported cases with a deletion in the 14q23q31 region, identified by conventional karyotyping, encompass this deleted region. These cases share with our patient renal and cardiac anomalies. The developmental delay in our patient was rather mild compared to previous reported cases.
P35: Evaluation of tripled repeat primed (TP)-PCR assay and Sizing PCR assay of the FMR1 gene for the molecular diagnosis of the fragile X syndrome

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Fragile X syndrome is caused by the abnormal expansion of the CGG repeat in the 5’ untranslated region of the FMR1 (Fragile X Mental Retardation 1) gene. Routine molecular analysis includes a combination of PCR, allowing accurate sizing of alleles up to 100 CGG repeats, and Southern blot analysis to identify longer alleles and evaluate the methylation status of the FMR1 promoter. However, Southern blot analysis largely constrains throughput and efficiency and requires large amounts of high quality DNA.

This study aims to evaluate if in a routine diagnostic setting Southern blot can be replaced by (two recently commercialized) PCR based assays.

The FMR1 TP (triplet repeat primed)-PCR assay (Abbott) contains a reverse primer hybridizing within the CGG repeat region and generates multiple amplicons of various sizes. As the number of CGG repeats increases, a characteristic PCR product profile (ladder motif) is generated by capillary electrophoresis and allows rapid distinction between normal and expanded FMR1 alleles. The FMR1 Sizing PCR assay (Abbott) contains primers flanking the CGG repeat and is intended for accurate sizing of normal, premutation and full mutation alleles.

In a pilot study we analyzed 6 samples (2 with normal alleles, 2 premutations and 2 full mutations). The results of the TP-PCR and the Sizing PCR were concordant with results previously obtained by PCR and Southern blot analysis.

Further evaluations on samples with a large variety of FMR1 CGG repeat lengths is currently ongoing and will allow exploring the limits of repeat length sizing with these kits and the ability for detection of mosaicism.
P36: An atypical case of pseudoxanthoma elasticum with abdominal cutis laxa: evidence for a clinical disease spectrum

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Introduction

Pseudoxanthoma elasticum (PXE), featuring papular skin lesions, a retinopathy and cardiovascular complications, results from elastic fibre fragmentation and mineralization due to ABCC6 mutations. The PXE-like syndrome (PXEL), caused by GGCX mutations, features generalized cutis laxa, mild retinopathy and a clotting deficiency. The GGCX carboxylase activates vitamin K (VK)-dependent calcification inhibitors (matrix gla protein (MGP), osteocalcin (OC)). We present a patient with a clinical and histochemical overlap phenotype between PXE and PXEL.

Methods & Results

The proband presented typical PXE features - yellowish skin papules and severe retinopathy - and marked abdominal cutis laxa, typical for PXEL. Dermal ultrastructural evaluation revealed mineralization in the periphery of the elastic fibres, typical for PXEL. Immunohistochemistry showed marked staining for uncarboxylated (uc) MGP and ucOC, seen in PXE and PXEL, though not confined to the middermis – as in PXE – but affecting the whole dermis as in PXEL. Measuring circulating levels of carboxylated (c) and ucMGP and OC revealed elevated ucOC/cOC ratios, as in PXEL (normal in PXE), but normal ucMGP/cMGP ratios, as in PXE (elevated in PXEL). Molecular analysis unveiled two previously reported ABCC6 mutations, while GGCX harboured a gain-of-function polymorphism. Circulating VK levels were severely decreased, possibly neutralising the effect of the latter.

Conclusion

This phenotype, reminiscent of PXE and PXEL, suggests that PXEL may represent a spectrum of ectopic calcification disorders who are clinically and pathogenetically related to PXE. The low VK serum levels suggest a pathophysiological role for VK deficiency in these disorders.
P37: Sotos syndrome associated with cutis laxa: a clinical and molecular puzzle

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Introduction
Cutis laxa, characterized by fragmentation of elastic fibres, has been associated with several syndromic phenotypes with heterogeneous mode of inheritance. Besides the skin symptoms, related systemic features are diverse, including skeletal, pulmonary, cardiovascular and metabolic symptoms.

Results & discussion
The proband, first child of healthy non-consanguineous parents, was born at 38 weeks’ gestation. During pregnancy, an isolated unilateral hydronephrosis was detected. At birth, dysmorphic features were noted, including macrocephaly with sparse frontal hair, a pointed chin and low-set, posteriorly rotated ears. In addition, redundant skin folds in the neck, hands and lower limbs were present. Skin biopsy confirmed the diagnosis of cutis laxa. Soon after birth, he developed recurrent episodes of hypoglycaemia and suffered severe feeding difficulties which required a feeding tube. Radiographies revealed an advanced bone age (4.5 months for 5 weeks of age) and advanced bone maturation.

The phenotype of this patient showed resemblance to Costello syndrome. However, these patients do not have advanced bone age and maturation. Micro-array analysis revealed a 5q35.2q35.3 deletion, compatible with Sotos syndrome. Cutis laxa is however not a classic feature. Three cases of alleged Sotos syndrome with cutis laxa have been previously reported; however, contrary to our patient, neither advanced bone age nor hypoglycaemia were present.

Conclusion
The phenotype of our patient does not fully comply with that of the three patients previously reported as Sotos syndrome with cutis laxa. This might suggest that this patient harbours a heterozygous mutation in one of the genes within the deleted 5q35 region which may explain the presence of cutis laxa.
P38: Pregnancy outcome in carriers of Robertsonian translocations

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Background
Robertsonian translocation carriers are at risk for infertility, miscarriages or chromosomally unbalanced offspring. Adequate reproductive counselling in these carriers remains a difficult issue.

Methods
A retrospective analysis was made of all prenatal diagnoses from Robertsonian translocation carriers during the period 01.01.1992 till 31.12.2007. Data on the carriers and the results of their prenatal analyses were retrieved as well as data on their previous pregnancies.

Results
Twenty-eight female and 20 male carriers of Robertsonian translocations were identified and a total of 79 prenatal samples were obtained. Female carriers had 10.3% unbalanced results at chorionic villus sampling and 5.9% at amniocentesis, whereas for male carriers this was 3.6% en 0% respectively. When taking into account all pregnancies of these carriers, 52.7% (female carriers) and 61.8% (male carriers) of the pregnancies led to the birth of a healthy child.

Conclusion
Pregnancies of carriers of a Robertsonian translocation are at increased risk for aneuploidy and prenatal chromosomal testing should be discussed. More than half of the pregnancies lead to the birth of a healthy child, but it remains difficult to prospectively determine which couples will be successful in obtaining a pregnancy with or without artificial reproductive techniques (ART) and/or embryo selection. The reason for ascertainment of the translocation should be taken into account when counselling these couples. In carriers diagnosed with the translocation because of infertility or recurrent miscarriages, PGD could be the first choice whereas in case of diagnosis because of a familial history, becoming spontaneously pregnant and performing amniocentesis could be encouraged.
**P39: Identification of miRNAs contributing to Neuroblastoma chemoresistance**

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**BACKGROUND**
The emergence of the role of microRNAs (miRNAs) in exacerbating drug resistance of tumours is recently being highlighted as a crucial research field for future clinical management of drug resistant tumours (1). The purpose of this study was to identify dysregulations in expression of individual and/or networks of miRNAs which may have direct effect on neuroblastoma (NB) drug resistance.

**METHODS**
Individual subcultures of chemosensitive SH-SY5Y and UKF-NB-3 cells were rendered chemoresistant to doxorubicin (SH-SY5Y, UKF-NB-3) or etoposide (SH-SY5Y). In each validated chemoresistance model, the parental and subcultured cell lines were analysed for miRNA expression profiling, using a high-throughput quantitative polymerase chain reaction (RT-qPCR) miRNA profiling platform (2,3) for a total of 668 miRNAs.

**RESULTS**
A total of seven miRNAs were found to be differentially expressed (higher than 2-fold change) within all three NB chemoresistance models. Four miRNAs were upregulated in the subcultured chemoresistant cell line. Three miRNAs were found to be downregulated in the chemoresistant cell lines for all models.

**CONCLUSIONS**
Based on the initial miRNA findings, this study elucidates the dysregulation of seven miRNAs in three separate NB chemoresistant cell line models, spanning two cell lines (SH-SY5Y & UKF-NB-3) and two chemotherapeutic agents (doxorubicin & etoposide). These seven miRNAs may thus be possibly linked to chemoresistance induction in NB. Such miRNAs are good candidates to be novel drug targets for future miRNA based therapies against aggressive tumours that are not responding to conventional chemotherapy.

P40: Co-occurrence of two rare autosomal recessive syndromes in a young patient.

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Wolfram syndrome is a rare neurodegenerative disorder characterized by type I diabetes mellitus, diabetes insipidus, optical atrophy and neurological signs. WFS is transmitted in an autosomal recessive manner and mostly associated with WFS1 mutations. Gitelman syndrome, also referred to as familial hypokaliemia-hypomagnesemia, is an inherited renal tubular disorder. Gitelman syndrome is transmitted as an autosomal recessive trait and associated with SLC12A3 mutations. Here we describe a young patient, born to non consanguineous parents, affected by these two autosomal recessive disorders. The patient presented the classical sign of Wolfram syndrome including optic atrophy, diabetes mellitus and neurogenic bladder. His brother was also affected and presented optic atrophy and diabetes mellitus. Following an hospital admission for diabetic acidocetosis, a persistent and unexplained hypokaliemic alkalosis with hypomagnesemia was observed, suggesting a renal tubular disorder. Because such tubulopathy is not typically observed in WFS, Gitelman syndrome was suspected. Both clinical diagnoses have been confirmed by molecular biology. Moreover, the patient is carrier of three causal mutations associated with Gitelman syndrome. Presence of multiple, rare and unexplained clinical finding suggests a single syndromic association. However, distinct autosomal recessive diseases can sometimes occur, even in the absence of consanguinity.
P41: Prevalence of BRCA1/2 mutations in sporadic breast/ovarian cancer patients and identification of a novel de novo BRCA1 mutation in a patient diagnosed with late onset breast and ovarian cancer: implic

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Purpose
In order to adequately evaluate the clinical relevance of genetic testing in sporadic breast and ovarian cancer patients, we offered comprehensive BRCA1/2 mutation analysis in patients without a family history for the disease.

Methods
We evaluated the complete coding and splice site regions of BRCA1/2 in 193 sporadic patients. In addition, a de novo mutation was further investigated with ultra deep sequencing and microsatellite marker analysis.

Results
In 17 patients (8.8%), a deleterious germline BRCA1/2 mutation was identified. The highest mutation detection ratio (3/7 = 42.9%) was obtained in sporadic patients diagnosed with breast and ovarian cancer after the age of 40. In 21 bilateral breast cancer patients 2 mutations were identified (9.5%). Furthermore, 140 sporadic patients with unilateral breast cancer were investigated. Mutations were only identified in patients diagnosed with breast cancer before the age of 40 (12/128= 9.4% vs. 0/12 with Dx>40). No mutations were detected in 17 sporadic male breast cancer and 6 ovarian cancer patients.

BRCA1 c.3494_3495delTT was identified in a patient diagnosed with breast and ovarian cancer at the age of 52 and 53 respectively, and was proven to have occurred de novo at the paternal allele.

Conclusion
Our study shows that the mutation detection probability in specific patient subsets can be significant, therefore mutation analysis should be considered in sporadic patients. As a consequence, a family history for the disease and an early age of onset should not be used as the only criteria for mutation analysis of BRCA1/2. The relatively high mutation detection ratio suggests that the prevalence of BRCA1/2 mutations may be underestimated, especially in sporadic patients that developed breast and ovarian cancer. In addition, although rare, the possibility of a de novo occurrence in a sporadic patient should be considered.
P42: Dickkopf-3 is regulated by the MYCN-induced miR-17-92 cluster in neuroblastoma

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Objectives: Neuroblastoma is a paediatric tumour with a remarkable diverse clinical behaviour ranging from spontaneous regression to widespread metastatic disease. About 50% of the high stage aggressive tumours are characterized by amplification of MYCN which is an important independent predictor for poor prognosis. In neuroblastoma tumours, MYCN expression was shown to be inversely correlated with expression of Dickkopf-3 (DKK3), an extracellular protein described as a tumour suppressor in several cancer types. Chromatin immunoprecipitation showed that there was no direct interaction between the MYCN transcription factor and DKK3, which lead us to hypothesize that DKK3 might be negatively regulated by MYCN induced microRNAs. MiRNAs are small non-coding RNAs that downregulate the expression of target genes through binding to their 3’UTR region.

Methods: We used online prediction databases in order to identify candidate miRNAs targeting the 3’UTR of DKK3. Direct interactions between miRNAs and the 3’UTR of DKK3 were investigated using luciferase assays. The effect of miRNAs on DKK3 mRNA and protein levels was evaluated after transient overexpression of selected pre-miRs in the MYCN non-amplified SH-EP neuroblastoma cell line. Levels of secreted DKK3 protein levels were measured using Enzyme-Linked ImmunoSorbent Assay (ELISA) and the effect of miR-17-92 overexpression on DKK3 mRNA and protein levels was evaluated using the tetracycline-inducible SH-EP-TR-miR-17-92 cell line.

Results: Several members of the MYCN-induced oncogenic miR-17-92 cluster (miR-19a, miR-19b and miR-92a) were predicted to interact with the 3’UTR of DKK3. Using SH-EP-TR-miR-17-92, we investigated the effect of overexpression of the entire miR-17-92 cluster on DKK3 levels and demonstrated a significant down regulation of DKK3 mRNA and protein levels after miR-17-92 overexpression. Furthermore, transiently overexpressing a single miRNA, miR-92a, induced similar effects, whereas the overexpression of only miR-19a or miR-19b did only effect the DKK3 protein levels. This is the first observation of a combined repressive effect on protein expression through targeting of mRNA stability and translation by different members of one miRNA cluster. Direct interaction between miR-19a, miR-19b or miR-92 and the 3’UTR of DKK3 was validated using luciferase assays.

Conclusions: In this study, we confirm the direct regulation of DKK3 through miR-17-92 components thus explaining the mechanistic basis for the consistent down regulation of DKK3 through MYCN in neuroblastoma cells. These results suggest a critical role for DKK3 as a tumour suppressor in neuroblastoma. This further links MYCN to another developmental pathway controlled by PHOX2B, which also regulates DKK3 expression. Further studies are warranted in order to unravel the role of DKK3 and on its potential as a possible therapeutic target.
P43: Mosaic 14q12 deletion affecting PRKD1 gene in a patient with psychomotor delay and dysmorphism

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The patient is a young girl born at term from consanguineous parents with low growth parameters: weight of 2kg240 (-3SD), size of 44 cm (-3.5 SD) and microcephaly with head circumference of 29 cm (-4.5 SD). Facial dysmorphism was also observed: upslanting palpebral fissures, epicanthus, narrow face with sloping forehead, dysplastic left ear, microretrognathia, large nasal bridge. Moreover this girl presented with congenital cardiopathy (VSD), anteposed anus, sacral dimple and atypical left palmar crease. She is now 3 year old, language is totally absent and she has feeding difficulties which required gastrostomy. She has severe psychomotor delay and MRI shows corpus callosum hypoplasia.

First genetic investigations showed a normal female karyotype, no 22q11 deletion and no subtelomeric deletion. CHARGE syndrome was investigated by sequencing CHD7 gene which gave no evidence of mutations.

CGH was performed using Agilent 44K array which showed a 412 kb deletion at 14q12. Average log ratio value (-0.56) was rather high and suggested a mosaic deletion which was confirm by FISH to be present in 55% of blood lymphocytes.

Three patients with similar 14q12 deletion have been reported in the literature (Bisgaard et al., 2006, Am J Hum Genet 140A :2180-2187 ; Papa et al., 2008, Am J Med Genet 146A :1994-1998 ; Mencarelli et al., 2009, Eur J Med Genet 52 :148-152). Deletions in these patients are larger than in our patient, overlapping at least PRKD1 and FOXG1 genes. This later gene, given its role in neurogenesis, has been proposed to explain developmental delay and is involved in some “RETT-like” syndromes. In our patient, only PRKD1 is deleted. However she has many phenotypic features in common with patients reported with 14q12 deletion reported in the literature: developmental delay observed at birth, severe psychomotor delay, microcephaly and several facial dysmorphic features. Two patients with a 150 kb deletion affecting PRKD1 have been reported in DECIPHER. Phenotypic features partly overlap with those observed in our patient and in the three cases of the literature.

These observations suggest that FOXG1 deletion is perhaps not the only candidate gene responsible of the phenotype presented by 14q12 deletion carriers. Despite these phenotypic similarities, it is not possible to unambiguously link the phenotype of our patient with this deletion because, on one hand, she is mosaic for this deletion and, on the other hand, we cannot preclude another genetic origin specially given the consanguineous familial context. This case illustrate the complexity of integrating molecular, clinical and literature data to achieve a clear and unambiguous diagnosis.
P44: MYCN pathway components are targeted by rare recurrent DNA copy number alterations in neuroblastoma

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Background
Neuroblastoma (NB) is an embryonal tumor arising from immature sympathetic nervous system cells. Despite multimodal therapies, aggressive subgroups of NB show a disappointingly low survival rate, illustrating the urgent need for alternative treatments. Apart from the discovery of large recurrent critical regions of gains and losses, only a handful of genes are known to be implicated in NB oncogenesis including MYCN, PHOX2B, ALK and NF1. Genetic alterations in ALK and NF1 showed that rare genomic events (amplification and deletion/disruption, respectively) can be so-called smoking guns hinting at a fundamental role in NB oncogenesis. Therefore we performed high-resolution array CGH analysis to screen for copy number alterations in order to uncover additional rare recurrent focal genomic imbalances affecting protein coding genes and loci encoding non-coding genes, miRNAs and T-UCRs.

Methods
188 NB samples and 33 NB cell lines were screened using a 44K/60K custom oligonucleotide array platform, enriched for critical regions in NB, miRNAs and transcribed ultra-conserved non-coding regions (T-UCRs). Circular binary segmentation (CBS) values were wave corrected and extracted from the in-house developed software tool arrayCGHbase. The statistical environment R was used to extract homozygously deleted (log2(CBS)<-2.0) and amplified regions (log2(CBS)>2.0) and to identify recurrent (≥ 2 samples) focal (<2Mb) aberrations. Loss and gain were defined by log2(CBS)<-0.3 and >0.3, respectively.

Results
We identified 67 chromosomal regions of interest ranging from 63 bp to 6.2 Mb. Strikingly, several DNA copy number alterations implicated genes in the MYCN pathway. hTERT, a known direct MYCN target gene, was affected by high level amplification (or copy number gain), leading to increased hTERT expression which is associated with poor survival. Other rare amplicons described in NB also harbour MYCN target genes, such as cell cycle regulators CDK4 and CCND1 and TP53 inhibitor MDM2. Furthermore, our screen revealed a targeted copy number gain of the oncogenic miR-17-92 cluster, another MYCN target. We also observed a homozygous deletion in the GTPase activator, RGS5, a known effector of angiogenesis in cancer. Strong inverse correlation between RGS5 and MYCN expression suggested MYCN regulation which was confirmed using a MYCN inducible system. Furthermore, the miR-17-92 cluster was predicted to target RGS5 and was validated in vitro using a miR-17-92 inducible system.
Finally, the MYCN upstream component ELAVL4 was targeted through small recurrent alterations. Likewise, DNA copy number alterations of other MYCN upstream regulators such as LIN28B have been reported.

Conclusions
Our comprehensive high resolution DNA copy number screening in NB uncovered the implication of multiple up- and downstream effectors of the MYCN pathway and established RGS5 as a new bona fide target gene with putative potential for anti-angiogenic therapy.
P45: WDR62 is a spindle pole protein and is mutated in MCPH2 primary microcephaly

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Autosomal recessive primary microcephaly (MCPH) is a genetic disorder of neurodevelopment resulting in a small brain, with no malformation and no neurological deficit except mild mental retardation. MCPH is genetically heterogeneous with more than seven loci known to date. MCPH2 is the second most common cause of MCPH. The MCPH2 gene has long eluded identification. We now report homozygous missense and frameshift mutations in the WDR62 gene in seven MCPH2 families, after homozygosity mapping and Illumina GAII sequencing of the array-captured critical region DNA from two families. Of note, the one patient with a predicted bi-allelic null allele had a more severe phenotype, including lissencephaly, suggesting some genotype-phenotype correlation. In human cell lines, WDR62 is a spindle pole protein, like ASPM and other MCPH proteins. Mutant WDR62 fails to localize to the mitotic spindle pole. In human and mouse embryonic brain slices, WDR62 expression was restricted to neural precursors undergoing mitosis. These data support the hypothesis that a centrosomal mechanism is crucial to neural precursors’ proliferation. This mechanism possibly involves the control of the cleavage furrow orientation during neural precursor cell mitosis. The more severe findings in patients with null mutations also suggest a mechanistic link between microcephaly and some brain malformations like lissencephaly.
P46: Identification of down stream components of the ALK signaling pathway in neuroblastoma

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Background
ALK is a tyrosine kinase receptor expressed in the developing nervous system and activated in several tumor entities. Recently, ALK activating mutations were detected in familial and sporadic neuroblastomas (NB). In sporadic NBs, 2 hotspot mutations were observed at residues F1174 and R1275. As a first step towards understanding the nature of signaling in ALK-driven neuroblastoma formation, we assessed oncogenicity of the different hotspot mutations. Moreover, we established an ALK expression signature in NB cell lines following RNAi mediated knockdown and pharmacological ALK inhibition.

Methods
Oncogenicity of F1174L and R1275Q mutations was tested using a Ba/F3 cell transformation assay. Gene expression data (Affymetrix HG133Uplus2) was obtained after stable ALK knockdown using shRNA and inhibition using the small molecule ALK inhibitor NVP-TAE684 in six NB cell lines with activating ALK mutations (F1174L (n=3), R1275Q (n=3)), amplification (n=1) and three wild type cell lines. Protein expression of ALK and phosphorylation status of ALK and downstream canonical signaling effectors was assessed in all these samples using Western Blotting.

Results
Stronger oncogenicity and increased phosphorylation was established for the F1174L as compared to the R1275Q mutant. In a next step, we determined an ALK pathway activity score based on mRNA expression data following knockdown and pharmacological inhibition of ALK in NB cell lines. Further analysis in a gene expression data set of 100 tumors revealed a very high ALK activity score for an ALK amplified and an F1174 mutant tumor as well as an increased score for MYCN amplified tumors. Finally, we explored the ALK mediated gene expression profiles in order to identify down stream modulators. Amongst the differential expressed genes we found enrichment for members of the MAPK signaling cascade. In accordance with this finding, we observed a decrease in ERK, MEK and PLCgamma phosphorylation and identified ETV5/ERM as an important putative effector of this pathway. Functional in vitro and in vivo assays are ongoing to determine the contribution to the oncogenic NB phenotype of ETV5/ERM.

Conclusion
We established the F1174 mutation as the most oncogenic ALK hotspot mutation. Next, we delineated an ALK pathway activity score. Finally, we have identified the MAPK/ERK pathway as an important component of down stream ALK signaling and propose ETV5/ERM as a key effector thus offering new therapeutic targets for molecular therapy.


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Recently, germline mutations in RAD51C were found to be associated with an increased risk for breast and ovarian cancer. Meindl et al. (2010) detected six monoallelic pathogenic mutations in RAD51C by screening 1,100 unrelated German women with gynaecologic malignancies (breast and/or ovarian tumors). Strikingly, all six deleterious mutations were exclusively found within 480 BRCA1/2 negative families with patients presenting with both hereditary breast and ovarian cancer and not in breast cancer only families.

With this study we aim to determine the prevalence of germline RAD51C mutations in Belgian/Dutch breast and ovarian cancer families, previously found to be negative for BRCA1&2 mutations. We performed mutational analysis in 302 index patients from families presenting with breast and ovarian cancer patients. Mutation detection was performed with High resolution melting curve analysis, followed by Sanger sequencing of the aberrant melting curves.

Besides some frequent single nucleotide polymorphisms and a missense variant (c.506T>C; p.Val169Ala) in exon 3, which was predicted to be clinically not important, we did not identify any deleterious mutation.

Based on the prevalence of germline mutations in the initial report, we had expected to detect at least three deleterious mutations. Our results are in accordance with the results of Zheng et al. (2010) and Akbari et al. (2010) who could not detect RAD51C mutations in respectively 92 and 454 families either. Therefore, deleterious germline RAD51C mutations may be extremely rare. To further increase the statistical power of our study, we are currently screening 100 additional families.

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Objectives
The principal objective of this implementation is to replace old strategies that are too much expensive and time-consuming in the routine diagnosis of Fragile X syndrome (FXS) and Y-Chromosome microdeletion (YCM). These strategies consist of the DNA amplification and GeneScan analysis Software followed by Southern blot in the 40% of non-informative cases in the diagnosis of FXS and of the DNA amplification with two different multiplex using 8 Y-chromosome markers and gel electrophoresis in the diagnosis of YCM. The new methods for both of these diseases are based on a PCR amplification and GeneScan analysis Software. The FMR1 TP-PCR and FMR1 Sizing PCR assays (Abbott) have been validated for the FXS, using the TP-PCR as screening and the Sizing-PCR as quantification methods. Devyser AZF (Devyser) has been validated for the detection of the YCM using a PCR amplification of 14 STS.

Patients and methods
For FXS, 160 samples have been tested including 46 known patients with permutated (27) and full mutation (9), 4 external DNA controls as size standards (Coriell), 12 mixing artificially normal and full mutation DNA to simulate different levels of mosaicism (ranging from to 5% to 100%), and 93 DNA patients analyzed in blind test for a prospective validation. For YCM, 40 samples have been tested including 8 mosaic samples, and 3 DNA from EMQN quality controls.

Results
For both syndromes, we obtained 100% concordance between old and new methods for the known positives patients and the external DNA controls. The concordance is of 100% for all DNA samples analyzed in blind test. The low level mosaicism of 10% can be also easily detected.

Conclusion
Both new methods provide a powerful and sensitive screening technique which can be useful for the routine diagnosis of FXS and YCM. Because they are faster and less expensive methods, they were accepted for the routine diagnosis.
P49: Severe end of the Shwachman-Diamond syndrome phenotypic spectrum: case report


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Introduction

Shwachman-Diamond syndrome (SDS, MIM 260400) is a rare autosomal recessive disorder characterized by exocrine pancreatic insufficiency, hematologic abnormalities with single- or multi-lineage cytopenia, susceptibility to myelodysplasia syndrome and acute myeloid leukaemia and skeletal abnormalities. Mutations occurring in the SBDS gene are found in most of the patients. The clinical spectrum varies between and within families. Commonly, SDS presents in infancy with failure to thrive and poor growth secondary to exocrine pancreatic dysfunction and recurrent infections secondary to neutropenia. Only a few cases with neonatal presentation have been reported until now. We report on here a newborn with SDS with nanism, narrow thorax and hematologic abnormalities.

Clinical report

The patient, a boy, was the first child of healthy, unrelated, Belgian parents. At 22 weeks gestational age a foetal ultrasound showed a narrow thorax and short limbs (<< 2SD). The MRI showed pulmonary hypoplasia (35% of the expected volume). Asphyxiating thoracic dysplasia (Jeune syndrome) was suspected. The boy was delivered at 38 weeks. His birth length was 41.5 cm (-5SD), weight 2490 g (P10) and head circumference 30.5 cm (-3SD). Apgar score was 7/8 at 1/5 min. Rapidly, he developed a respiratory distress with tachypnea; a CPAP was necessary. Since birth, he had signs of pancytopenia: neutropenia (360/μL), thrombocytopenia (20.000/μL) and anaemia (Hb 10.3g/dl). The bone marrow examination revealed hypocellularity. The skeletal survey showed a metaphyseal dysplasia: short limbs with wide and cupped metaphyses of the long bones, narrow chest with short ribs and cupped posterior ends. An absence of ossification of the sacral wings and a delayed ossification of the ischiopubic and iliopubic rami were observed. The ultrasound of the pancreas was normal. Given the clinical and radiological features, the diagnosis of SDS was made. The analysis of the SBDS gene is ongoing.

Discussion

If haematological and gastrointestinal findings are observed in most of the children with SDS at the time of the diagnosis, at the most severe end of the spectrum the patients present with nanism and narrow thorax. In our patient the initial prenatal working diagnosis was asphyxiating thoracic dysplasia (Jeune syndrome). After birth the presence of a pancytopenia allowed us to make the diagnosis of SDS syndrome. A precise diagnosis is important to orientate the management, the molecular testing and the genetic counselling.
P50: Cancer gene prioritization for targeted resequencing

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Background
Due to the high cost, whole genome or exome sequencing using next-generation sequencing (NGS) is currently typically performed on only a limited number of cancer samples. The low number of samples is often problematic in downstream interpretation of the significance of variants. Targeted resequencing of candidate cancer genes is an alternative method that can partially circumvent this problem; by restricting the number of genes to sequence, more samples can be included in the screening, hence resulting in substantial improvement of statistical power. Here, a successful strategy of prioritizing genes for targeted resequencing of cancer genomes is presented.

Methods
Positive predictive values (PPV) and cumulative distribution plots were generated for the evaluation of four different strategies to prioritize genes: fitSNP DER (differential expression ratio) values, standard deviation (SD) of gene expression, correlation between gene copy number and expression, and frequency of gene copy number alterations. We also explored whether combinations of any of the above prioritization strategies could improve the initial results. The strategies were evaluated on publically available copy number, gene expression and mutational data sets from six different cancer types: breast cancer, colon cancer, pancreas cancer, ovarian cancer, glioblastoma and medulloblastoma.

Results
Using the different prioritization strategies, genes were ranked and the mutation frequency within the top-ranked genes was determined. If a strategy is successful in identifying mutated cancer genes, the top genes in the prioritized lists should be enriched for mutated genes, as shown in an increase in PPV value. For the colon cancer data set for example, the mean PPV value for the fitSNP approach showed an increase from the baseline value of 5% to 21% when sequencing the top 50 genes. Similar results were seen for the other data sets and strategies, with variable improvements in enrichment. Differences in best prioritization methods were noticed across data sets, and depending on the number of top genes that were examined.

Conclusions
A gene prioritization strategy based on the fitSNP DER value appears to be most successful in identifying mutated cancer genes, with SD as a good second best. One single method to prioritize genes in all cancer genomes could not be found, most likely due to the fact that different cancer types display diverse mutational landscapes.
P51: Identification of a new rare recurrent 1q42.2-qter deletion in neuroblastoma

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Background

Neuroblastoma (NB) is an aggressive embryonal tumor that accounts for approximately 15% of childhood cancer death. To date, our molecular knowledge about this tumor remains limited, with only a few causal genes identified so far. Hence, detailed genomic characterization of this tumor is necessary to improve our understanding of the underlying molecular pathogenesis and variable clinical behavior. In this study, high resolution array comparative genome hybridization (CGH) was performed in order to search for previously undetected DNA copy number gains and losses.

Methods

Array CGH was performed on 188 primary NB tumors and 33 NB cell lines using a 44K or 60K custom oligonucleotide array platform, specifically enriched for genomic regions known to be recurrently implicated in NB, as well as miRNA loci and transcribed ultra conserved non-coding regions (T-UCRs). Circular binary segmentation ratio values were wave profile corrected and extracted from the in-house developed database arrayCGHbase (http://medgen.ugent.be/arraycghbase). The statistical software R was used to define the frequency of aberrations and demarcate the shortest regions of overlap for gains and losses.

Results

A new recurrent 17.9 Mb deletion at the distal end of the long arm of chromosome 1 (1q42.2-qter) was identified. This deletion was found in 3% of the total tumor cohort with similar breakpoints as determined at a 10kb resolution. Further screening of more NB tumor profiles revealed 11 additional distal 1q deletions with comparable breakpoints. The recurrent 1q deletions are typically found in 11q deleted tumors without MYCN amplification. Using ultra-high resolution (~115bp resolution) custom arrays covering the breakpoints on 1q a clustering of the breakpoints was observed within a 1.4 Mb segment. The commonly deleted region contains 134 protein coding genes, 1 miRNA and 4 transcribed ultra conserved region elements. Some of the genes in this region are known tumor suppressor genes in cancer.

Conclusions

We identified a new rare recurrent deletion in NB at the distal end of the long arm of chromosome 1. This finding contributes to the delineation of the genomic profile of this aggressive tumor and offers future perspectives for the identification of new causal genes in NB. Further analyses are ongoing to uncover the role of potential candidate genes residing in this region.
PS2: Identification of sporadic colorectal cancers with high instability of microsatellites and loss of MLH1 protein expression

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Introduction
Deficiency of DNA mismatch repair (MMR) causes microsatellite instability (MSI) in a subset of colorectal cancers (CRC). Patients with these tumours have a better prognosis and may have an altered response to chemotherapy. Some of the tumours are caused by inherited mutations (hereditary nonpolyposis colon cancer or Lynch syndrome). However, ~85% are epigenetic changes of sporadic origin, especially in cases with loss of expression of the MMR MLH1 protein. This inactivation is due to promoter hypermethylation. Recently, the BRAF V600E activating mutation has been shown to be associated with sporadic MSI-high CRC.

Objective
To develop a sensitive algorithm to select patients with germline MLH1 mutated Lynch syndrome among those with loss of MLH1 expression by determination of MLH1 promoter hypermethylation and BRAF V600E mutation.

Material
Paraffin embedded-tissue (PET) from 26 patients with MSI-high MLH1- CRC, frozen tissue was available for 10 cases.

Methods
Methylation status of MLH1 promoter was studied by methylation sensitive-Multiplex ligation–dependent Probe Amplification (MS-MLPA) (MRČ-Holland) and compared to the MS-PCR after sodium bisulfite. The BRAF V600E mutation was analyzed by mutant-allele-specific-PCR (MASA), real-time PCR and compared to DNA sequencing of BRAF exon 15.

Results
MLH1 promoter hypermethylation was detected in 19 out of 26 tumors (73%) by MS-MLPA.positive These results were confirmed by MS-PCR in a selection of 10 cases. The BRAF V600E mutation was detected in 4 out of 10 randomly selected tumors (40%) by MASA. These results were confirmed by real-time PCR and DNA sequencing. No discrepancies were observed between the results obtained from the DNA extracted from PET vs frozen tumor.

Conclusion
We conclude that MS-MLPA is a robust and reliable method to study MLH1 promoter hypermethylation on paraffin embedded tissue in MSI-high MLH1 negative CRC. In a second step, we propose to look for BRAF V600E mutation by MASA-PCR or real-time PCR. Only a small fraction of MLH1- CRC should be offered genetic counseling with search for MLH1 gene mutations.
INTRODUCTION
Fragile X syndrome is the most common form of inherited mental retardation and the second most frequent cause of mental retardation (MR) after Down’s syndrome. It results from mutations in a (CGG) \text{n} repeat found in the FMR1 gene. Confirmation of the diagnostic is based on the Southern Blot analysis. As this method is long and tedious, several PCR-based screening methods have been reported with variable results.

MATERIAL AND METHODS
In this present study, we performed a systematic screening of the Fragile X syndrome for 300 patients with syndromic MR. This screening was based on two distinctive methods. The Fluorescent PCR was applied for the whole samples. When no amplified fragments were obtained, Methylation Specific PCR (MSP), using both primers specific for the methylated and unmethylated alleles, was performed. Males, which treated DNA just amplified with the methylated allele specific primers, will need further analysis by Southern Blot to confirm the diagnosis of Fragile X syndrome.

RESULTS
In our cohort, only five males (1.6\%) presented this MSP profile. Comparatively to the data of the literature, the diagnosis of fragile X syndrome, in our cohort, is relatively rare.

CONCLUSION
These results will allow us to review this strategy of systematic screening of the fragile X syndrome for all patients with syndromic MR and to propose clinical criteria to select candidate patients for this screening.
PS4: Congenital brain malformations and cognitive disorders: the contribution of the array-CGH in understanding how copy number variations shape our brain

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Objectives
To investigate the genomic imbalances that can cause congenital brain malformations and cognitive disorders and delineate candidate genes which dosage is critical for the regular function of the central nervous system (CNS).

Methods
Patients with congenital brain malformations w/o cognitive disorders of unknown origin are referred for genetic investigations. Array comparative genomic hybridisation (aCGH) based on BACs (Sanger 3k BAC aCGH) or oligonucleotides (OGT 105k or 180 k oligoarrays) is performed and validated by FISH, qPCR or higher resolution arrays (Sanger 32K-chromosome-specific arrays, OGT oligoarrays). The rearranged regions are further investigated in-silico. Candidate genes, when strongly inferred, are Sanger-sequenced for mutation screening.

Results
Copy number variations (CNVs) can result in a wide spectrum of brain malformations ranging from: a) brain size disorders as illustrated by a familial microcephaly that segregates with a deletion 1q34 disrupting the AKT3 gene, b) structural anomalies (e.g. agenesis of the corpus callosum (ACC)) where we report 2 children presenting with mental disability and ACC with a de novo deletion 13q34. ARHGEF7 arises as the best candidate for ACC since we detected no mutation in SOX1 gene, c) migration anomalies (e.g. pachygyria) analyzed in a 6-year-old-Pakistani girl presenting with severe mental disability, seizures and dysmorphic gestalt resulting from an inv dup(x)(p22.2) that disrupts the ARHGAP6 gene.

The in-silico analyses of the genomic content of the rearranged regions enabled: a) assessing the evidence for causality, b) the detection of the most likely mechanism generating the CNVs and c) the prioritization of the respective candidate gene(s).

Conclusions
This study demonstrates that both deletions and duplications can result in congenital CNS malformations. Further investigation of patients with brain malformations by aCGH should detect other novel CNVs and help finemapping loci for candidate genes.
**P55: Microlissencephaly with very short stature in kindred with congenital fibrosis of extraocular muscles**

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Microlissencephaly is an extremely rare condition probably autosomal recessive characterized by congenital microcephaly and severe gyration abnormalities. Patients with microlissencephaly have frequently other brain malformations, epilepsy and usually die in the neonatal period.

Congenital fibrosis of extraocular muscles type 3 (CFEOM3) is characterized by ophthalmoplegia and ptosis. It is caused by heterozygous mutations in TUBB3 or KIF21A genes.

Genes for alpha and beta tubulin (TUBA1A, TUBB2B and more recently TUBB3) are described as responsible for different types of neuronal migration abnormalities. Stronger evidence is shown for genes encoding microtubules associated proteins (e.g. LIS1, DCX).

We report on a consanguineous Moroccan family in which the father and eldest daughter presented a CFEOM3. They also have myopathic facies, fatigue and limbs weakness. Prenatal diagnosis of microlissencephaly and short stature was performed in the second fetus of this couple. The child, now aged 6 years old, was born at term. Her present weight and height are -6SD and head circumference is -10SD. She has severe psychomotor retardation but is otherwise in good health and remains seizure-free. MRI showed extreme microlissencephaly, agenesis of the corpus callosum and hypoplasia of the left cerebellar hemisphere. We sequenced TUBB3 and ARIX genes and hotspots regions of mutations of KIF21A gene but we didn’t find any mutation. We speculate that the association of two very rare conditions in this consanguineous family may not be spurious.
P56: Prenatal autosomal recessive polycystic kidney (ARPKD) disease: does genotype improve genetic counselling?

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ARPKD represents a well characterized phenotype and genotype condition with a prevalence of 1/40,000 living births. Molecular progress mapped candidate gene, ARPKD gene, to 6p21-centromere (Zerres 1994) and by positional cloning to precisely identify PKHD1 gene (Ward 2002). This gene encoding polycystin contains 86 exons whereas the longest open reading frame transcript is made of 67 exons with no hotspot mutation. Genotype determination is a considerable challenge since the gene extends over 470kbs and alternative splicing generates multiple transcripts. The detection rate reaches 85% for both mutations. Since then a database listing the identified mutations (more than 130) was created and identification of the two truncating mutations was associated with the most severe (lethal) form (Bergmann 2004). We report on the natural history of a 3 month-old boy who developed ARPKD. Prenatally, mother was referred at the age of 29 weeks for bilateral enlarged kidneys, no anatomic malformation nor hepatic lesion. Decreased amniotic fluid volume was recorded but no oligo nor anamnios. The pregnancy was the first from consanguineous Turkish parents without any personal history. Kidney ultrasound was normal to both of them. Phenotype allowed to postulate on ARPKD. Homozygosity mapping and microsatellites study identified homozygous region on PKHD1 gene (6p21) DNA from the fetus, heterozygous in both parents. Mutation screening then identified two missense mutations to the fetus namely, c.4292 G>A (protein level: p Cys1431Tyr). Both parents were heterozygous carriers. Prenatal genetic counseling was offered to parents who decided not to terminate the pregnancy although aware on the pejorative prognosis. Delivery took place at 36 2/7 weeks, birth weight was 2700 g and height 47 cms. The newborn developed pulmonary hypertension requiring high frequency mechanical ventilation and inhaled Nitric oxide for the first six days. Arterial hypertension was diagnosed. Renal function was altered (absence of spontaneous diuresis for the first days). This last was overcome through continuous intravenous Furosemide infusion for six days and maintained by oral administration. Repetitive ultrasound showed enlarged polycystic kidneys but absence of hepatic echogenic anomaly. At the age of 12 weeks, this boy is treated with furosemide and amloidipine.

This so far unreported mutation is associated with a moderate phenotype. De Namur et al. recent reported on a cohort of 54 fetuses whereas the 16 who were diagnosed with two missense mutations showed more severe phenotype then expected. Our recent history once again underlines the true challenge ARPKD represents.
References:
PS7: Molecular diagnosis of autosomal recessive retinal dystrophies by homozygosity mapping with SNP arrays

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Background
Homozygosity or identity-by-descent (IBD) mapping has been instrumental in gene discovery of several autosomal recessive (AR) retinal dystrophies (RDs). The increasing use of genomewide single-nucleotide polymorphism (SNP) arrays makes IBD mapping an accessible tool in a clinical context. The goal of this study was to demonstrate the efficacy of SNP array based IBD mapping as a molecular diagnostic tool in consanguineous families with a variety of RDs.

Subjects and Methods
In total, 35 patients out of 29 families with various RDs were subjected to IBD mapping using genomewide 250K SNP arrays. Candidate genes associated with RDs located within major IBD regions were screened for mutations by Sanger sequencing. In addition, the clinical record was revisited in patients in whom a mutation was found.

Results
Homozygous sequence variants were identified in 9 families. Novel variants were detected in the following genes: RDH12, USH2A, CNNM4 and ABCA4. The clinical diagnosis was revisited in all cases in which the molecular cause was elucidated, which yielded important clinical insights for three families. First, the identification of a homozygous CNNM4 mutation as a molecular cause in a family with CRD was instrumental for diagnosing amelogenesis imperfecta and a reappraisal of the clinical diagnosis (Jalili syndrome). Second, the presence of a homozygous IQCB1 mutation in a patient with non-syndromic LCA implied a substantial risk of developing nephronophthisis later in life. Last, an infarct visual prognosis was revealed in an asymptomatic younger sibling of a girl affected with LCA, as he was also homozygous for the nonsense RDH12 mutation identified in his sister.

Conclusion
In this study we applied IBD mapping as a diagnostic tool in 29 families segregating RDs. Following SNP genotyping and sequencing of only one to two candidate genes per proband, the causal mutation was identified in 9 families (31%) so far, thereby overcoming the need for laborious gene-to-gene testing of all known disease genes and the need for a well established phenotype prior to testing. Indeed, we demonstrated that IBD mapping allowed the identification of disease genes that would not have been selected by routine first-pass screening based on the patient’s initial clinical diagnosis. Finally, IBD mapping can also be used in families with a single affected individual.
P58: Haplotype analysis to determine founder effect in 150 Belgian hemophilia A patients

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The genetic basis of haemophilia A (HA) is well-established and factor VIII genetic testing for HA patients is increasing available in Belgium. No study has however so far evaluated the significance of founder effect in patients with HA living in Belgium.

Mutation screening of factor VIII (F8) gene was performed in 150 apparently unrelated Belgian patients with HA living in different parts of Belgium and attending regularly our Centre. Seventy different point mutations spread throughout the 26 exons of the F8 gene were identified in 95 affected families with different severities (severe-moderate-mild). Eleven recurrent mutations were identified repeatedly in a total of 37 families: c.1648G>A, c.3637delA, c.4379_4380dupA, c.5305G>A, c5398C>T and c.5954G>A were found in two families, c.1293G>T in 3 families, c.6932C>A and c.6533G>A in 4 families, c.6089G>A and c.6532C>T in 7 families. Haplotype analysis was used to determine whether these recurrent mutations occurred independently or were related through a founder effect.

The primary aim was to construct a haplotype by genotyping 17 single-nucleotide polymorphisms (SNPs) located in the F8 locus in affected males from these 37 families. Six different haplotypes reported in the reference European population HapMap were identified in our Belgian HA patients giving a first indication on the membership to the same family. To increase the capacity of the discrimination, five highly polymorphic microsatellites close and intragenic of the F8 disease-causing mutation were analysed in a second step to corroborate the SNP haplotypes analyses.

We found that for several mutations apparently unrelated HA families originate from the same ancestor. For others, it is clear that the causing genetic variation was inherited independently from two or three distinct ancestors. This study provides new insight into the molecular aetiology of HA in Belgium and the important contribution of founder effect.
P59: Novel genomic imbalances in familial epilepsy syndromes: implications for genetic counseling

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Objectives
To study the genetic bases of some familial epilepsy syndromes and discuss some genetic counseling issues.

Methods
Three families where several members presented seizure, mental disability of various degree w/o dysmorphic features or other neurological findings were investigated by genetic linkage analysis and/or array-comparative genomic hybridization. Results were validated by higher resolution aCGH, FISH and/or qPCR. Sanger-sequencing of candidate genes or reverse-transcriptase-qPCR for functional validation where applied when possible.

Results
In family #1, five male sibs presented mental retardation, hypotonia and behaviour disorders. Their two sisters and their father were clinically normal. However, their mother was mildly affected. By aCGH, we detected in all affected boys and their mother a 1 Mb interstitial duplication of chromosome 9p22.1-p21.3 spanning AF9 gene and disrupting SLC24A2 and KIAA1797. Using highly polymorphic microsatellites covering entire chromosome X and chromosome 9p we excluded an XLMR disorder and confirmed linkage to the segregating 9p region. AF9 was overexpressed and mice model studies identified AF9 as a developmental active epigenetic modifier during the generation of the cortical plate and up-regulates transcription of epithelial Na+ channel subunit alpha.

In family #2, we detected a paternally inherited 251 kb interstitial dup(3)(p25.3) encompassing the VHL and IRAK2 genes in a patient with mental retardation/multiple congenital anomalies, benign childhood epilepsy with occipital paroxysms (BCEOP) and ectomorphic habitus whose mother and maternal uncles had centrottemporal epilepsy. IRAK2 can cause epilepsy. Even though we excluded linkage to 3p, this dup(3)p(25.3) likely have a genetic modifier effect.

In family #3, a mild-mentally-disabled boy presenting a maternally inherited spinocerebellar ataxia (SCA) and a paternally transmitted epilepsy had a 2,2 Mb dup(17p12.1) of paternal origin and a maternally inherited mutation (c.736 G>C) in the CACNA1A gene causing SCA6.

Conclusion
This study provides further evidence for the involvement of novel genomic imbalances in epileptic syndromes and the power and limits of molecular genetics tools to assist clinicians to provide genetic counseling.
P60: Clinical and cytogenetic study of Rubinstein-Taybi Syndrome: a review of 12 Tunisian cases

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The Rubinstein-Taybi syndrome is a rare genetic disease responsible for 1.6‰ intellectual disabilities of the child. It is defined clinically by four major signs include characteristic facial dysmorphism, broad thumbs and big toes and a constant growth and mental retardation. Several congenital anomalies may be associated. This syndrome is usually sporadic but rare familial forms are described suggesting an autosomal dominant mode. To date, two genes, CREBBP and EP300 are involved in this syndrome. Microdeletion of 16p13.3 region, present in 10% of cases is one of the genetic mechanisms involved.

Object: The aim of this work is to do a clinical study of a series of 12 tunisians patients with the syndrome of Rubinstein-Taybi and achieve a cytogenetic study by the technique of fluorescence in situ hybridization (FISH) in search of a 16p13.3 microdeletion.

Methods: A clinical analysis of the cardinal signs and the various associated anomalies in our patients with a comparison to literature data. The application of FISH technique was based on the use of probes specifics of CREBBP locus at 16p13.3 region.

Results: All our patients had the cardinal signs of the syndrome of Rubinstein-Taybi namely facial dysmorphism with microcephaly, antimongoloide obliquity of palpebral fissures, a beaked nose and a microrétrognatisme; abnormalities of the extremities and psychomotor retardation. However, growth retardation occurs in 50% and cardiac complications, ophthalmic, renal, skeletal and neurological disorders are less frequent in our series. The FISH study provided confirmation of the molecular diagnosis of Rubinstein-Taybi syndrome in 8.3% of cases. This rate is consistent with the literature data. In our study, the familial form with an impairment of a pair of siblings was found. It is very likely due to a gene mutation;

Conclusion: This syndrome is heterogeneous clinically and genetically. For sporadic cases, the recurrence risk is <1%. However, prenatal diagnosis can be proposed to the possibility of germline mosaicism.
P61: Rapid, sensitive and discriminatory HbS and HbC mutation detection using High Resolution Melting Analysis

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Background
Hemoglobinopathies are common monogenic diseases forming a major public health problem due to their severity and disabling nature. Genetic identification of the two most frequently observed missense mutations in the beta-globin gene, HbS and HbC, are important epidemiologically and aid in prevention of the sickle cell trait and other serious hemoglobin disorders.

Aim
Our increasing patient population of Mediterranean, African and Middle Eastern origin urge for the need of a rapid, inexpensive and high-throughput genetic testing for HbS and HbC variants.

Results
Classical high resolution melting analysis with an optimized PCR amplicon of 110 bp allowed a sensitive and reliable identification of the two neighbouring HbS (c.20A>T) and HbC (c.19G>A) mutations. Discriminatory melting profiles were observed for all possible combinations of mutations: HbAA, HbAS, HbAC, HbSS, HbCC and HbSC, and confirmed the results obtained by Hb-chromatography and PCR followed by restriction digestion. Within the wild type control population tested, mainly consisting of Belgian and North African individuals, two other aberrant melting patterns were observed. Sequencing of all samples with aberrant melting patterns revealed one polymorphism at position c.9 either in heterozygous or homozygous state. Further testing including samples from over 35 other nationalities did not disclose other melting profiles.

Conclusion
This HbS and HbC HRM seems a promising, inexpensive and high-throughput alternative to PCR and restriction digestion analysis, although further validation is needed prior to implementation in a post- and prenatal diagnostic setting.
P62: A novel mutation of NDUFV1 associated with complex I deficiency by a genome-wide approach in consanguineous siblings

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Deficiency of complex I of the mitochondrial respiratory chain is a frequent cause of encephalopathy in children, but few mutations have been reported in each of its subunits. Furthermore, in the absence of families large enough for linkage, and of robust functional testing of the mutations, it is difficult to unequivocally demonstrate the causality of the observed mutations and to delineate genotype-phenotype correlations, making additional observations of these very rare genetic defects necessary. We observed two siblings with an early onset encephalopathy with medulla, brainstem and mesencephalon lesions on brain MRI and death before eight months of age caused by a complex I deficiency. The parents were first cousins and unaffected. Assuming that the disease was caused by homozygosity for an ancestral mutation in a nuclear-encoded gene, we used homozygosity mapping and identified 4 genomic regions that were homozygous and concordant in the two patients, encompassing 4 complex I genes, one of which only, NDUFV1, showing a missense mutation. The mutation, p.Arg386His, affects a highly conserved residue, contiguous to a cysteine residue known to coordinate a Fe ion. Another mutation of the same codon had been reported in another patient, yielding strong additional evidence for causality. Our observation adds to our understanding of complex I deficiency. It validates the functional role of Arg386, and therefore supports the current model of iron-sulfur clustering in NDUFV1.
P63: The microRNA body map: dissecting microRNA function through integrative genomics

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Background
MicroRNAs are tiny regulators of protein coding gene expression. While a growing body of evidence implicates deregulated microRNA expression in various human diseases, including cancer, our understanding of individual microRNA function remains limited. Here, we present the microRNA body map, an interactive online compendium and mining tool of microRNA expression profiles that forms the basis of novel functional miRNA annotation.

Methods
high-dimensional miRNA and mRNA expression profiles were generated or collected from human, murine and rat miRNA profiling studies.

Results
Functional miRNA annotation was inferred through integrative transcriptomics and gene set enrichment analysis. For gene sets that negatively correlate with the expression of a given miRNA, further analysis is performed to determine if the gene set is significantly enriched with predicted miRNA targets or targets of a transcription factor that, in itself, is predicted to be under negative regulation of the miRNA. In this way, integrating multiple levels of information allows to dissect miRNA function with higher resolution and accuracy as compared to approaches that rely on miRNA target prediction alone and enables the assessment of tissue or disease specific miRNA functions. Through this approach, we could infer validated miRNA functions and gain insights in the mechanisms by which miRNAs regulate signaling pathways. The functional annotation catalogue is complemented by a miRNA expression data analysis tool for selection of differentially expressed miRNAs or tissue/disease specific miRNAs from a collection of 22 datasets representing 750 human and rodent samples.

Conclusion
The microRNA body map enables prioritization of candidate microRNAs based on functional annotation and expression profile across tissue or disease subgroups. The microRNA body map project has great potential to become a community resource.
P64: NXTVAT: a sample centric variant annotation resource

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Background
As genetic variation data is being generated at an unprecedented scale, assessment of functional consequences of the variants in a given patient or patient cohort is a challenging task, both from a computational as from a data management perspective. It is expected that in this new era of personalised genomics, a clinical sample may need to be re-annotated repeatedly as new annotation information on the genome becomes available and new insights on variant interpretation accumulate. While various initiatives emerge to collect the overwhelming amount of genomic variants currently generated, a central system to manage and store the annotation of genomic variants as well as determining the functional effects is still missing.

Description
Here we present our efforts to create a centralised and standardised variant functional annotation repository called ‘NeXT-generation Variant Annotation Tracker’ or ‘NXTVAT’. In this repository a genomic variant, its annotation and in silico functional effect interpretation is stored only once, and can be linked to multiple samples that are organised in projects. Updated annotation information through new releases of annotation databases or improved variant interpretation algorithms evokes a specific alert to relevant users without the need for re-analysing samples. Variant annotation is done using Ensembl API [1] and ANNOVAR [2]. A plug-in style organisation of these resources allows easy modification of the annotation pipeline or to extend it with additional tools. The user friendly web interface enables submission of variants in various formats, including the emerging standard format VCF version 4.0 from the 1000 genomes consortium [3, 4].

References
P65: Selection of candidate colorectal cancers for germline MLH1 mutation among those associated with high instability of microsatellites and loss of MLH1 protein expression.

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Introduction: Deficiency of DNA mismatch repair (MMR) causes microsatellite instability (MSI) in a subset of colorectal cancers (CRC). Patients with these tumours have a better prognosis and may have an altered response to chemotherapy. Some of the tumours are caused by inherited mutations (hereditary nonpolyposis colon cancer or Lynch syndrome). However, ~85% are epigenetic changes of sporadic origin, especially in cases with loss of expression of the MMR MLH1 protein. This inactivation is due to promoter hypermethylation. Recently, the BRAF V600E activating mutation has been shown to be associated with sporadic MSI-high CRC.

Objective: To develop a sensitive algorithm to select patients with germline MLH1 mutated Lynch syndrome among those with loss of MLH1 expression by determination of MLH1 promotor hypermethylation and BRAF V600E mutation.

Material: Paraffin embedded-tissue (PET) from 20 patients (Med : 73yr [46-89]) with MSI-high MLH1- CRC, frozen tissue was available for 10 cases.

Methods: Methylation status of MLH1 promotor was studied by methylation sensitive-Multiplex ligation-dependent Probe Amplification (MS-MLPA) (MRC-Holland) and compared to the MS-PCR after sodium bisulfite treatment. The BRAF V600E mutation was analyzed by mutant-allele-specific-PCR (MASA), real-time PCR and compared to DNA sequencing of BRAF exon 15.

Results: MLH1 promotor hypermethylation was detected in 19 out of 20 tumors (95%) by MS-MLPA. These results were confirmed by MS-PCR in a selection of 13 cases. The BRAF V600E mutation was detected in 6 out of 9 randomly selected tumors (67%) by MASA, all of them exhibited a MLH1 promotor hypermethylation. These results were confirmed by real-time PCR and DNA sequencing. No discrepant results were observed with DNA extracted from PET vs frozen tumor. The only CRC without MLH1 methylation nor BRAF mutation and therefore good candidate for MLH1 germline mutation occurred in the youngest patient (46yr).

Conclusion: We conclude that MS-MLPA is a robust and reliable method to study MLH1 promotor hypermethylation on paraffin embedded tissue in MSI-high MLH1 negative CRC. In a second step, we propose to look for BRAF V600E mutation by MASA-PCR or real-time PCR. Only a small fraction of MLH1- CRC should be offered genetic counseling with search for MLH1 gene mutations in patients older than 50 yr.
P66: Preimplantation Genetics Diagnosis using Single Cell 60-mer oligo arrayCGH platform

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Preimplantation Genetic Diagnosis with Aneuploidy Screening (PGD-AS) is used for the selection of genetically normal embryos before implantation, thus increasing both baby take home rate and health of the fetuses. Array CGH technologies have been employed to detect chromosomal aneuploidies in single blastomeres on a genome-wide level. The aim of this study was to explore whether the use of high resolution Agilent 244K Human CGH arrays would improve the detection of segmental aneuploidies in single cells compared to the previously used BAC and SNP arrays (Vanneste et al., Nat Med, 2009).

In brief, 8 Epstein Barr Virus (EBV) transformed lymphoblastoids and 43 blastomeres derived from human embryos were analyzed. Data were normalized by the new developed channel based method. Subsequently, mean-median calculation, Circular binary segmentation (CBS) algorithms have been optimized and employed to analyze the accuracy of the data to call for a priori known and/or de novo whole chromosome and segmental aberrations in single blastomeres.

We succeeded in developing a new normalization method to preprocess Agilent 244K human microarray data, enabling single cell data analysis on oligo platforms. We proved that this channel based method can effectively correct for artifacts/biases introduced by single cell amplification while maintaining a similar dynamic range of the log-ratios as compared to unamplified genomic DNA (Cheng et al.). The mean-median calculation method can identify a priori known aberrations while the CBS and the Haarseg algorithm can detect both a priori known and de novo aberrations.

In conclusion, we present a novel research to detect copy number variations of single cell DNA derived from blastomeres. This research is helpful for the future study to improve the selection of high quality human embryos before implantation. Whole chromosome and segmental imbalances can be detected at the single cell level using Agilent 244K human CGH microarrays. However, the aberration calling accuracy of the three detection algorithms optimized for Agilent is in the same order of magnitude as compared to BAC and SNP based arrays. Further validation studies are needed before any array CGH technology can be introduced in the clinic as a standardized assay for PGD.
P67: Dominant GDAP1 mutations cause predominantly mild CMT phenotypes

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Background
Ganglioside-induced differentiation associated-protein 1 (GDAP1) is commonly mutated in autosomal recessive Charcot-Marie-Tooth (ARCMT) neuropathy, resulting in demyelinating, axonal and intermediate phenotypes with an early onset and severe course. In rare instances, GDAP1-mutations also cause autosomal dominant CMT (ADCMT).

Methods
Mutation analysis was performed in a large cohort of ADCMT patients by means of bidirectional sequencing of coding regions and exon-intron boundaries of GDAP1. Intragenic GDAP1 deletions were excluded using an allele quantification assay. The pathogenic character of one sequence variant was confirmed by in vitro experiments assaying mitochondrial morphology and function.

Results
In eight CMT families we identified four pathogenic heterozygous GDAP1 mutations, three of which are novel. Three of the mutations displayed reduced disease penetrance. Disease onset in the affected individuals was variable, ranging from early childhood to adulthood. Disease progression was slow in most patients and overall severity milder than typically seen in autosomal recessive GDAP1 mutations. Electrophysiological changes are heterogenous but compatible with axonal neuropathy in the majority of patients.

Conclusions
With the present study we broaden the phenotypic and genetic spectrum of autosomal dominant GDAP1-associated neuropathies. We demonstrate that cell-based functional assays can be reliably used to test the pathogenicity of unknown variants. We discuss the implications of phenotypic variability and the reduced penetrance of autosomal dominant GDAP1 mutations for CMT diagnostic testing and counseling.
P68: Parental insertional balanced translocations are an important cause of apparently de novo CNVs in patients with developmental anomalies.

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Array CGH screening is becoming the first tier diagnostic test for the identification of copy number changes in patients with mental retardation and/or congenital anomalies. The identification of a causative CNV is not only important to make a proper diagnosis but also to enable the accurate estimation of the recurrence risk to family members. Upon identification of a de novo interstitial deletion or duplication the risk recurrence is considered very low. Nevertheless, apparently de novo imbalances in a patient can be the consequence of the unbalanced transmission of a derivative chromosome involved in an insertional translocation (IT) in one of the parents. To determine the frequency with which insertional balanced translocations would be the origin of submicroscopic imbalances in patients with developmental abnormalities, we investigated the potential presence of an IT in a consecutive series of de novo interstitial CNVs. Using the whole genome array method we have analyzed 10459 index patients with MR/DD referred for clinical diagnostics, and found 498 interstitial de novo aberrations. We were able to performed 419 FISH analyses, amongst which we found 6 imbalances resulted by the presence of IT in one of the parents. Our results show that insertional translocations underlie approximately 1.5% of the apparently de novo interstitial CNVs, indicating that submicroscopic ITs are much more frequent than cytogenetica visible. This risk estimate should be taken into account during counselling family members and warrant parental testing in cases with de novo interstitial aberrations.
P69: Neural tube defects in Cornelia De Lange Syndrome (CDLS): a new Moroccan case

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Cornelia de Lange Syndrome (CdLS) is a multiple congenital syndrome which affects between 1/10,000 and 1/60,000 neonates. The genetic bases are still not clear. Its principal clinical characteristics are distinctive facial features, upper limb malformations, growth and cognitive retardation. The diagnosis of the syndrome is based on the distinctive clinical features that are very characteristic (arched eyebrows, synophrys, depressed nasal bridge, longphiltrum, downturned angles of the mouth). Other clinical features like gastrointestinal, renal abnormalities and cardiac defects were described. Here we present a case of CdLS with "neural tube defects" to confirm that the features of this disorder vary widely among affected individuals and range from relatively mild to severe.
P70: Phenotypic heterogeneity of Kabuki (Niikawa-Kuroki) syndrome: about three cases

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Kabuki syndrome (KS, Niikawa–Kuroki syndrome, MIM:147920) is a rare multiple congenital anomaly/mental retardation syndrome described simultaneously by Niikawa et al and Kuroki et al. The estimated frequency of this syndrome is about 1/32 000 in Japan. KS is characterized by postnatal growth retardation, distinctive facial features, dermatoglyphic anomalies, skeletal dysplasia, and mental retardation. The molecular basis remains unknown.

In our report, we present three boys with different clinical features. The first is 12 years old, with a hypogammaglobulinemia A and G, a perception deafness, cryptorchidism, facial dysmorphism, joint hyperlaxity and fetal pads. The two others present only a mild mental retardation with joint hyperlaxity, facial features and fetal pads. Through the study of these three cases we confirm the phenotypic heterogeneity of the kabuki syndrome which makes it underdiagnosed.
P71: Detection of mitochondrial DNA depletion using relative real-time PCR

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Mitochondrial depletion syndrome (MDS) is a disorder characterized by a severe reduction of the total amount of mitochondrial DNA. This disorder is clinically heterogeneous and autosomal recessive inherited. MDS manifests either as a myopathic, encephalomyopathic or hepatocerebral form. At the moment, pathogenic mutations in at least 9 nuclear encoded genes are known to cause depletion. All genes are involved in the mitochondrial DNA biosynthesis and in the maintenance of deoxynucleotide pools. However, these genes explain only a small percentage of the MDS cases hence it can be expected that others will be discovered. It is hard to identify the underlying molecular defect in a MDS patient relying only on the clinical spectrum because of the unclear genotype-phenotype correlations. Therefore, a simple method would be helpful to determine mitochondrial DNA depletion before performing sequencing analysis. In the past mitochondrial DNA content was estimated by Southern blot analysis, which is a time consuming, laborious technique requiring high amounts of DNA. Real-time PCR is one of the more recent developed molecular techniques and is now commonly used for quantification. Using the Roche Lightcyler 480 we optimized a relative real-time PCR quantification assay to determine the mitochondrial DNA content in liver and muscle tissue of patient and control samples. Relative real-time PCR quantification seems a good technique to determine mitochondrial DNA depletion rapidly and accurately in a routine setting.

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For functional studies a sufficient amount of globine proteins, wild type and mutants, is required. Therefore the focus is placed on the optimization of the parameters during gene expression and protein purification. The recently discovered globine protein, neuroglobin seems to be involved in the modulation of hypoxic/ischemic brain damage. However the exact mechanisms of neuroglobin induction and the protection of the neuron are still unclear. To unravel the function of neuroglobin, biochemical characterization and functional studies are necessary. Brain damage caused by cerebral hypoxia/ischemia can be limited by endogenous defence mechanisms such as the biosynthesis of neuroprotective proteins. A better understanding of these mechanisms may lead to new therapeutic strategies for the treatment of stroke and related disorders.

Specifically, the objective is to optimize the neuroglobine production in E. coli (flask or fermentation-scale) and the purification via FPLC.

During fermentation experiments different parameters (i.e. temperature, pH, stirring conditions, time of induction and addition of heme-precursors) were adjusted for optimal production. Although there was a gain in cell density, no increase in protein production (in comparison to the flask-level) was obtained.

For protein purification different steps in sample preparation were performed. After sonication, sample preparation includes ammoniumsulphate precipitation (60%) followed by dialysis. Bulk separation is performed using DEAE Sepharose Fast Flow matrix (GE Healthcare). Red fractions contain neuroglobin and are collected in a volume of 50-100ml. As elution was done by 300mM NaCl, desalting (dialysis) is needed. Next, ionchromatography and gelfiltration were performed using the Akta purifier 100 (GE Healthcare).

First a HiTrap DEAE Fast Flow (5ml; GE Healthcare) column was evaluated using a 10ml superloop, linear gradient from 10-30% 5mMTris pH 8.5-1M NaCl buffer and a pre-centrifugale concentration of the sample. Fractionation of the neuroglobine (17kDa) is obtained by programming the UV-1 at 412nm. The red fractions are confirmed using SDS-PAGE and are pooled for the next step.

The assessment of the gelfiltration using the HiPrep 26/60 Sephacryl S-200 High Resolution (GE Healthcare) included two assays resulting in purified neuroglobin.
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