European School of Genetic Medicine

28th Course in

Medical Genetics

Bertinoro, Italy, May 17-21, 2015

Bertinoro University Residential Centre
Via Frangipane, 6 – Bertinoro

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ABSTRACTS OF LECTURES

Sunday, May 17

Medical Genetics Today

Dian Donnai
University of Manchester, Manchester Centre for Genomic Medicine
St Mary's Hospital, UK

Genomic Medicine today is built on a distinguished history of clinical, scientific and technological contributions. Over the 60 years since the discovery of the structure of DNA and the ~ 50 years since the introduction of chromosome analysis for diagnostic purposes an increasing range of services has been available to benefit patients with genetic disorders and their families.

Whereas in the past the application of medical genetics was limited to diagnosis and risk assessment for patients with a relatively small range of rare diseases, the vast explosion in knowledge and technologies has allowed medical genetics to have a much greater impact on medicine from a vastly increased range of diagnostic tests, even therapies for some conditions (Dietz 2010) as well so-called 'precision medicine' where a specific treatment is given on the basis of a germ line or somatic mutation (for example in a tumour) or a drug prescribed in doses based on a genotype i.e. pharmacogenetics.

Many clinical observations and hypotheses formulated many years ago have now been proven by our ability to investigate them with more powerful techniques e.g.

- Clinical observations suggested that conditions with asymmetry and localized overgrowth or with skin lesions were likely to be mosaic disorders and over the past few years this has been confirmed in Proteus syndrome, melanocytic nevus, linear sebaceous nevus, hemimegalencepahly syndromes, Ollier and Maffucci syndromes. Interestingly all these conditions involve mutations in genes from pathways which also are well described in common cancers such as RAS-MAPK, PI3K-AKT-mTOR and IDH1/IDH2 .
- Similarly the concept of syndrome families (now known to closely match developmental pathways) was based largely on clinical observation (Spranger 1985,). The examples usually given are the disorders associated with FGFR mutations (achondroplasia group of skeletal dysplasias) and disorders of the RAS-MAPK pathway (Noonan syndrome disorders) (Denayer et al. 2008).

The new technologies enabling targeted capture and massively parallel sequencing of individual genomes/exomes have resulted in major discoveries initially on small *clinically* well characterised patients (Ng et al 2010, Hood et al 2012). As these genes have been identified new developmental

pathways have been elucidated and many disorders with overlapping clinical features shown to be due to mutations in functionally related genes perhaps amenable to treatment by similar molecules.

Over the past four years the emphasis has shifted from discovery to diagnostic applications. Families of individuals with unknown disorders are being offered exome sequencing of trios (mother, father, child) (Veltman, Brunner 2012 and the UK 12,000 patient DDD study (*Nature* 2015) http://www.ddduk.org/intro.html) or targeted testing using large panels of appropriate genes being offered to patients with specific disorders such as retinal dystrophy, cataract, epilepsy etc. (O'Sullivan, 2012 Rehm 2013). Interestingly results of diagnostic applications of NGS indicate that there is a much wider phenotypic spectrum associated with mutations in many genes than was suspected from initial clinical definition and Sanger sequencing. Recognising that exome sequencing may miss pathogenic mutations some centres are now introducing whole exome sequencing into diagnostic practice (Gilissen 2014). More frequently, however, WGS is being used for large scale research studies that may have some individual patient benefits but are being used to generate so-called 'Big Data' www.genomicsengland.co.uk/the-100000-genomes-project. Concerns have been expressed about the ethical aspects of NGS but as experience deepens most centres are finding ways of addressing these in conjunction with patient groups (Bredenoord et al 2013).

Medical Genetics as a clinical specialty is constantly changing. The last 15 years has seen a massive increase in referrals of conditions generally regarded as common complex disorders such as breast and bowel cancer and some cardiac diseases. The first challenge has been to separate out those families with a 'monogenic subset' of the disease which are the group which our current services can best help. Meanwhile large scale research efforts such as the Wellcome Trust Case Control Consortium (http://www.wtccc.org.uk) have been making progress looking for genetic variations – generally of small effect – which contribute to the pathogenesis of common disorders and the new technologies are rapidly contributing to this research too. The new molecular knowledge about the basis of disease has challenged current understanding and classification of disease and some have called for a new taxonomy to take account of recent discoveries (National Research Council 2011).

Also set to greatly change the practice of genetic medicine is the introduction of non-invasive prenatal testing (NIPT) for a greater range of chromosomal and single gene disorders (Bianchi 2012, 2014 Chitty 2013)

Some may argue that Medical Genetics as a clinical specialty is not needed and that systems specialists and pathology laboratories can provide all that is needed. However I would argue that there are skills that we bring which considerably enhance patient care which are not available in other specialist clinics. We offer services for patients and families, for all age groups, for all body systems and over generations and time. We have knowledge of rare disorders – diagnosis, natural history and complications. We can offer or advise on screening, monitoring, prevention of complications (anticipatory care) and therapies. We offer genetic counselling to affected and apparently healthy people and are a major source of information to families, support groups and to other professionals in social care and in education. However the roles

of clinicians and scientists in Medical Genetics will change. Certainly we will be called upon to educate our colleagues in other specialties. Clinically we should ensure our expertise in deep phenotyping is recognised, as well as our expertise in interpreting sequencing data in the light of the phenotype. In addition there are already treatments for some of the conditions we see, and we are likely to be involved in disease stratification as part of multidisciplinary teams involved in clinical trials. The time has certainly come to consider Genomic Medicine as a specialty with real expertise and an important future.

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Genotypes and phenotypes

Han G. Brunner

Radboud UMC, Department of Human Genetics, Nijmegen, and Maastricht University Medical Center, Department of Clinical Genetics, The Netherlands

Much of human and medical genetics concerns the relationships that exist between human genes, the variation and mutations that occur within these genes, and the phenotypes that result from these mutations. At least 5000 human phenotypes have been documented in the Online catalogue of Mendelian Inheritance in Man. Many still remain to be described. The number of disease genes increases all the time and now totals well over 1000.

So what do we know of the relationships between genes and phenotypes?

I shall discuss the following categories:

1. One gene causes multiple phenotypes
    a. allelic series occur when the mutations vary in severity, and a graded series of phenotypes results. This is evident in the case of achondroplasia, its less severe variant hypochondroplasia, and the lethal condition thanatophoric dysplasia. All three conditions are due to mutations of the FGFR3 gene.

       Similar allelic variation is present for cystic fibrosis, for spinal muscular atrophy, for hemophilia, and for many other genetic diseases. This means that in some families who have a milder or more severe form of a genetic disease the prognosis may be very different from what the textbooks say.
    b. Opposite phenotypes may occur if some mutations activate, and others inactivate the same gene. As an example I shall discuss activating mutations of the luteinizing hormone receptor gene which cause early puberty in males, and inactivating mutations which cause Leydig

cell hypoplasia. Activating mutations of the RET gene cause thyroid tumors (FMTC, and MEN2B), while inactivating mutations cause Hirschprung's disease.

c. Sometimes, mutations affect different functional domains within a gene. If this is the case, then the resulting phenotypes may be markedly different.

   An interesting example occurs for the P63 gene, where mutations in the DNA-binding domain cause EEC syndrome, including split-hand-foot malformation, and mutations in the SAM domain of the gene cause Hay-Wells syndrome without hand malformations, but severe skin problems, and a fusion of the eye-lids. A similar situaton has been reported for other genes, such as the Gli3 gene (mutations cause either Pallister Hall syndrome, or Greig syndrome), and the FGFR2 gene (Apert syndrome and Crouzon syndrome).

2. Two or more genes cause the same phenotype. This is called genetic heterogeneity.It appears to be very common, and is usually due to the fact that different genes encode components of a multiprotein complex, or a receptor and its ligand, or different components of a biochemical or cellular pathway.
   a. As an example, several genes that cause Fanconi anemia encode proteins that form part of a single complex that functions in DNA repair. Many other examples exist. It is likely that all Usher syndrome genes interact with each other in the cell.
   b. The Walker Warburg syndrome can be caused by mutation of either the POMT1, POMT2, FUKUTIN, or FKRP genes. All genes encode proteins that function in glycosylation of target proteins in brain and mucle such as alpha-dystroglycan. Here, the phenotypic similarity is explained by the loss of the same biochemical function in the cells.

3. Overlapping phenotypes may involve different genes. Yet, their products will still often affect the same function within the cell or the organism. As an example, I shall discuss how mutations of the Collagen genes encoding the type 2, 11A1, and 11A2 collagen chains cause recognizable variants of the Stickler syndrome. These 3 collagen chains together for a heterotrimeric triple helix collagen protein.

The overall conclusion is (1) that phenotypic differences between patients with a single genetic disease are important as they may point to relevant genotypic variation.
At the same time, (2) phenotypic overlap between different genetic diseases indicates that the gene products share a function at the cellular level.

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Introduction in Next Generation Sequencing technologies and applications

Joris A. Veltman

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Department of Clinical Genetics, Maastricht University Medical Center, Maastricht, The Netherlands.

There is considerable variation between the genetic code of two individuals, both at the single nucleotide and at the structural level. Identifying and studying the consequences of these variations, a core activity in human genetics research, is driven by technological innovations. Currently we are in the midst of one of the greatest technological revolutions in genomics. Novel DNA sequencing methods are dramatically increasing sequencing throughput to a level where it is soon possible to rapidly sequence an individual genome for an affordable price. If properly established, whole genome sequencing will have a major impact on the entire field of medicine; All genomic variation that can be linked to disease is detectable in a single experiment!
In this presentation I will introduce next generation sequencing technology, discuss its development and advantages over traditional sequencing technologies, illustrate the use of this technology for rapid identification of disease causing genes in rare and common disease and discuss briefly its potential for implementation in the clinic.

Recommended reading:

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How to deal with next generation sequencing output

Christian Gilissen

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Next Generation Sequencing (NGS) technologies have revolutionized the field of medical genetics research by generating large numbers of DNA sequences within a matter of days at very low cost. Next generation sequencing is being used extensively to search for Mendelian disease genes in an unbiased manner by sequencing the entire protein-coding sequence, known as the exome, or even the entire human genome.[1] Increasingly, NGS is also being applied for the diagnosis of patients with genetically heterogeneous disorders, where sequencing of all individual disease genes in infeasible.[2,3]

Because of the large amounts of data that are being generated, bioinformatics plays an increasingly important role. In this talk I will focus on the basic bioinformatic concepts, data formats and pitfalls of analyzing NGS data from resequencing experiments for applications in research and diagnostics.[4]

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[3] A post-hoc comparison of the utility of Sanger sequencing and exome sequencing for the diagnosis of heterogeneous diseases. Neveling K, Feenstra I, Gilissen C, Hoefsloot LH, Kamsteeg EJ, Mensenkamp AR, Rodenburg RJ, Yntema HG, Spruijt L, Vermeer S, Rinne T, van Gassen KL, Bodmer D, Lugtenberg D, de Reuver R, Buijsman W, Derks RC, Wieskamp N, van den Heuvel B, Ligtenberg MJ, Kremer H, Koolen DA, van de Warrenburg BP, Cremers FP, Marcelis CL, Smeitink JA, Wortmann SB, van Zelst-Stams WA, Veltman JA, Brunner HG, Scheffer H, Nelen MR. Hum Mutat. 2013 Dec;34(12):1721-6. PMID: 24123792

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Monday, May 18

Complex Disordes

Andrew Read

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Linkage is a relation between *loci*, association is a relation between *alleles* or *phenotypes*. However, both depend on identifying shared ancestral chromosome segments. Linkage analysis is performed in families, where shared chromosomal segments are large, so that a genomewide linkage study can be conducted using only a few hundred markers. Genomewide association studies look for ancestral segments shared by very distantly related people. Because many meioses separate such people, the shared segments are very small, and a GWAS requires huge numbers of markers.

Historically, linkage was one of the earliest techniques to be used in genetic analysis. Already in the 1930s JBS Haldane and others had attempted linkage analysis of human conditions. Lack of suitable markers restricted progress until the 1980s, when the identification of large numbers of DNA variants (restriction fragment length polymorphisms) spread across the whole genome made genomewide linkage studies possible. Later work moved to panels of microsatellites and then SNPs, making 'mapping before lunch' a real possibility by the early 2000s.

Linkage has been extremely successful with mendelian conditions, but despite much effort, it largely failed for complex conditions. Risch and Merikangas (*Science* **273:** 1516-17; 1996) showed that, given certain assumptions, association is in principle more powerful than linkage for detecting weak susceptibility factors. Despite the success of genomewide association studies in identifying hundreds of susceptibility factors for complex diseases (http://www.genome.gov/gwastudies), much of the heritability of complex conditions remains unaccounted for. I will discuss possible reasons why both linkage and association studies of complex disease have been disappointing.

Arrays and CNVs

Eva Klopocki

Institute for Human Genetics, Biozentrum, University of Würzburg, Germany

Genetic variation is due to different types of variants i.e. single nucleotide variations/polypmorphisms (SNVs/SNPs) or larger copy number variations (CNVs). CNVs belong to the class of structural genomic variants. These variants contribute to human phenotypic variation as well as Mendelian and complex diseases, including developmental delay/intellectual disability, autism, schizophrenia, and epilepsy. The development of molecular karyotyping technologies like microarray based comparative genomic hybridization (array CGH) and SNP microarrays enabled genome-wide detection of CNVs. These technologies and their application in research as well as diagnostics will be presented.

In the last ten years the role of CNVs in human disease became obvious by the discovery of numerous novel microdeletion and microduplication syndromes. The underlying molecular mechanisms leading to CNVs such as non-allelic homologous recombination (NAHR), non-homologous end-joining (NHEJ) and a DNA replication-based mechanism, fork stalling and template switching (FoSTeS), are discussed. In addition, this lecture will provide an overview of clinical consequences of CNVs.

Literature

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Molecular syndromology in the NGS-era: which phenotype, which family, which strategy? Applications to aging research

Bernd Wollnik

Center For Molecular Medicine, University of Cologne , Germany

Novel sequencing technologies as well as adopted conceptual strategies can dramatically speed up gene identification in medical genetics. There was little doubt that massive parallel sequencing would have a great impact on studying causative genes for rare syndrome in the future, and the last years have impressively shown that this future has already started. We currently do see a huge wave of gene identification studies

using these novel sequencing technologies. It is important to note that only together with subsequent functional work on identified proteins and pathways these novel technologies will elucidate underlying pathogenic mechanisms. This talk will present our recent experiences in using whole-exome-based approaches in medical genetics and show successful examples, which shed light into the pathogenesis of selected syndromes as well as specific syndromes with accelerated aging phenotypes. These progeria syndromes are rare congenital disorders, which share an overlapping premature-aging phenotype including among others alopecia, wrinkled skin, lipoatrophy, and cardiovascular abnormalities. These diverse progeria syndromes differ with regards to their time of manifestation, the severity of the symptoms, and the life expectancy of the affected patients. The genetic cause has been identified for some progeria syndromes, e.g. *de novo* dominant mutations in the *LMNA* gene cause Hutchinson-Gilford progeria syndrome. Our strategy is to use NGS-based technologies for the identification of novel genes causing human congenital disorders associated with premature aging phenotypes and to investigate mechanisms responsible for premature aging. Disturbance of genomic integrity and accumulation of DNA damage seems to have an important impact on accelerated aging processes in these patients. Examples will be given and molecular mechanisms discussed.

Bottlenecks in understanding mitochondrial diseases & Preventing and treating mitochondrial diseases

Patrick Chinnery

Institute of Genetic Medicine
Newcastle University, UK

What are mitochondrial diseases?

Mitochondrial disorders are a group of inherited diseases that are principally caused by a biochemical defect of the respiratory chain. As a group they affect ~ 1 in 5000 of the population.

Mitochondrial biogenesis

The respiratory chain is a group of enzyme complexes situated on the inner mitochondrial membrane that is the main source of adenosine triphosphate (ATP), which the principal source of energy within the cell. A bioenergetic defect causes cell dysfunction and affects organs that are critically dependent on energy metabolism such as a nervous system, neuromuscular system, heart, endocrine organs and the eye.

The mitochondrial respiratory chain consists of over 100 proteins forming five enzyme complexes. Thirteen components are synthesised from small circles of DNA present within the mitochondrion (the mitochondrial genome, mtDNA, 16569bp). MtDNA also codes for the 24 RNAs required for intra-mitochondrial protein synthesis. The remaining respiratory chain sub-units and key factors in the maintenance of mitochondrial DNA, the transcription and translation of the mitochondrial genome, and assembly of the respiratory chain,

are all encoded by nuclear genes. These proteins are synthesised in cytosol, usually with a mitochondrial targeting pre-sequence, allowing delivery of the peptides through the mitochondrial membrane involving a dedicated translocation machinery.

Genetic basis of mitochondrial disorders

The dual genetic origin of the respiratory chain means that mitochondrial disorders can arise from mutations of either mtDNA or the nuclear genome. MtDNA mutations fall into two groups: deletions and point mutations. Deletions usually cause sporadic diseases, and point mutations are usually inherited down the maternal line. Some point mutations are homoplasmic (all the genomes are mutated) and some are heteroplasmic (a mixture of mutated and wild type mtDNAs). The proportion of mutated and wild type genomes determines whether a cell expresses a biochemical defect. In patients with mitochondrial disease the inherited mutation load determines whether an individual is affected, and the severity of the phenotype. Siblings can have very different levels of a heteroplasmic mutation. This is thought to be due to a germ-line genetic bottleneck. Mutations in nuclear encoded mitochondrial proteins can be inherited as a dominant trait, an autosomal recessive trait or an X-linked disorder.

Clinical features of mitochondrial disease

The clinical discipline of mitochondrial medicine was founded on the description of classical clinical syndromes diagnosed by muscle pathology or the measurement of an underlying biochemical defect. Examples include Leber hereditary optic neuropathy (LHON), mitochondrial encephalomyelopathy with lactic acid and stroke like episodes (MELAS), myoclonic epilepsy with ragged red fibres (MERRS) and chronic progressive external ophthalmoplegia (CPEO). However, with the widespread use of molecular diagnostics it is becoming clear that both biochemical defects in mitochondrial function and mutations in relevant disease genes cause a wide range of overlapping phenotypes. Some of these phenotypes are organ specific (for example, causing an optic neuropathy, or inherited deafness), whereas others cause multisystem disorders that can be different in different affected family members.

Nuclear-mitochondrial disorders

Initial genetic linkage studies, candidate gene studies and more latterly whole exome and whole genome sequencing has led to a profusion in the number of different diseases caused by mutations in nuclear-encoded mitochondrial proteins. Remarkably, some of these appear to have distinct phenotypes, despite being due to common underlying mechanisms.

A major group of nuclear-encoded mitochondrial disorders are caused by defects of mtDNA maintenance. Mutations in nuclear gene encoding the mtDNA polymerase γ (*POLG*) are the most common cause. Autosomal dominant *POLG* mutations can cause chronic progressive external ophthalmoplegia. Autosomal recessive *POLG* mutations can cause a severe childhood encephalopathy called Alpers-Huttenlocher syndrome characterised by intractable epilepsy and liver failure. Autosomal recessive *POLG* mutations can

also cause a late onset recessive ataxia syndrome. *POLG* mutations cause both the loss of mtDNA (mtDNA depletion) and the formation of secondary mutations of mtDNA (both deletions in point mutations), which accumulate in tissues over time and therefore explain the phenotype. Other genes involved in mtDNA maintenance disorders include *POLG2, ANT1, Twinkle, OPA1* and more latterly *SPG7*.

The second major group of nuclear-encoded mitochondrial disorders are associated with defects of mtDNA transcription and translation. These disease genes cause biochemical defects affecting multiple respiratory chain complexes. Diverse molecular mechanisms have been implicated, and these disorders can present at any age with an overlapping spectrum of phenotypes.

The other major group of nuclear-encoded mitochondrial disorders are caused by defects of nucleotide metabolism. These are predominantly autosomal recessive enzyme defects including thiamine phosporylase deficiency (*TP*) which causes mitochondrial neurogastrointestinal encephalomyelopathy (MNGIE); mutations in *TK2* (thymidine kinase) which cause a pure myopathy syndrome usually presenting in childhood; and mutations in *DGUOK* (deoxyguanosine kinase) mutations which present with a combination of myopathy, liver failure and occasionally encephalopathy.

Investigation of mitochondrial disorders

Occasionally it is possible to identify the specific mitochondrial phenotype based on the clinical presentation. This can immediately suggest a molecular genetic blood test leading to a precise diagnosis. However, often patients who not fit neatly into specific phenotypic group and a more systematic approach is required. This usually involves biopsy of an affected tissue or organ and histological, histochemical and biochemical analyses. Abnormal histochemistry or biochemistry can confirm the presence of a mitochondrial disorder and guide subsequent molecular genetic analyses. It is important to note that some heteroplasmic mtDNA mutations cannot be detected in blood, and the molecular defect may be restricted to clinical affected tissues. This means that in certain circumstances blood DNA analysis is not a reliable means of making a diagnosis.

Whole exome and whole genome analysis has the potential to identify both nuclear and mtDNA disorders through the analysis of target reads in exome datasets, and the rich mtDNA sequence coverage found in whole genome sequence libraries. It is likely that whole genome sequencing will transform the way that clinicians approach the diagnosis of mitochondrial disease in the future.

Clinical management of mitochondrial disease

At present there is no known treatment of mitochondrial disorders. As a consequence the main stay of treatment is in preventing disease and the management of complications. Preventing disease involves genetic counselling. This can be difficult, particularly for heteroplasmic mtDNA disorders, where it is difficult to predict the proportion of inherited mutated genomes in an individual patient. Prenatal and preimplantation diagnostic approaches can help define disease current risks, but are not easy to interpret in

many instances. MtDNA mutations are only inherited down the maternal line. Nuclear disorders have a variety of different inheritance patterns as explained above.

Supportive management includes the monitoring of patients with the development of complications, such as diabetes mellitus, cardiomyopathy and epilepsy, which are managed using conventional approaches.

Although a variety of different vitamins, food supplements and minerals have been tried in patients with mitochondrial disease, there is no objective evidence that any of these agents work. There are a few exceptions to this general rule, including the replacement of co-enzyme Q10 in patients with specific genetically determined biochemical defects of co-enzyme Q10 biosynthesis, and the use of bone marrow transplantation in patients with MNGIE caused by thiamine phosphorylase deficiency.

International consortia have established large cohorts of patients that are currently being studied to chart the natural history of the different phenotypes caused by the different genetic defects. This is a critical step in the development of new treatments. A large randomised controlled trial involving over 100 patients was published in 2011 and shows promise for the drug idebenone in the treatment of Leber hereditary optic neuropathy, but the trial failed to provide definitive evidence that the treatment is beneficial. Five other trials are currently being sponsored by industry for mitochondrial myopathy in 2015.

In recent years there has been great interest in the development of mitochondrial transfer techniques to preventing the transmission of mtDNA diseases. A number of different apporaches have been proposed with varied levels of reported efficacy in preclinical studies using mice, primates and in-vitro human embryos. The UK Parliament voted to allow mitochondrial transfer as a treatment to prevent mitochondrial DNA disease in 2015, but the treatment is not currently in clinical use in Europe.

Further reading

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Tuesday, May 19

Epigenetics and disease – lessons from imprinting disorders

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Epigenetics

Different cells in the body are characterised by different functions and different levels of gene expression despite each sharing the same genetic code. This variation in gene activity from cell to cell is achieved by mechanisms and processes that are collectively termed epigenetics. These epigenetic changes alter gene expression without altering the DNA sequence. One epigenetic mechanism that is readily measured is DNA methylation. It is potentially reversible and heritable over rounds of cell division. Furthermore such epigenetic modification of DNA can be influenced by environment, gene interaction or by stochastic error and there is a higher rate of epimutation than DNA mutation.

Variation in DNA methylation is a well-recognised cause of human disease and is likely to play a pivotal role in the cause of complex disorders. The challenge is to identify consistent epigenetic alterations of aetiological significance, given that epigenetic modification of DNA differs between tissues, occurs at different times of development within the same tissue and is sensitive to continual environmental factors. This makes it difficult to determine whether epigenetic mutations are a primary cause or secondary to the disease process.

Genomic imprinting is one of the best understood examples of epigenetic regulation of gene expression. The expression patterns of imprinted genes are characterised by expression from only one allele (of the pair) in a consistent parent of origin manner. The pattern is set by targeted methylation within the male or female germ line that resists the post fertilisation waves of demethylation of the zygote. Imprinted genes are thought to play an important role in fetal growth and their carefully regulated expression is important for normal cellular metabolism and human behaviour.

Imprinting Disorders

Several well- known disorders of imprinting are known including Beckwith Wiedemann syndrome, Transient Neonatal Diabetes, Temple syndrome, Wang Kagami Ogata syndrome, Russell Silver syndrome, Angelman syndrome Prader Willi syndrome and Pseudohypoparathyroidism type 1B. Only a proportion of people with these syndromes have a true epigenetic error, as uniparental disomy (inheritance of both chromosome homologues from one parent with no contribution from the other) and copy number variation are more common underlying causes. Studies to determine the cause of seemingly ‘true’ epigenetic aberrations, identified in imprinting disorders, may provide helpful insights into the causes of epigenetic

mutations in general. For example the work on imprinting disorders has led to the identification of *ZFP57*, as a gene essential for DNA methylation maintenance.

Disease	**Prevalence**	**Main diagnostic clinical features**	**Additional clinical features (may develop with time)**	**Frequency of 'epigenetic' aberration**	**Reference**
Prader Willi syndrome	1 in 17,500	Low birth weight Hypotonia, Hyperphagia Developmental delay	Hypogonadism Diabetes Obesity	Approximately 1%	(Williams, Driscoll, and Dagli)
Angelman syndrome	1 in 16,000	Severe developmental delay No speech Epilepsy Ataxia	Microcephaly	4%	(Cassidy and Driscoll)
Beckwith Wiedemann syndrome	1 in 13,700	Macrosomia/overgrowth Macroglossia Umbilical defect	Increased risk of Wilms tumour Hypoglycaemia	60%	(Weksberg, Shuman, and Beckwith)
Silver Russell syndrome	1 in 50,000 Likely underestimate	Intrauterine growth retardation Faltering growth Short stature	Relative macrocephaly Genital abnormalities Hypoglycaemia	50%	(Wakeling et al.)
Transient neonatal diabetes	1 in 400,000	Intrauterine growth retardation Neonatal diabetes with remission	Macroglossia Umbilical hernia Developmental delay Diabetes	26%*	(Docherty LE, et al.)
Temple syndrome (maternal UPD 14 associated syndrome)	unknown	Intrauterine growth retardation Hypotonia, Scoliosis Developmental delay Early puberty ,Short stature	Hydrocephalus Cleft palate	uncertain	(Kotzot)
WKO syndrome (Paternal UPD 14 associated syndrome)	unknown	Bell shaped chest Hypotonia Developmental delay	Umbilical defects Larger birth weight	uncertain	(Kagami et al.)
Pseudohypoparathyroidism 1B	unknown	Hypocalcaemia due to Parathryoid resistance (tetany/parasthesia)	Obesity	>90%+	(Bastepe et al.)

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Long range effects

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Complex developmental processes require tightly controlled regulatory networks which ensure correct temporal and spatial gene expression during development. Gene expression programs are guided by cis-regulatory elements including promoters, enhancers, repressors and insulators. Some of these elements are located at large distances from the target gene itself and are therefore termed “long distance” or “long-range” regulatory elements. Disruption of long-range gene regulation can cause tissue- and stage-specific effects some of which have become recognized as a significant cause of human disorders. Different mechanisms underlie disruption of long-range gene regulation. These can give rise to phenotypes that differ from those associated with mutations in the coding regions of the affected genes.

Structural aberrations of the human genome contribute to phenotypic variation as well as pathogenic conditions. Copy-number variations (CNVs) constitute one group within these structural aberrations that arise from deletions (loss) or duplications (gain), and as a consequence result in a copy-number change of the respective genomic region. CNVs may include entire genes, parts of transcripts, or only noncoding sequences. By now it is well accepted that structural aberrations affecting coding regions can have pathogenic effects i.e. due to changes in gene dosage. Noncoding variants which may encompass *cis*-regulatory elements, however, have only recently come into focus as disease-associated variants. The consequences of CNVs in noncoding sequences are less obvious, although, the so far described phenotypes associated with alterations in noncoding elements with regulatory potential are striking and at the same time confined to a certain tissue/organ. Excellent clinical examples for this are duplications encompassing potential enhancer

elements which cause limb malformations i.e. brachydactyly, polydactyly, and mirror-image duplications.

Besides CNVs in non-coding sequences structural aberrations such as inversions and translocations may disturb the regulatory landscape and chromatin architecture and have been associated with human disorders. One of the underlying mechanisms is known as "enhancer adoption" indicating a gene which is driven by an enhancer that is not its own potentially causing ectopic expression. Structural variants may also disrupt regulatory boundaries i.e. deletion of insulator elements resulting in aberrant gene regulation.

In addition to congenital anomalies non-coding regulatory mutations have been identified in somatic disease conditions i.e. cancer (Weinhold et al. 2014). Examples will be presented in this lecture.

In conclusion genetics changes affecting regulatory elements are expected to be higher among conditions which are due to disturbance of complex developmental processes. Integrating data from patients with the recently published data from the ENCODE project will broaden our view of genes and their regulation and contribute to our understanding of pathomechanism underlying human disease and in general phenotypic traits.

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ENCODE project - https://www.encodeproject.org/V

MaoA and Behavioural Genetics

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In 1993, we described a single large family with a truncating mutation in MAOA that was associated with various impulsive behaviours, including abnormal sexual behaviours and impulsive aggression.

Only a handful of families and patients with complete MAOA deficiency have been described since then all with behavioural problems. It has been established that mice with a knock-out mutation of MAOA also show abnormal behaviour including aggressive behaviours. A landmark study from 2003 demonstrated that a frequent polymorphism in the MAOA promoter leads to antisocial behaviours but only in the presence of severe maltreatment in childhood. This study has been influential in supporting a nature AND nurture paradigm. This polymorphism has been invoked in court cases, and on at least one mutation the presence of the minor allele (which occurs in 1/3 of all males) was reason to reduce the sentence of a killer. Most recently, two studies supported the hypothesis that convicts with the minor allele for the MAOA polymorphism might be more likely to use aggression in their criminal acts.

All of this has implications for the ever-continuing nature-nurture debate in society.

Wednesday, May 20

Inherited cancer and prospects for therapy

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Life is dependent on cell division. Without it we die; with it we are at constant risk of cancer. Many cancers are driven by the chance accumulation of genetic errors so in most cases they show no evidence of familial aggregation. (Welch JS et al Cell 2012;150:264-78).

We can learn much from rare events; naked mole rats produce excess hyaluran to ease their movement underground and reduce their cancer risk (Gorbunova V Nature Reviews Genetics 2014;15:531-540). Chromothrypsis (a shattered chromosome) in a patient with a rare blood disorder WHIM syndrome removed an activation mutation and revealed a gene which can be down regulated to enhance clonal expansion in the marrow (McDemott DH et al Cell 2015;160:686-699).

Studying families with rare cancer combinations can shed light on mechanism and focus clinical efforts to prevent cancer. Around 3% of solid tumours, excluding lung cancer, are attributable to a germline susceptibility, typically resulting from an autosomal dominant loss of function in a tumour suppressor gene. Around 100 genes have been identified where useful predictive statements can be based on sequencing and where preventive intervention is possible (Rahman N, Nature 2014;505:302-8). The mainstay of therapy is to identify premalignant change or early cancer and ablate or remove it. Laser therapy to early retinoblastomas is a classic example. In some cases, such as hereditary thyroid and colorectal cancer it is possible to remove the "at risk" organ. In Familial Adenomatous Polyposis the whole colon is resected in early adulthood.

As molecular pathways become better understood, therapeutic and preventive drug treatments become feasible. The most exciting recent development is the emergence of the PARP inhibitors which block single strand DNA repair forcing cells to rely on homologous recombination. This pathway requires functional BRCA1 and 2. Where gene carriers have lost the second gene copy and developed a cancer, HR is compromised and PARP inhibitors become lethal. The first, olaparib, is now licensed for use in HR deficient ovarian cancer in relapse.

When drugs are to be used in a preventive mode, the risk of side effects becomes pre-eminent. Extensive data supports the view that anti-inflammatory agents prevent solid tumours especially of the gastrointestinal tract. Selective COX2 inhibitors, developed as safer alternatives to aspirin because they do not cause peptic ulceration, were trialed and shown to prevent polyps. They were withdrawn, however, when it became clear

that there was an excess of heart attacks among the healthy people using these drugs to prevent future cancers.

A review of early trials of aspirin to prevent cardiovascular disease has revealed fewer cancers in the following decade among those randomised to aspirin. Two trials examined the effects of aspirin on cancer prevention. The women's Health Study gave alternate day low very dose (100mg) aspirin or placebo to 18,000 women and found after 10 years that the incidence of colorectal cancer fell by 18% in those on aspirin (Cook NR et al Ann Int Med 2013; 159:77-85.). The CAPP2 trial randomized 1009 carriers of a mismatch repair gene defect, at risk of Lynch syndrome or hereditary non-polyposis colorectal cancer, to daily 600mg aspirin or placebo for 2-4 years. Analysis in those who completed the target of 2 years treatment revealed a 63% reduction in colorectal cancer at 5 years and a similar fall in other cancers such as endometrial cancer.(Burn et al Lancet 2011;378:2081-87). Several lines of evidence suggest part of the effect is attributable to suppression of inflammation. Aspirin may also enhance apoptosis of pre malignant cells, analogous to effects of salicylates in plants. CaPP3 will test different doses of aspirin in 3000 MMR gene defect carriers commenced in 2014. Aspirin may be combined with other lifestyle interventions to reduce the burden of hereditary cancers, even in the presence of a highly penetrant gene defect.

Spinal muscular atrophy: from gene to therapy

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SMA is a devastating neuromuscular disorder that leads to progressive muscle weakness and atrophy and that represents the most common lethal genetic disease in infants. SMA is an autosomal recessive disorder with an incidence of 1:6000 to 1:10.000. The carrier frequency in the general population lies between 1:35 and 1:125 depending on the ethnicity (1, 2). Patients with SMA are generally divided into clinical sub-categories (termed SMA type I, II, III and IV) based on disease onset and severity, with SMA type I having the earliest onset and most severe phenotype (3). Although SMA is considered to be a motor neuron disorder, additional organs can also be impaired, albeit mainly occurring in severely affected SMA mice and patients (4).

SMA is caused by homozygous absence (or rarely subtle mutation) of *SMN1*, whereas disease severity is influenced by the number of *SMN2* copies and other SMA modifying genes (5-7). Since *SMN2* mRNA is mainly alternatively spliced lacking exon 7 due to a single translationally silent variant, 90% of SMN protein is truncated and unstable. The remaining 10% of transcripts, however, are full-length and produce protein identical to that encoded by *SMN1* (5, 8). Since the SMN protein has a housekeeping function in snRNP biogenesis and splicing the multi-organ impairment mainly associated with very low SMN levels found in severely affected SMA mice and patients is an obvious consequence of SMN expression levels that fall under a certain critical threshold (9). At present, there is no curative treatment available for patients with SMA, but impressive progress has recently been made towards the development of new therapies.

The main focus of translational SMA research at present is the development of SMN-dependent therapies. These efforts include strategies directly targeting SMN protein stability, endogenous SMN2 mRNA transcription, or splicing by using small-molecules (antisense oligonucleotides, AONs) or drugs, and approaches based on SMN gene replacement using self-complementary serotype 9 adeno-associated virus vectors (scAAV9) expressing SMN1.

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Marfan syndrome and related disorders: from gene to therapy

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The recent study of different connective tissue diseases and their homologous mouse models have dramatically altered our understanding of their pathogenesis. A major breakthrough was realized with the study of mouse model of Marfan syndrome (MFS). The study of emphysema development in a fibrillin-1 deficient Marfan mouse model pinpointed altered TGFbeta signaling as the culprit in the pathogenesis. The role of TGFbeta pathway was also proven in the study of aortic walls of fibrillin-1 mouse models. This central role of TGFbeta in aortic aneurysm formation was confirmed by the identification mutations in the *TGFBR1/2* genes (transforming growth factor beta receptor 1 or 2) as the cause of a new aortic aneurysm syndrome (Loeys-Dietz syndrome, LDS). This syndrome is characterized by the triad of hypertelorism, cleft palate/bifid uvula and widespread aneurismal disease with arterial tortuosity. Increased TGFbeta activity was demonstrated in aortic walls of both LDS and MFS patients. Interestingly, in two rare autosomal recessive connective tissue disorder, the arterial tortuosity syndrome, caused by deficiency of a glucose transporter, GLUT10 and in the cutis laxa type 1B, caused by fibulin-4 deficiency, both also complicated with arterial aneurysms, we also showed TGFbeta upregulation in vascular smooth muscle cells.

Most recently, mutations in other components of the TGFbeta signaling pathway, including *SMAD3, TGFB2, TGFB3* and *SKI* have been associated with LDS-like phenotypes and Shprintzen-Goldberg syndrome.
As such, these human diseases and different mouse models have offered the opportunity to unravel the complex interaction between aortic integrity and extracellular matrix regulation of TGFbeta activity. There is increasing evidence indicating that misregulation of TGFbeta signaling owing to defects in extracellular proteins is centrally important to the development of aortic aneurysms. This view has now replaced the previous idea that aortic aneurysms were simply due to a structural deficiency of the microfibrillar matrix in the aorta. Moreover, this new view offers excellent targets for therapeutic interventions. A large study in pediatric MFS population confirmed that angiotensin blocker losartan (with known TGFbeta blocking effect) is equally effective to high dosis of beta-blocker, the current standard treatment.

The ciliopathies: model disorders to study epistasis and total mutational load

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The overwhelming majority of human genetic disorders manifest substantial inter- and intra-familial variability, in part due to the action of second-site modifiers on the primary causal locus[1]. Advances in genomic technologies have hyper-accelerated gene discovery. However, in contrast with the identification of *bona fide* causal genes and alleles, the dissection of second-site phenomena in humans is lagging significantly, in part due to the lack of genetic power and a general paucity of functional information. This, in turn, leads to the limited utility of genetic information at the primary disease locus in terms of prognosis, patient management and, potentially, therapeutic strategies. Among the models proposed to explain genetic modulation phenomena, one attractive hypothesis is that of mutational load, in which the amount and distribution of alleles in a discrete cellular module can influence variability.

The ciliopathies, a phenotypically and genetically heterogeneous group of disorders that manifest overlapping phenotypes such as retinal degeneration; skeletal and limb defects; central and peripheral nervous system defects; and renal, pancreatic and biliary cysts and fibrosis; have emerged over the past decade as a useful model to study the effect of *trans* alleles on primary disease loci[2,3]. This is because a) they represent a severity/pleiotropy continuum with imperfect genotype-phenotype correlations at the primary locus; b) they are caused by structural and/or functional defects at the primary cilium, a semi-closed system whose constituent protein components are largely known; and c) the effect of ciliary dysfunction can be captured quantitatively using downstream *in vivo* functional assays and *in vitro* reporters derived from an improved understanding of this organelle in morphogenetic signaling[4]. Although each ciliopathy is individually rare, collectively their contribution to the overall genetic disease burden in humans approaches

population frequency similar to that of common defects such as Down syndrome, with a minimal estimated collective incidence of ~1:1,000 conceptuses[5]. To understand how *trans* mutations in a functional system can contribute to clinical variability in this disease group, we previously conducted unbiased medical resequencing of candidate ciliary genes in a large patient cohort across the ciliopathy severity spectrum. These studies have lead to the identification of both new causal genes and also a number of *trans* and *cis* alleles with a potential exacerbating or ameliorating effect on penetrance and/or expressivity. In this lecture, we will discuss several examples, including:

1) *TTC21B*, a retrograde intraflagellar transport protein is not only a novel cause of the phenotypically discrete ciliopathies, isolated nephronophthisis (NPHP) and Jeune asphyxiating thoracic dystrophy (JATD), but it is also a significant contributor to mutational load in ciliopathies[6]. Mutational screening of *TTC21B* was the first large-scale analysis of an axonemal protein-encoding locus across the ciliopathy spectrum ranging from mild (isolated NPHP), moderate (Bardet-Biedl syndrome (BBS), JBTS) to severe (MKS, JATD), and sequencing data combined with functional assays revealed a five-fold enrichment of pathogenic *TTC21B* variants in the ciliopathy cohort in comparison to healthy controls. Moreover, genetic interaction studies demonstrated that genetic sensitization of retrograde intraflagellar transport (IFT) probably exacerbates at least 13 different primary ciliopathy loci. Together, this locus contributes to the mutational burden of 5% of individuals with ciliopathies. Further, this work provided a foundation for further investigation into the mutational contribution of the IFT complex in ciliopathies, thereby offering the opportunity to explore human genetic disease using a systems biology modular approach.

2) Cis-complementation, a phenomenon in which a second, co-evolving allele contains a protective effect on mutations, is widespread in the genome and can be validated experimentally using ciliary models[7]. As part of our ongoing work to determine the consequences of variation on ciliary function, we have performed *in vivo* complementation assays of >500 alleles found in ciliopathy patients, with a primary focus on mutations in the BBSome[8] and the IFT particle[9]. Cross-comparison across *in vivo* and *in vitro* ciliary assays yielded >90% concordance for both pathogenic and benign alleles[6,10], suggesting high specificity and sensitivity for these assays. However, based on discordance between our experimental data and popular prediction algorithms such as SIFT[11], PolyPhen2[12], and MutationTaster[13] (sensitivity and specificity of 70%-80%), we hypothesized that mutant residues with poor evolutionary conservation are more likely to be discordant among predictions. A systematic comparative genomics analysis of human disease-causing missense variants showed that an appreciable fraction of disease-causing alleles are fixed in the genomes of other species, suggesting a role for genomic context. We developed a model of genetic interactions that predicts most of these to be simple pairwise compensations. Functional testing of this model on two known human ciliopathy genes revealed discrete *cis* amino acid residues that, although benign on their own, could rescue the human mutations *in vivo*.

Despite this progress, the fundamental challenge of predicting phenotype and or clinical progression based on single locus information remains incompletely understood. We anticipate that saturated knowledge of allele quality and quantity across the ciliopathy phenotypic spectrum can be synthesized further to generate improved models that can explain both causality and also improve our resolution of the mechanisms underscoring variable penetrance and expressivity. These findings will in turn, likely be applicable toward explaining phenotypic variability in other forms of Mendelian disease.

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Thursday, May 21

Ethics of Medical Genetics

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In 2013 the American College of Medical Genetics and Genomics issued recommendations on the reporting of incidental findings obtained from exome or genome sequencing (*Genet Med* 15: 565-74; 2013). They recommended that laboratories should pro-actively seek variants in a specified list of around 50 genes (see Table below) and report these back to the referring physician in every case, regardless of the age, indication and other circumstances of the patient. The proposal attracted considerable controversy. Robert Green, who was to have delivered this talk, was a prime mover in developing the proposal. In my talk I will examine the rationale and background behind the ACMG proposal and consider alternative views and approaches to handling incidental findings, both in genomic research and clinical genetics services.

Table 1 Conditions, genes, and variants recommended for return of incidental findings in clinical sequencing

KP: known pathogenic, sequence variation is previously reported and is a recognized cause of the disorder;
EP: expected pathogenic, sequence variation is previously unreported and is of the type that is expected to cause the disorder.
Note: The recommendation to not report expected pathogenic variants for some genes is due to the recognition that truncating variants, the primary type of expected pathogenic variants, are not an established cause of some diseases on the list.

Phenotype	MIM disorder	PMID-Gene Reviews entry	Typical age of onset	Gene	MIM gene	Inh	Variants to report
Hereditary breast and ovarian cancer	604370	20301425	Adult	*BRCA1*	113705	AD	KP and EP
	612555			*BRCA2*	600185		
Li–Fraumeni syndrome	151623	20301488	Child/adult	*TP53*	191170	AD	KP and EP
Peutz–Jeghers syndrome	175200	20301443	Child/adult	*STK11*	602216	AD	KP and EP
Lynch syndrome	120435	20301390	Adult	*MLH1*	120436	AD	KP and EP
				MSH2	609309		
				MSH6	600678		
				PMS2	600259		
Familial adenomatous polyposis	175100	20301519	Child/adult	*APC*	611731	AD	KP and EP
MYH-associated polyposis;	608456	23035301	Adult	*MUTYH*	604933	AR	KP and EP
adenomas, multiple colorectal,	132600						
FAP type 2; colorectal adenomatous polyposis, autosomal recessive, with pilomatricomas							
Von Hippel–Lindau syndrome	193300	20301636	Child/adult	*VHL*	608537	AD	KP and EP
Multiple endocrine neoplasia type 1	131100	20301710	Child/adult	*MEN1*	613733	AD	KP and EP
Multiple endocrine neoplasia type 2	171400	20301434	Child/adult	*RET*	164761	AD	KP
	162300						
Familial medullary thyroid cancer	1552401	20301434	Child/adult	*RET*	164761	AD	KP
PTEN hamartoma tumor syndrome	153480	20301661	Child/adult	*PTEN*	601728	AD	KP and EP
Retinoblastoma	180200	20301625	Child	*RB1*	614041	AD	KP and EP
Hereditary paraganglioma–pheochromocytoma syndrome	168000 (PGL1)	20301715	Child/adult	*SDHD*	602690	AD	KP and EP
	601650 (PGL2)			*SDHAF2*	613019		KP
	605373 (PGL3)			*SDHC*	602413		KP and EP
	115310 (PGL4)			*SDHB*	185470		
Tuberous sclerosis complex	191100	20301399	Child	*TSC1*	605284	AD	KP and EP
	613254			*TSC2*	191092		
WT1-related Wilms tumor	194070	20301471	Child	*WT1*	607102	AD	KP and EP
Neurofibromatosis type 2	101100	20301380	Child/adult	*NF2*	607379	AD	KP and EP
Ehlers–Danlos syndrome, vascular type	130050	20301667	Child/adult	*COL3A1*	120180	AD	KP and EP
Marfan syndrome, Loeys–Dietz syndromes, and familial thoracic aortic aneurysms and dissections	154700	20301510	Child/adult	*FBN1*	134797	AD	KP and EP
	609192	20301312		*TGFBR1*	190181		
	608967	20301299		*TGFBR2*	190182		
	610168			*SMAD3*	603109		
	610380			*ACTA2*	102620		
	613795			*MYLK*	600922		
	611788			*MYH11*	160745		
Hypertrophic cardiomyopathy, dilated cardiomyopathy	115197	20301725	Child/adult	*MYBPC3*	600958	AD	KP and EP
	192600			*MYH7*	160760		KP
	601494			*TNNT2*	191045		KP and EP
	613690			*TNNI3*	191044		KP
	115196			*TPM1*	191010		
	608751			*MYL3*	160790		
	612098			*ACTC1*	102540		
	600858			*PRKAG2*	602743		
	301500			*GLA*	300644	XL	KP and EP
	608758			*MYL2*	160781	AD	KP
	115200			*LMNA*	150330		KP and EP
Catecholaminergic polymorphic ventricular tachycardia	604772			*RYR2*	180902	AD	KP
Arrhythmogenic right-ventricular Cardiomyopathy	609040	20301310	Child/adult	*PKP2*	602861	AD	KP and EP
	604400			*DSP*	125647		
	610476			*DSC2*	125645		
	607450			*TMEM43*	612048		KP
	610193			*DSG2*	125671		KP and EP
Romano–Ward long QT syndrome types 1, 2, and 3, Brugada syndrome	192500	20301308	Child/adult	*KCNQ1*	607542	AD	KP and EP
	613688			*KCNH2*	152427		
	603830			*SCN5A*	600163		
	601144						
Familial hypercholesterolemia	143890	No Gene	Child/adult	*LDLR*	606945	SD	KP and EP
	603776	Reviews		*APOB*	107730	SD	KP
		entry		*PCSK9*	607786	AD	
Malignant hyperthermia susceptibility	145600	20301325	Child/adult	*RYR1*	180901	AD	KP
				CACNA1S	114208		

PGD AND PGS: is this the future?

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Preimplantation genetic diagnosis (PGD) involves the use of assisted reproductive technologies, such as in vitro fertilisation (IVF), to produce embryos from couples at high-risk of transmitting serious inherited conditions to their offspring. A minute amount of tissue (usually a single cell) is removed from each embryo and subjected to genetic analysis, thus revealing the embryos that are affected by the familial disorder. Only healthy embryos are transferred to the uterus and consequently the issue of pregnancy termination is avoided. It has been 25 years since the first PGD cases were performed and in that time it has become an accepted reproductive strategy for patients carrying single gene mutations and chromosome rearrangements. However, a large amount of patient-specific work-up is necessary for each case, leading to delays in treatment and high costs. New methodologies may finally be able to solve this problem. The introduction of methods that permit comprehensive chromosome analysis (e.g. microarray comparative genomic hybridisation [aCGH], quantitative PCR, and most recently next-generation sequencing) now allow almost all chromosome abnormalities to be tested using a single protocol. This has reduced costs and eliminated waiting lists for patients that carry a chromosome rearrangement (e.g. translocation). A generic protocol for patients with single gene mutations is also now available, in the form of Karyomapping.[1] Rather than focusing on the detection of disease-causing mutations, which are often unique to an individual patient and require extensive customisation of protocols to allow accurate detection in single cells, Karyomapping uses a microarray to genotype hundreds of thousands of single nucleotide polymorphisms (SNPs) distributed across the genome. Preimplantation diagnosis then employs linkage analysis, tracing the inheritance of disease-associated SNP haplotypes from the parents to their embryos. Karyomapping has the additional benefit of providing information on the chromosomal content of the embryos tested, which may be of particular value to couples having PGD where the woman is of advanced reproductive age and therefore at increased risk of an aneuploid conception.

Although the use of PGD for the avoidance of single gene disorders is growing in popularity, the most common reason for the genetic testing of embryos remains preimplantation genetic screening (PGS). The purpose of PGS is to test embryos for aneuploidy and ensure that those transferred to the uterus are chromosomally normal. Aneuploidy is extremely common in human preimplantation embryos and its incidence is closely related to female age. For patients in their early thirties more than one-third of embryos at the blastocyst stage are typically aneuploid, whereas for women over forty more than two-thirds of blastocysts are usually affected. Aneuploidy is almost always lethal and consequently the transfer of such abnormal embryos to the uterus typically results in failure to implant or miscarriage. The aneuploid embryos produced during an IVF cycle are indistinguishable from their chromosomally normal counterparts using the routine morphological assessments carried out in IVF laboratories, hence the need for genetic assessment. As

with PGD, embryos analysis involves the biopsy of one or more cells (depending on the developmental stage at which testing is carried out) from the embryos produced during an IVF cycle. The cells may be tested for chromosome abnormalities using any of several methods, the most widely used of which is aCGH. In theory the identification and transfer of chromosomally normal embryos to IVF patients should dramatically reduce the risk of Down syndrome, reduce the incidence of miscarriage and significantly increase pregnancy rates.

In the past the clinical use of PGS has been considered controversial. Despite the strong underlying theory, several clinical trials failed to demonstrate any of the expected benefits of chromosome screening. However, it is now apparent that these disappointing results were primarily due to technical limitations of the methods for genetic analysis that were available at the time. Modern technologies, such as aCGH, have overcome these limitations and there are now several randomized controlled trials showing significantly improved pregnancy rates following PGS applied at the blastocyst stage (five days after fertilisation of the oocyte).[2-4]

Most recently, next-generation sequencing (NGS) has been introduced for the purpose chromosome screening, providing the most cost-effective option for comprehensive aneuploidy detection in single cells from human embryos.[5] Currently, the use of NGS to investigate human embryos focusses on a 'low-pass' strategy, in which less than 0.1% of the genome is sequenced. However, there remains the theoretical possibility of deeper sequencing, revealing the entire genome of embryos. Of course, the potential for such detailed genetic analysis to be carried out prior to transfer of embryos to the uterus raises significant ethical questions.

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ABSTRACTS OF STUDENTS POSTERS

A novel ANO10 mutation confirms the role of TMEM16K protein in autosomal recessive cerebellar ataxias.

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Background: The hereditary cerebellar ataxias are a heterogeneous group of conditions that is commonly classified according to their mode of inheritance. The Autosomal-recessive cerebellar ataxias (ARCAs) comprise a clinically and genetically heterogeneous group of neurodegenerative disorders. At least 20 different forms of (ARCAs) are known to date. Vermeer et al. (2010) was the first to report and identify 4 different mutations in the *ANO10* gene in 8 affected members from 3 unrelated families with autosomal recessive spinocerebellar ataxia-10 (SCAR10).

Methods: We investigated a large consanguineous family of Arab-Christian ancestry that live in northern Israel with multiple affected individuals exhibiting variable phenotypes of Slowly progressive cerebellar ataxia which diagnosed before 20 years of age, associated with cerebellar dysarthria, saccadic pursuit, ocular alterations, nystagmus, cerebellar atrophy seen on brain MRI or CT, intellectual disability, tortuosity of conjunctival vessels, and vascular changes.

We employed whole genome homozygosity mapping and targeted gene Sanger sequencing. Five affected individuals and five healthy members were included in this study.

Results: We identified a novel homozygous mutation c.139+1G>T that was detected at the first nucleotide of the consensus donor splice site of exon 2, fully segregated in this family. The c.139+1G>T mutation creates a new splicing donor site (GT) in intron 2. Therefore, the last base of exon 2 (G) joins the newly formed 1st base of intron 2 (T) to become the new donor site (GT). This change leads to a frame shift and to a formation of stop codon after 9 amino acids p.G47Efs*9 in the TMEM16K protein.

Conclusions: Our data further support ANO10 gene as causing autosomal recessive ataxia suggesting that this gene may be an important cause of ARCAs in different ethnic groups, and underscore the important role of the TMEM16K protein in normal brain tissue.

This study allows us accurate genetic counseling to family members and other members in the village, which is considered a key factor for informed decision regarding family planning

Spectrum of mutations in NF1 gene in Polish population witch NF1 and NFNS clinical diagnosis.

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Background and aim

Both neurofibromatosis type I (NF1) and neurofibromatosis-Noonan syndrome (NFNS) are clinical entities belonging to the RASopathies – disorders related to the dysfunction of RAS/MAPK signaling pathway. The NF1 is characterized by the presence of café-au-lait (CAL) spots, neurofibromas, freckling, optic nerve gliomas, Lisch nodules, specific bone lesions and neurological problems like learning and intellectual disabilities. The NFNS clinical suspicion is based on the presence of clinical features typical for Noonan syndrome (NS), including craniofacial dysmorphy and/or congenital heart defect, and for NF1 (e.g. CAL spots and freckling).

Both entities are inherited in autosomal dominant manner and are caused by the presence of pathogenic mutation in NF1 gene (17q11.2) that encodes neurofibromin – a protein involved in the regulation of the activity of several signaling pathways including RAS/MAPK, cAMP/PKA and PI3K/AKT pathways that regulate cell proliferation and differentiation.

The aim of the study was to identify the molecular defect underlying NF1 and NFNS phenotype in Polish patients.

Patients and methods:

The study group included 79 Polish patients with a clinical diagnosis of NF1 (70 patients) or NFNS (9). The analysis of NF1 gene was based on the analysis of the deletion presence with MLPA technique (MLPA SALSA: P122-C1 NF1 AREA Kit and P081-B1/P082-B1 NF1 MLPA kits, 70 patients) followed by the sequence analysis of NF1 gene with Sanger sequencing (performed for 28 NF1 patients and all NFNS cases). Novel variants were tested in silico using Alamut package (Interactive Biosoftware) and on-line available tools (eg. Human Splicing Finder).

Results and discussion

In 4 patients with NF1 clinical diagnosis, the large deletion (≈1,29Mb) of chromosome 17 encompassing NF1 and neighboring genes was detected. A smaller deletion of exons 1-12 of NF1 gene was found in one patient and her affected relatives. The NF1 sequence analysis revealed the presence of 23 variants in 19 NF1 and 4 NFNS patients. Nine variants have not been previously described, but the co-segregation analysis in families and/or in silico tests supported their pathogenic character.

Conclusion

The sequencing analysis revealed the presence of point mutation in 19/28 (68%) NF1 and 4/9 (44%) NFNS patients. Although the sequencing method followed by in silico analysis seem to be effective in the mutation identification, further in vitro analysis of novel variants, especially these affecting splicing, is necessary to verify their functional effect. In 5 out of 70 cases (7.14%) the NF1 deletion was detected suggesting that MLPA is valuable method in NF1 diagnosis.

The work was supported from the National Science Centre (UMO-2011/01/D/NZ5/01347 and UMO-2013/09/B/NZ2/03164).

Role of the SAM domain of ANKS6 and its interacting partners in cystic kidney disease

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BACKGROUND:

We have demonstrated that a missense mutation in the SAM domain of Anks6 induces renal cysts in the cy/+ rat. Homozygous rats died at 4 weeks. Heterozygous rats displayed a slow progression of the disease leading to death after 1 year. Approximately 75% of the cysts derived from the proximal tubule. Cysts in the liver and pancreas were also observed in about half of old-affected females. The objective of this study is to further investigate the role of Anks6 in the pathogenesis of PKD by using a mouse model.

METHODS:

We identified in a library of ENU treated mice a mouse carrying a mutation in the SAM domain of Anks6, six amino acid away from the PKD-causative mutation in the cy/+ rat strain. We rederived the mouse, transferred the mutation on a C3H background by 7 successive backcross breedings and analysed the mutant mouse phenotype.

RESULTS:

Anks6 was detected in cilia of normal and cystic tubules. A very slow progression of the disease is observed in homozygous mutant mice which die after 16 months. Mice heterozygous for the mutation do not display any cysts. Cysts are detected in glomeruli and also in different nephron segments in cortex, medullary and papilla. Immunohistochemical markers showed that cysts derive from collecting ducts and thick ascending limb of Henle's loop, whereas only few cysts were observed in proximal tubules. We could not detect cysts in other organs, such as liver and pancreas. No differences in cyst origin, cyst size and cyst number were noticed between males and females. we provide the first evidence *in vivo* of an interaction between ANKS6, ANKS3 and BICC1 in the rat and in the mouse. We show in vivo defective interaction of ANKS6I747N with BICC1 in the mouse and ANKS6R823W with ANKS3 in the rat, which most likely explains the different kidney phenotype observed in the two models.

CONCLUSIONS:

This new mouse model provides unambiguous evidence of the role of Anks6 mutations in PKD. Comparison of PKD/Mhm(Cy/+) rat and of our Anks6 (I747N) mouse model further shows that the two models display noticeably different PKD phenotypes and that cystic enlargement is due to defective interaction with different protein partners, ANKS3 and BICC1, respectively in the rat and in the mouse. Our work suggests important roles for ANKS6, ANKS3 and BICC1 interacting molecules and provides the basis for future investigation on the function of the SAM domain of ANKS6.

Multiple CNVs identified in a patient with speech and psychomotor development delay, short stature, underweight and dysmorphic features.

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We present a 4-year-old boy, born spontaneously at 39 weeks of gestation to non-consanguineous healthy parents. The pregnancy was complicated by the intraamniotic infection. His birth weight was 2530 g (<5 centile), body length 51 cm (75 centile) and Occipito-Frontal Circumference (OFC) 31 cm (<5 centile).

He presented at age of 2 years with subtle dysmorphic facial features (thin, arched eyebrows, prominent glabella), speech and psychomotor development delay, failure to thrive and four café au lait spots. Brain MRI showed pons hypoplasia. In addition, neuronal migration disorder was suspected.

On physical examination he presented growth retardation with body height 82,6 cm (-2,3SD), body weight 8,9 kg (-2,7SD), and head circumference 46,5 cm (-2,3SD).

A karyotype analysis in peripheral blood lymphocytes revealed a suspicion of structural aberration in chromosome 5p15.3 region.. Array-CGH (CytoSure, ISCA 8 x 60 K v2.0, OGT) analysis showed a ~4,5 Mb terminal deletion of chromosome bands 5p15.33p15.32, ~4,3 Mb duplication of chromosome bands 9p24.3p24.2 and ~800 kb deletion at chromosome 3p26.3 containing the *CNTN6* gene. The *CNTN6* gene may play a role in the formation of axon connections in the developing nervous system. 3p26.3 deletion was found in patient`s mother, who is asymptomatic. The deletion at chromosome 5 and duplication at chromosome 9 arose *de novo* which confirms relationship with ascertained pathology.

In addition, the terminal deletion at 5p15.33p15.32 overlaps the critical region of Cri du Chat syndrome, that explains patient`s speech delay.

So far, no other patient with the same chromosomal aberrations has been reported.

Two cases of sizable deletions in the 11qter and 6q24q25 chromosomal regions inherited from parents with no major health issues

Ana Blatnik, Andreja Zagorac, Danijela Krgovic, Faris Mujezinovic and Nadja Kokalj-Vokac

Jacobsen syndrome (OMIM #147791) and 6q24-q25 deletion syndrome (OMIM #612863) are both well-defined contiguous gene deletion syndromes. Jacobsen syndrome is caused by a 5-20 Mb deletion of 11qter and is typically associated with growth retardation, developmental delay, trigonocephally, typical facial features (epicanthus, hypertelorism, ptosis, broad nasal bridge, short nose with anteverted nostrils, carp-shaped upper lip, retrognathia, and small low-set ears), various heart, skeletal and genitourinary malformations as well as thrombocytopenia. Patients with deletions in 6q24q25 often present with a heart defect, prenatal IUGR with postnatal short stature, redundant skin and dysmorphic facial features including a triangular face, frontal bossing, short palpebral fissures, thin upper lip and long and flat philtrum. In both syndromes the vast majority of causative deletions arise de novo or from balanced parental rearrangements, with rare instances of deletions inherited from parents with a similar phenotype. Here, we report on two cases of pathogenic deletions in the 11q24.2q25 and 6q24.3q25.2 chromosomal regions, both inherited from mothers with mild dysmorphic features, no major malformations or health issues related to the disorders and no history of serious developmental difficulties. The 7.7 Mb deletion involving 11q24.2q25 was detected in a new-born boy with a low birth weight, dysmorphic features (abnormal head shape, epicanthus, low-set and dysplastic ears), renal pelvis dilatation and thrombocytopenia. The 6 Mb deletion of 6q24.3q25.2 was found prenatally in a fetus with tetralogy of Fallot. No additional malformations were seen on ultrasound. Cases such as these highlight the importance of testing seemingly unaffected parents even in cases of large, cytogenetically detectable deletions/duplications. Information regarding parental status might prove crucial

particularly in cases of prenatally established diagnoses. Also, our cases clearly demonstrate the great phenotypic variability that exists between carriers of identical chromosomal aberrations within the same family.

Homozygosity mapping using SNP microarray as a useful diagnostic tool in consanguineous populations

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Introduction: Consanguinity increases the coefficient of inbreeding and the likelihood of the presence of pathogenic mutations in homozygotic state. An SNP-based microarray is a useful tool not only for studying copy number variants but also for the detection of uniparental disomy and regions of homozygosity (ROH) throughout the genome. Evaluation of ROH can guide medical geneticists to focus on a single specific gene in order to achieve molecular diagnosis in families with common parental ethnic background or consanguinity. In addition, ROH analysis can eliminate genes as possible candidates in highly heterogeneous recessive conditions.

Methods: We describe six cases in which analysis of SNP-based microarrays (Illumina HumanOmniExpress-24 v1.0 BeadChip) has assisted us reaching molecular diagnosis in consanguineous families by sequencing a single candidate gene that was localized within homozygosity region.

Results: We identified a disease-causing mutation in all the families. All the genes are related to metabolic or neurological disorders and include: CRLF1 (Crisponi syndrome/CISS1 syndrome), PHKG2 (Glycogen storage disease IXc) NPC1 (Niemann-Pick C disease), MPV17 (hepatocerebral mitochondrial DNA depletion), MAN2B1 (Alpha-mannosidosis), and MOCS2 (Molybdenum cofactor deficiency).

Conclusions: These examples demonstrate the great diagnostic value of SNP microarray in deciphering molecular defect in consanguineous families by minimizing the need for massive parallel sequencing and reducing the cost of the molecular work-up.

Impact of late amniocentesis in the era of genomic technology

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Introduction: Amniocentesis is a conventional tool for obtaining fetal genetic information. The traditional timing for amniocentesis is between 17-23 weeks of pregnancy. This timing enables decisions related to future of pregnancy before viability. Less frequently, third trimester amniocentesis is utilized due to late onset of abnormal sonographic findings.

The uprising genomic technologies introduce far more detailed information about the fetus compared to the number and structure of choromosomes, as obtained by traditional karyotyping.

Aim: To assess the indications for late amniocentesis, complications, genetic results and decision making process of couples undergoing such procedures.

Methods: : The medical records of 67 pregnant women who underwent third trimester amniocentesis since June 2013 till September 2014 were analyzed and followed by characterizing of newborn outcome.

Results: Sixty seven women (68 fetuses) underwent late (28+3 till 38+3 weeks) amniocentesis. The genetic test used was chromosomal-microarray-analysis (CMA). Main indications included newly-appearing abnormal sonographic findings (51 pregnancies, 76%), 5 suspected CMV infections (0.7%), 2 cases with soft markers (0.3%) and 2 cases with abnormal biochemical screening (0.3%). Most of the abnormal sonographic findings were cardiovascular, IUGR, macro/microcephaly and poly/oligohydramnion or combination of two of the above. Complications included 4 (6%) preterm deliveries. There were no reported cases of chorioamnionitis nor intrauterine fetal death (IUFD). Results: Most women (54, 78%) received normal array results. Fourteen (20%) fetuses had abnormal array result. Of these, 6 fetuses had trisomy 21. The other 8 fetuses had copy number changes which could not have identified using traditional karyotyping (including 15q (Prader Willi/ Angelman syndrome), 1q21 microdeletion syndrome, 1q41 microdeletion syndrome, and Xq23 microduplication). In two cases (3%) the significance of the finding was unknown (VOUS) , one of which was paternally inherited from a healthy parent.

Conclusions: Third trimester amniocenteses, although safe, pose a great challenge for the treating physician, and women undergoing testing, given the complexities and uncertainties unraveled. in light of uprising genomic technology. Pre-test genetic counselling is of utmost importance, to ensure a comprehensive preparation of women/parents for the test's potential findings and setting the stage so the type of results may meet their expectations . Due to CMA's increased detection rates and shorter turnaround time late amniocentesis in the era of genomic technology may serve as an extremely helpful tool for detecting

abnormalities and reassure parents in light of late appearing sonographic findings, yet may unravel uncertainties for which the couple should be prepared.

More clinical overlap between 22q11.2 deletion syndrome and CHARGE syndrome than often anticipated

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CHARGE and 22q11.2 deletion syndromes are variable, congenital malformation syndromes that show considerable phenotypic overlap. We further explore this clinical overlap and propose recommendations for the genetic diagnosis of both syndromes.

We describe two patients clinically diagnosed with CHARGE syndrome, who were found to carry a 22q11.2 deletion and additionally found 3 patients in the literature. The other way round, we identified typical 22q11.2 deletion features in 3,7% of our cohort of *CHD7* mutation carriers (n=30/802). And we analysed *CHD7* in 20 patients with phenotypically 22q11.2 deletion syndrome, but without haploinsufficiency of *TBX1* and identified pathogenic mutations in five of them.

These results indicate CHD7 and TBX1 share a molecular pathway or have common target genes in affected organs. Clinical and molecular overlap between CHARGE and 22q11.2 deletion syndrome isn't an isolated observation, we know examples of other syndromes through literature.

We conclude that differentiating between CHARGE and 22q11.2 deletion syndromes can be challenging. Based on our results we strongly recommend performing *CHD7* analysis in patients with a 22q11.2 deletion phenotype without *TBX1* haploinsufficiency, and conversely, performing a genome-wide array in CHARGE syndrome patients without a *CHD7* mutation.

ANALYSIS OF TGFB1 C-509T AND G-800A PROMOTER POLYMORPHISMS IN ASTHMA

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BACKGROUND

Asthma is a complex lung disease with onset and development resulting from interactions between genetics and environmental factors. Transforming growth factor-beta 1 (TGFB1) is a multifunctional cytokine that plays an essential role in asthma airway inflammation and remodelling. The role of TGFB1 gene promoter polymoprhisms in asthma remains unclear. The aim of this study was to analyze TGFB1 gene promoter polymorphisms C-509T and G-800A in Serbian asthmatics.

METHOD

The study has encompassed two groups of patients, 102 adults and 37 children, as well as control group of 58 healthy individuals. DNA extracted from peripheral blood was analyzed for the presence of TGF-B1 promoter polymorphisms by PCR and DNA sequencing. Statistical analysis was performed using Chi-square test (SPSS 20.0). P value less than 0.05 was considered statistically significant.

RESULTS

The distribution of C-509T and G-800A genotypes was similar in all analyzed groups (Table 1).

Table 1. Distribution of TGFB1 C-509T and G-800A polymorphism genotypes in Serbian asthmatics and controls

Genotype	Adults	Children	Controls
-509CC	0.35	0.29	0.22
-509CT	0.40	0.29	0.36
-509TT	0.25	0.42	0.42
-800GG	0.89	0.92	0.83
-800GA	0.09	0.05	0.13
-800AA	0.02	0.02	0.04

The frequencies of-509T and -800A alleles were slightly increased in control group (0.60 and 0.10) in comparison to both patients groups, adults (0.45 and 0.06) and children (0.55 and 0.05), but the differences were not statistically significant.

CONCLUSION

In Serbian asthmatics and healthy controls similar distribution of genotypes was found for both polymorphic TGFB1 sites, C-509T and G-800A. The role of TGFB1 gene promoter polymorphisms as risk factors for asthma should be investigated in larger cohort of patients and controls.

Identifying different mechanisms underlying de novo mutations in humans

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De novo point mutations in humans have been recognized as an important source of genetic variation, contributing to genetic diversity and evolution, as well as a cause for disease.

Here we studied the mutational mechanism that give rise to de novo mutations (DNMs) in humans using Whole genome Sequencing data of 832 child-and-parent-trios. After applying a rigid filtering classifier we identified 40,323 high-confidence de novo mutations. We confirmed that increased paternal age gives rise to more DNMs, and also found a significant effect of maternal age. For 7,926 out of the 40,323 mutations we successfully performed phasing and determined the parental origin. Based on these phased mutations we again confirmed the parental age-effects. Mutational showers are multiple DNMs within the same individual within close proximity, likely caused by a single event. We identified 324 of these putative shower events. Comparison of nucleotide substitution frequencies showed significantly different profiles for shower events, supporting the idea of a different mutational mechanism. Our study suggests that different mutational mechanism may underlie the occurrence of de novo mutations.

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Design and optimization of a bioinformatics tool for the detection of large deletions and insertions (even CNVs) and large insertions/deletions in targeted next generation based panels

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It is well known that intragenic large deletions and insertions and copy number variation (CNVs) are a critical part of genetic variations that can lead to rare diseases. The next generation sequencing technologies have been evolved into the optimal strategy for unmasking variants underlying human genetic disorders. The advantages of the NGS approach include higher coverage and resolution, more precise detection of variants and the capability to identify, at the same time, large deletions and insertions. Taking these advantages into account, our unit (UDyTEMC) at Clinical University Hospital in Santiago de Compostela (Spain) has introduced 17 multigene panels based in NGS technology that enable simultaneous interrogation of dozens to hundreds of genes associated with pediatric Mendelian diseases. These panels are being offered to specialists

of complex paediatric patients from different paediatric units of our country with a turnaround time of 4-8 weeks. As part of this program, software for the automatic detection of this kind of mutations is being designed and optimized based on features extracted from NGS data (deep of coverage), for which there is no feasible software nowadays.

This informatics tool aims to compare the coverage of a test sample with control samples in some specified regions. For this, we get the coverage log-ratio for each base-pair in each region for both test and control samples. The null hypothesis follows a normal distribution with N(0,1) and we have considered a p-value <0.05.

In our study we have analyzed 195 samples and it was used 10 controls. To compare the samples between them it was used the same enrichment kit for both control and test samples, and they are both from the same sequencing run so the coverage profile can be as homogenous as possible. All the insertions-deletions from the control sample were identified, and further, we identified 6 novel deletions and 2 insertions of one to several exons in 8 patients; in one of them the insertion corresponded to a large duplication of a cluster of sodium channels genes previously identified through CGH array in patients with epilepsy. With these results, we want to demonstrate the importance of the development of these tools in the detection of critical genomic variants and understanding human CNVs in disease conditions will be a worthy endeavour for the development of personalized medicine in the future. Surely, more controls samples should be tested and quality tests must be applied to assess the diagnostic efficacy of this bioinformatic tool.

Use of Genetic panels for the Diagnosis of Neurometabolic disorders in paediatric patients

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Genomic research in neurodegenerative and metabolic disorders is essential for: 1) diagnosis, prognosis and treatment of these disorders, 2) the application of new preventive treatments and/or their inclusion in clinical trials of new drugs, 3) provide, within a reasonable period of time, a good family genetic counselling, and 4) avoid diagnostics odyssey.

The new sequencing technologies require us to leap toward a new medication and to a new paradigm in which genomic tools will be essential in the diagnostic process of the patient and, in many cases for early diagnosis of diseases that could eventually only be diagnosed by clinical signs and symptoms developed by the patient.

In recent years, there has been an exponential growth of knowledge about the genetic basis of disorders. This exponential growth of knowledge is now one of the biggest challenges faced by health systems and physicians to their patients. For this reason, the construction of personal genomic skills is essential,

especially in a reference unit, and is based on already developed projects (diagnosis of lysosomal disorders, and monogenic diabetes by massive sequencing).

Since October 2012 we have developed and implemented in our unit a total of 17 genetic panels based on massive sequencing technology primarily concerned with the diagnosis of neuropaediatric and metabolic disorders. So far we have analyzed a total of 205 patient samples from up to 20 different hospitals in the country, reaching a diagnosis rate between ~40-45%. In this communication we intend not only to relate the results obtained with the application of these paediatric diagnostic panels, but also the challenges we need to face to achieve increase speed, efficiency and safety of these diagnostic tools.

Keywords: metabolic and neuropaediatric disorders, massive sequencing

Is cffDNA an adequate material for NGS study?

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Contemporary prenatal diagnosis is mainly based on the invasive methods and is associated with the risk of miscarriage. Maternal peripheral blood may be a new, noninvasive source of information about fetal genom. It includes the fetal cells and fragments of DNA, which leads to the formation of the natural microchimerism state during each pregnancy.

Cell free fetal DNA (cffDNA) consist of very short nucleic acid fragments circulating in the blood of the pregnant women. Its origin is not clear. This represents an average of 3-7% of the pool of free DNA in maternal plasma. cffDNA analysis is technically difficult. The method must detect trace amounts of fetal DNA in maternal mixture plasma. Current methodologies are not able to complete separate the fetal from the maternal DNA in one sample.

Recently, NGS sequencing technologies open new opportunities in cffDNA analysis. DNA molecules from maternal plasma are sequenced at random. It allows for relative quantification of DNA fragments. The proportional DNA molecules sequenced from the chromosome of interest are compared with those estimated from different chromosomes. It is possible to detect the extra material from fetal chromosome trisomy within the maternal plasma DNA.

Whole exome sequencing detected a novel PHF6 mutation as the cause of Börjeson-Forssman-Lehmann syndrome in four males in a Danish pedigree

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We report a Danish family with four boys with developmental delay aged 3-12 years. They were related as brothers/cousins with mothers being sisters. All four boys had normal birth weight, poor suck, hypotonia, developmental delay, no language, big ears, small penis and testes, tapered fingers, and broad distance between 1st and 2nd toes. The pedigree was consistent with X-linked inheritance. Whole-exome sequencing was performed by exome capture (AgilentSureselect) followed by sequencing of all exons and exon/intron boundaries at the X chromosome. A hemizygous mutation p.M1V in the PHF6 gene was found in all four boys and in heterozygosity in the two mothers and the grandmother. The mutation alters the start codon where a p.M1T mutation has been reported previously. Mutations in the PHF6 gene causes Börjeson-Forssman-Lehmann syndrome (BFLS). The mutation found in this family caused BFLS in the four affected boys with three unaffected carrier females.

BFLS is a rare X-linked mental retardation syndrome. BFLS was first described in 1962. Approximately 20 cases have been reported with mutations in the PHF6 gene. At least 12 different PHF6 mutations have been found in both familial and sporadic cases, predominantly missense and truncation mutations. Five recurrent mutations have been reported, which arose independently.

The main clinical features are normal birth weight, feeding problems and hypotonia in infancy, developmental delay, mild/moderate mental retardation, large ears, short toes, small genitalia, gynaecomastia, truncal obesity, tapered fingers, and coarsening of facial features.

BFLS might be underdiagnosed due to its rather unspecific phenotypic characteristics.

Evaluating the performance of clinical criteria to predict mismatch repair gene mutations in Lynch syndrome: A comprehensive analysis of 3671 families

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Introduction

Lynch syndrome is an autosomal dominant tumour predisposition syndrome caused by mutations in one of four mismatch repair (MMR) genes (MLH1, MSH2, MSH6 and PMS2). Carriers of MMR gene mutations have a high lifetime risk for colorectal (CRC) and endometrial cancer as well as other malignancies. As mutation analysis is costly and time consuming, clinical criteria and tumour tissue analysis are used as prescreening methods. The commonly applied clinical criteria are the Amsterdam criteria and the (revised) Bethesda guidelines. The aim of our study was to evaluate the performance of different clinical criteria to predict MMR gene mutations.

Patients and methods

We included 3671 families from the German HNPCC Registry fulfilling the Amsterdam I or II criteria or the original or revised Bethesda guidelines. The families were divided into nine mutually exclusive groups. Groups 1 and 2a-c comprised families with single affected individuals. Groups 3a-b, 4 and 5 are composed of families with a history of Lynch syndrome related cancer and group 6 consists of families with at least one colorectal adenoma under the age of 40 years without family history of cancer.

Results

A total of 680 families (18.5%) were found to have a pathogenic MMR gene mutation. Among all 1284 families with MSI-H the overall mutation detection rate was 53.0%. Mutation frequencies and spectrum were significantly different between clinical groups ($p<0.001$). The highest frequencies were found in families fulfilling the Amsterdam criteria (45.5% in group 3a and 53.8% in group 3b). Families with loss of MSH2 expression had a higher mutation detection rate (69.5%) than families with loss of MLH1 expression (43.1%). In the clinical groups with a positive family history as well as young age of onset (groups 3 and 4) mutations in MLH1 and MSH2 were predominant, while the proportion of MSH6 and PMS2 mutations was

increased in isolated tumour cases (group 2) and families with a higher age of onset (group 5). MMR mutations were found significantly more often in families with at least one MSI-H small bowel cancer ($p<0.001$). No MMR mutations were found in group 6 (colorectal adenoma under 40 years of age).

Conclusions

Familial clustering of Lynch syndrome related tumours, early age of onset, a positive MSI finding, and familial occurrence of small bowel cancer are good predictors for Lynch syndrome.

Calcineurin homologous protein 1, a Plastin 3 Binding Partner and Potential Modifier of Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA) is the second most frequent autosomal recessive disorder in humans (1) and the leading hereditary cause of infant mortality (2). Besides SMN2, whose copy number inversely correlates with SMA severity (3), plastin3 (PLS3) has been identified to be a phenotypic modifier of SMA (4). PLS3 overexpression (OE) has been found to protect individuals carrying the same homozygous deletion of SMN1 and identical numbers of SMN2 as their affected siblings. In in vivo and in vitro studies the beneficial effect of PLS3 OE on the SMA phenotype has been studied extensively. PLS3 has been shown to rescue axon length, improve neuromuscular junctions (NMJ), increase motor neuron soma size, increase levels of acetylcholine receptors and improve neurotransmission (5). Recently our group found that reduced SMN levels impair calcium influx and endocytosis (Riessland et al., revised version in preparation). Most interestingly, PLS3 OE in murine embryonic fibroblasts (MEFs) derived from SMA embryos rescued the impaired endocytosis (Peters et al., unpublished data). Although the rescue effect of PLS3 OE had been studied extensively the molecular mechanism behind it remains elusive. Binding partners of PLS3 were identified by mass spectrometry and yeast-two hybrid assay (Hosseini Barkooie et al., unpublished data). Co-immunoprecipitation and pull-down assays showed that the multifunctional Ca2+ binding protein calcineurin homologous protein 1 (CHP1) was a promising candidate, which is strongly interacting with PLS3. Detailed interaction studies revealed that the actin-binding domains of PLS3 are essential for the interaction with CHP1, whereas all domains of CHP1 are interacting with PLS3. Colocalization studies showed that CHP1 and PLS3 colocalize at lamellipodia of cells. CHP1 is a ubiquitously expressed protein (6). However, deficiency of CHP1 causes ataxia in mice (7). In line with that, a homozygous mutation in CHP1 (p.K18del) was found to be the underlying cause of autosomal recessive ataxia in a consanguineous family (Mendoza Ferreira et al., unpublished data). Since SMA is a neurodegenerative disease, we are mostly interested in the interface of neuronal and muscular systems. We found a high expression of CHP1 in neuronal tissue and a tendency towards increased CHP1 expression in brains of SMA mice. Motor neuron stainings showed a broad expression in the soma and the axon as well as a strong expression in the growth cone. To study the

modifying effect of CHP1 on the SMA phenotype we performed knockdown (KD) studies in NSC34 motor neuron-like cells. We found that Chp1 KD rescued the decreased neurite outgrowth in Smn depleted NSC34 cells. Since our studies indicate that the endocytosis machinery is impaired in SMA, endocytosis assay for clathrin-independent and clathrin-dependent receptor-mediated endocytosis were performed. Chp1 KD resulted in an immense increase of FITC dextran uptake via clathrin-independent endocytosis compared to control cells. Most outstandingly, it restored the impaired FITC dextran endocytosis in Smn depleted cells. Instead Chp1 KD had no effect on the clathrin-dependent endocytosis, suggesting that CHP1 restores endocytosis in SMN-deficient cells in a clathrin-independent manner. Taken together, our data confirm CHP1 as a potential modifier for SMA, and thereby help to unravel the molecular mechanism behind the rescue effect of PLS3 OE on the SMA phenotype.

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Mutation spectrum of GJB2 in central Iran

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Hearing loss is one of the most heterogeneous human diseases and occurs in 1 to 3 of 1,000 live births. Mutations in the *GJB2* gene and two deletions in the *GJB6* gene, del(GJB6-D13S1830) and del(GJB6-D13S1854), account for up to 50% of recessive deafness. In the present study we have identified the spectrum of *connexin 26* gene mutations in Iranian patients with moderate to profound nonsyndromic hearing loss (NSHL). The *GJB2* mutations were observed in 23.3% of the patient. Seventeen different mutations in *GJB2* gene were detected including two novel variants, p.I30L and p.W133G. p.I30L was identified in both healthy and affected members in two unrelated families. Therefore, it might not be pathogen. Compound heterozygous mutation of p.W133G /p.R184P was identified in two members of a family. *In silico* analysis predicted p.W133G mutation has deleterious effect on protein structure. The present study demonstrates that mutations in the *GJB2* gene, particularly 35delG, are important causes of NSHL in central Iran. In addition, it is shown structural analysis of novel mutations is useful in predicting the

pathogenicity of the novel mutation in medical genetics labs without equipment for functional assay of mutations.

Allelic Imbalance of Gene Expression of Multiple Sclerosis Susceptibility Genes IKZF3 and IQGAP1 in Human Peripheral Blood

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Multiple sclerosis (MS) is a chronic inflammatory, demyelinating disease of central nervous system (CNS). The cause of MS is largely unknown, but genetic and environmental factors, as well as interaction of these contribute to disease development. Recent genome-wide studies have revealed 110 single nucleotide polymorphism (SNPs) associated with susceptibility to MS, but their functional contribution to disease development is not known. Measure of relative expression levels from two SNP alleles of a gene in the same sample is a powerful approach for identification of *cis*-acting regulatory variants. We selected three genes, *CD69, IKZF3* and *IQGAP1*, with an MS associated SNP in their coding region or in complete linkage disequilibrium (LD) with a coding SNP and performed allelic imbalance (AI) expression analyses in whole blood samples from individuals heterozygous for the studied SNPs. AI was consistently observed for rs90709 in *IKZF3* and rs11609 in *IQGAP1*, which are in strong LD with the MS associated rs12946510 and rs3539, respectively. The MS risk allele correlated with increased *IKZF3* and reduced *IQGAP1* expression both in samples from MS cases and healthy controls. Individuals homozygous for the risk allele had a significantly reduced expression of *IQGAP1* compared to individuals homozygous for the protective allele. Our data indicate a possible gene regulatory role for MS-associated *IKZF3* and *IQGAP1* gene variants. This study highlight the usefulness of AI measurements to identify disease-associated SNPs or SNPs in LD with gene variants with *cis*-acting roles in gene regulation and suggests that the expression level of *IQGAP1* and *IKZF3* could explain the influence of their MS associated SNPs for MS susceptibility.

Fusion genes in Acute Myeloid Leukemia in children. Next-generation RNA sequencing - the way to the accurate diagnosis of the rare variant.

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Acute Myeloid Leukemia (AML) in children is a rare and heterogeneous disease, with an incidence of 7 cases per million children younger than 15 years. Despite major improvements in outcome over the past decades, it remains a life-threatening malignancy in children, with current survival rates of about 70%.

Collaborative research in childhood and adult AML has identified several prognostic factors variably used for risk stratification, among which one of the most relevant are chromosomal abnormalities and gene mutations. Properly conducted diagnostics of genetic abnormalities occurring in leukemic blasts is the basis for confirming the diagnosis and for selection of the appropriate treatment in many European therapeutic protocols. Besides the presence of the most common chromosomal rearrangements like t(8;21)(q22;q22)/RUNX1-RUNX1T1, inv(16)(p13.1q22)/CBFB-MYH11, t(15;17)(q22;q21)/PML-RARA, t(9;11)(p22;q23)/MLLT3-MLL that occur in a total of about 33-39% cases of pediatric AML, there is a large number of rare genetic aberrations, not detectable by routine cytogenetic and molecular genetics tests. This AML cases are classified into a normal-karyotype AML subgroup. Different outcomes of this AML subgroup suggest that it could be genetically heterogeneous. But lack of genetic markers makes it difficult for further study.

Next-generation RNA sequencing (RNA-Seq) is a helpful method for searching for potential aberrations in normal karyotype AML at the level of transcription. It is an alternative approach to genetic aberrations examination at the level of genomic DNA. RNA-Seq involves direct sequencing of complementary DNAs (cDNAs) using high-throughput DNA sequencing technologies followed by the mapping of the sequencing reads to the genome. Although RNA-Seq is still a technology under active development, it offers several key advantages over existing technologies. Unlike hybridization-based approaches, RNA-Seq allows identification of novel transcripts, including fusion genes transcripts. The importance of well-know as well as newly found fusion genes in myeloid leukemogenesis should be extensively studied both for their biological meaning and clinical utility.

Comparison of exome and genome sequencing platforms for the complete capture of protein coding regions

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For next-generation sequencing technologies sufficient base-pair coverage is the foremost requirement for the reliable detection of genomic variants. We investigated whether whole genome sequencing (WGS) platforms offer superior coverage of coding regions compared to whole exome sequencing (WES) platforms, and compared single-base coverage for a large set of different exome and genome samples (24 Agilent V4 (at 78x and 160x coverage), 12 Agilent V5 (100x), 12 NimbleGen V3 (95x), 24 Complete Genomics (44x and 87x), 11 Illumina HiSeq (28x), 12 Illumina X-Ten (40x)).

We find that WES platforms have improved considerably in the last years, but at comparable sequencing depth, WGS outperforms WES in terms of covered coding regions. At higher sequencing depth (95x-160x) WES successfully captures 95% of the coding regions with a minimal coverage of 20x, compared to 98% for WGS at 87 fold coverage. A comparison to published gene panel studies shows that these perform similar to WES and WGS in terms of coverage. Three different assessments of sequence coverage bias showed consistent biases for WES but not for WGS. We found no clear differences for the technologies concerning their ability to achieve complete coverage of 2,759 clinically relevant genes.

We show that WES performs comparable to WGS in terms of covered bases if sequenced at 2-3 times higher coverage. This does, however, go at the cost of substantially more sequencing biases in WES approaches, which may impact applications such as the identification of copy-number variants and somatic variation. Our findings will guide laboratories to make an informed decision on which sequencing platform and coverage to choose.

Exome sequencing in patients with Circumferential skin creases Kunze type: Evidence for locus heterogeneity

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Congenital circumferential skin creases are extremely rare and children born with this feature are referred to as 'Michelin tyre babies' based on the similarity with the mascot of the French tyre manufacturer. Some of these children have additional abnormalities including typical facial dysmorphism, cleft palate, short stature and intellectual disability. For this syndrome, our group proposed the term 'Circumferential skin creases Kunze type' (Wouters et al., 2011). So far, less than 10 cases have been described in the literature and all occurrences are sporadic. In an international collaboration we collected DNA samples from 8 patients with Circumferential skin creases Kunze type.

Exome sequencing was performed on the HiSeq2000 platform for two case-parent trios as well as two additional patients with this syndrome. Data analysis revealed the presence of pathogenic mutations in either one of two interacting genes, providing evidence for genetic heterogeneity. Three additional patients with the same phenotype have also been found to carry a mutation in one of these genes. While some patients carry a heterozygous de novo mutation, others present with homozygous mutations. Accurate genotype-phenotype correlations are being investigated. In addition, we are performing functional analyses at the protein level to elucidate the pathogenic mechanism of the mutations.

Further insight into the phenotype of ectodermal dysplasia associated with de-novo mutation of the EDAR gene

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Ectodermal dysplasia (ED) is a heterogeneous group of (nearly 20) disorders, characterized by lack or dysgenesis of at least two of the ectodermal derivatives, including hair, nails, teeth or sweat glands. Hypohidrotic ED (HED) is the most common form of ED, and is characterized by clinical triad of hypotrichosis (sparse hair), abnormal or missing teeth (anodontia or hypodontia) and deficient sweating (hypohidrosis or anhidrosis.

We present a 4 year old female, referred to our genetic clinic at the age of 2 years, due to lack of teeth development, sparse hair and fever episodes, which raised a question of Ectodermal Dysplasia (ED). Physical examination revealed two unusual clinical features: posterior cleft palate and hypoplastic uvula, and prominent natural course of ectodermal phenotypic improvement, in which at the age of 4.5 years, the scalp hair and eyebrows texture and density were less affected.

Her parents are distantly consanguineous, of Moslem Arab origin, and no other cases of ED reported in this family.

Genetic testing was performed using Whole exome sequencing, revealing a novel homozygous c.77C>T substitution mutation in the EDAR (EctoDysplasyn associated Receptor) gene, resulting in p.A26V change of the protein. This genomic change is suggested to result in disruption of the normal protein activity, using bioinphormatic prediction programs. Both parents were found to be heterozygote for this mutation.

NGS enabled the diagnosis of a rare form of ED in a single patient in a family. This allows targeted genetic counseling and testing other family members at risk. This finding allows us to speculate the association of c.77C>T mutation of EDAR with posterior cleft palate and significant phenotypic improvement as natural course of the disease, thus maybe expanding the spectrum of features of EDAR associated HED. Consequently, more diagnoses might be obtained in other affected individuals, yielding even better understanding of the phenotype-genotype correlation.

Carriers of V-LH among 1593 Baltic men have significantly higher serum LH

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Background: Luteinizing hormone (LH) is a pituitary heterodimeric glycoprotein essential in male and female reproduction. Its functional polymorphic variant (V-LH) is determined by two missense mutations (rs1800447, A/G, Trp8Arg; rs34349826, A/G, Ile15Thr) in the LH β-subunit encoding gene (LHB; 19q13.3; 1111 bp; 3 exons). Among women, V-LH has been associated with higher circulating LH and reduced fertility, but the knowledge of its effect on male reproductive parameters has been inconclusive.

Objective: To assess the effect of V-LH on hormonal, seminal and testicular parameters in the Baltic young men cohort (n = 986; age: 20.1 ± 2.1 years) and Estonian idiopathic infertility patients (n = 607; 35.1 ± 5.9 years).

Methods: V-LH was detected by genotyping of the underlying DNA polymorphisms using PCR-RFLP combined with resequencing of a random subset of subjects. Genetic associations were tested using linear regression under additive model and results were combined in meta-analysis.

Results: No significant difference was detected between young men and infertility patients for the V-LH allele frequency (11.0 vs. 9.3%, respectively). V-LH was associated with higher serum LH in both, the young men cohort ($p = 0.022$, allelic effect = 0.26 IU/L) and the idiopathic infertility group ($p = 0.008$, effect = 0.59 IU/L). In meta-analysis, the statistical significance was enhanced ($p = 0.0007$, resistant to Bonferroni correction for multiple testing; effect = 0.33 IU/L). The detected significant association of V-LH with increased serum LH remained unchanged after additional adjustment for the SNPs previously demonstrated to affect LH levels (FSHB -211G/T, FSHR Asn680Ser, FSHR -29A/G). Additionally, a suggestive trend for association with reduced testicular volume was observed among young men, and with lower serum FSH among infertility patients. The V-LH carrier status did not affect sperm parameters and other circulating reproductive hormones.

Conclusion: For the first time, we show a conclusive contribution of V-LH to the natural variance in male serum LH levels. Its downstream clinical consequences are still to be learned.

B4GALT7 Deficiency Causing Progeroid Ehlers Danlos Syndrome: Two New Patients Expand The Phenotype'

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Proteoglycans are components of the extracellular matrix with diverse biological functions. Defects in proteoglycan synthesis have been linked to several human diseases with common features of short stature, hypermobility, joint dislocations and skeletal dysplasia. B4GALT7 encodes galactosyltransferase-I that catalyses the addition of a galactose moiety on to a xylosyl group, in the tetrasaccharide linker of proteoglycans. Mutations in B4GALT7 are associated with the rare progeroid form of Ehlers Danlos syndrome (PEDS) and have been recently found to underlie Larsen of Reunion Island syndrome (LRS). The literature contains reports of nine cases of PEDS, four of whom have had molecular characterization, showing homozygous or compound heterozygous mutations in B4GALT7. We report two new, unrelated UK patients with compound heterozygous mutations in B4GALT7 expanding the phenotypic spectrum. Key features of this rare disorder include short stature, joint hypermobility, radioulnar synostosis, and severe hypermetropia. PEDS patients share a phenotypic spectrum with those with LRS although the key features of osteopenia and hypermetropia have not been reported in patients from Reunion Island. Progeroid features are not present in any of the reported patients with galactosyltransferase-I deficiency, questioning whether this is an appropriate name for this rare condition.

Identification of a synonymous variant as disease causing mutation in a DMD carrier

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A fifty year old woman was referred to our laboratory for analysis of the dystrophin gene. Her brother had died from Duchenne muscular dystrophy with unknown molecular cause. Based on elevated CK-levels in 1988 she had been identified as probable DMD carrier. After exclusion of a large deletion/duplication sequencing of the DMD coding region revealed the novel heterozygous variant: c.1329C>T. The substitution is synonymous, but as it is located near the end of exon 11, an effect on splicing was suspected. In order to prove or exclude such an effect transcript analysis was essential. A muscle biopsy was not feasible and the possibility of creating a lymphoblastoid cell line was lacking. Therefore RNA extraction from buccal and nasal epithelial cells was attempted. Even though full length dystrophin is not expected to be expressed in

these tissues, nasal epithelial cells, but not buccal cells, proved to be a good source of DMD mRNA for our purpose. An aberrant transcript not present in a normal control was identified, reamplified and sequenced. The aberrant transcript was found to lack the four last bases of exon 11 (r.1328_1331delGCAA) thus leading to a frameshift and a premature stop codon, p.(Ser443Ilefs*5). The results were in accordance with several in silico analyses. Taken together our findings yield convincing evidence, that c.1329C>T indeed is a disease causing mutation. Furthermore, nasal epithelial cells, which in contrast to muscle biopsies and blood samples can be obtained in a non-invasive way, proved to be a good alternative material for transcript analysis.

Identification and characterization of genetic modifiers of spinal muscular atrophy

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Proximal spinal muscular atrophy (SMA) is a common autosomal recessive neuromuscular disease and the most frequent genetic cause of infant death. A hallmark of this disease is the progressive degeneration of α-motorneurons in anterior horns of the spinal cord. Genetic causes of SMA are homozygous deletions or mutations of the *survival motor neuron 1* gene (*SMN1*). However, all patients carry a partially functional copy gene (*SMN2*) that mainly influences the severity of the disease (SMA type I (severe) to IV (mild)). In rare cases, siblings or parents of SMA patients carry homozygous *SMN1* deletion and only 3-4 *SMN2* copies, but are clinically asymptomatic, suggesting influence of protectivegenetic modifiers.

In a large discordant SMA family our group identified five asymptomatic and two SMA affected individuals all carrying homozygous SMN1 deletions. Transcriptome and linkage analysis led to the identification of a novel SMA protective modifier - Modifier 2 (*MOD2*) -, which was significantly downregulated in asymptomatic versus symptomatic or control individuals. We confirmed the protective effect of downregulated Mod2 on SMA background through *in vitro* approaches and SMA models of zebrafish, worm, and mouse. SMN depletion leads to defects in axonal outgrowth and also affects the maturation and maintenance of the neuromuscular junction (NMJ). Strikingly, *Mod2* downregulation itself led to increased axonal outgrowth and thus rescued S*mn* deficient neuronal cells in cell culture, as well as in mouse and zebrafish models. Moreover, immunofluorescence stainings of the *Transversus abdominis* (TVA) muscle of mice revealed that MOD2 is primarily localized at the presynaptic site at the NMJ suggesting MOD2 directly acting at misregulated structures in SMA.

Interestingly, a functionally closely related gene to *Mod2*, further referred to as *Mod3*, was also able

to restore axonal outgrowth in *Smn*-deficient murine NSC34 cells. A morpholino-mediated knockdown of *mod3* in zebrafish showed a mild axonal phenotype with increased axonal branching. *Mod3* downregulation in *smn* depleted zebrafish rescued the severe truncation phenotype comparable to the *mod2* rescued phenotype. Further studies will focus on molecular mechanisms of these modifiers rescuing the SMA phenotype and thus will also give further insight into which pathways are affected in SMA.

POMT2–related congenital muscular dystrophy – report of a clinical case and prenatal diagnosis in a Portuguese family

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Introduction:

Congenital muscular dystrophies are a group of rare genetic conditions presenting in infancy with a combination of muscular, central nervous system and ophthalmological manifestations. Clinical variability and genetic heterogeneity with incomplete genotype-phenotype correlation complicate molecular diagnosis of these conditions, and a complex diagnostic workflow is warranted in most cases.

Clinical case:

Twenty months-old female patient, first child of a non-consanguineous healthy couple, with an ongoing nine weeks pregnancy. Family history is irrelevant. The patient presented with delayed motor milestones and was referred to a Paediatric Neurology Service. No other clinical problems were apparent. Clinical examination revealed hypotonia, diminished muscle strength and reflexes, muscle atrophy except in calves, mild growth retardation with microcephaly and mild dysmorphic facial features. Previous initial investigation showed increased blood creatine kinase, twenty times above reference value. Muscle pathology showed dystrophic changes, predominance of type 1 fibres and absence of alpha-dystroglycan immunostaining. No major brain anomalies were detected. Given its frequency and the ongoing pregnancy, *LAMA2* sequencing was requested; the family was referred to a Genetics consultation. Because microcephaly has been described as being prevalent in *POMT1* and *POMT2*-related congenital muscular dystrophy patients, sequencing of both genes was additionally requested. A previously described pathogenic missense mutation was found in apparent homozigosity. Heterozigosity was confirmed in both parents and molecular prenatal diagnosis was performed revealing an affected foetus. Parents elected to terminate the pregnancy. To our knowledge this is the first *POMT2*-related congenital muscular dystrophy patient diagnosed in Portugal.

Discussion:

This case reflects the utility of establishing genotype-phenotype correlations in genetically heterogeneous diseases, when possible, to help guide genetic testing. Although new sequencing techniques are now becoming the main tool to identify the underlying gene mutated in each patient in these and other heterogeneous conditions, detailed clinical evaluation in a multidisciplinary context is very important and allows for a targeted approach to molecular diagnosis in some cases. Mutation identification enables patients and families to avoid recurrence of severe genetic conditions through genetic counselling, carrier testing, prenatal and preimplantation genetic diagnosis.

Integrating genomics across medical specialities

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Recent developments in genomics (such as the Genomics England 100,000 genomes project) mean that the medical workforce will need to acquire the relevant knowledge, skills and competence to effectively deliver care using genomic information. The Genomics in Mainstream Medicine Working Group was established in the UK under the auspices of the Joint Committee on Genomics in Medicine and a number of other organisations to raise awareness and to promote the integration of genomics into clinical practice across a wide range of clinical specialties. To facilitate this, 'clinical champions' have been recruited via the Royal College of Physicians speciality committees to help develop introductory resources as well as an action plan for promoting genomics within their own specialties. Work to date has included the development of a document 'template' for champions to adapt and use as an introductory resource as they see fit, and the 'mapping' of the educational landscape to identify genomics education initiatives for physicians that are already in place within the UK. Progress of this work so far and future objectives will be reported in the poster.

The better understanding of the molecular events associated with childhood cancers with molecular karyotyping.

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Cytogenetic analysis provides valuable diagnostic and prognostic information for the evaluation of cancer cells. Traditional and new techniques have been applied in clinical oncology with a variable range of resolution and sensitivity.

Conventional karyotype can evaluate the entire genome for changes in chromosome structure and number, but the resolution is relatively low. The detection limit for conventional banding techniques is 4 Mb, but for neoplasm cells this resolution is particularly low and rarely exceeds 5-10 Mb. Some structural chromosomal rearrangements are invisible in conventional karyotype. One of the many limitations of classical cytogenetics is the requirement for good quality metaphase preparations. Molecular karyotype has significantly higher, more than 10 times resolution than conventional karyotype. Additionally it can be done on fresh or formalin-fixed paraffin-embedded tissue without cell culture.

Molecular karyotyping is based on patient's genomic DNA and oligonucleotide microarray enabling genome-wide evaluation. Microarray may use non-polymorphic oligonucleotide probes or probes containing SNPs. Non-polymorphic probes can provide only copy number information, while SNP arrays can provide both copy number and loss-of-heterozygosity (LOH). Additionally, SNP array can detect also uniparental disomy (UPD). Acquired UPD is quite common in both hematologic and solid tumors and is reported to be responsible for 20 to 80% of the LOH seen in human tumors. UPD can be regarded as biological equivalent of a deletion. Molecular karyotyping has also limitation. It cannot detect balanced translocations, inversions and low-level mosaicism or small subclones of abnormal cells.

The higher coverage and resolution, more accurate estimation of copy numbers, more precise detection of breakpoints, and higher capability to identify novel CNVs (copy number variations) gives NGS (next-generation sequencing). NGS is a technology that parallelly sequences massive amounts of short DNA strands from randomly fragmented copies of a genome. A typical NGS run generate millions to billions of reads, which are assumed to be random representations of the targeted regions or the whole genome.

Increase levels of information on the genetic aberrations and the locations of important cancer genes can be clinically useful in cancer diagnosis and classification. In addition, it can contribute to our understanding of the molecules and processes that initiate childhood cancer.

Megalocornea associated with homozygous LTBP2 mutation p.R299X : a case report

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A 6-month-old boy, first child of healthy Romanian parents, was referred to our clinic because of megalocornea, spherophakia and suspected congenital glaucoma. No previous cases of eye abnormalities, glaucoma or blindness had been reported in the family. The child had been otherwise healthy, except for severe feeding difficulties requiring tube feeding for more than 3 months. CHRDL1 mutation analysis revealed no pathogenic mutations but LTBP2 analysis revealed a homozygous truncating mutation c.895C>T (p.R299X). This mutation has been previously described by Azmanov et al. (2011) as a founder mutation in the Roma/Gypsy population and it is believed to associate with a more severe phenotype and poorer outcome. The number of reported cases with this particular mutation is still relatively small, and to our knowledge, this is the first case ever diagnosed in Finland.

Discovering mobile element and gene retrocopy insertions in human genomes.

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Mobile elements (MEs), also known as transposons, are DNA sequences that can be autonomously copied or moved through the genome. MEs are major evolutionary drivers in changing the genomic architecture. One class of MEs – retrotransposons – still contain active copies in human genomes and the L1 subfamily can retropose transcripts of MEs or genes creating both mobile element insertions (MEIs) and gene retrocopy insertions. These insertions can directly result in pathogenic variation in a number of human diseases by inserting into functionally important regions and disrupting gene function, or indirectly by mediating copy number changes.

Polymorphic MEIs and gene retrocopy insertion polymorphisms (GRIPs) represent an interesting class of genomic variation, however detection of these elements is non-trivial because of their repetitiveness in the human genome. Therefore we have developed a method, called Mobster, to identify and genotype both MEIs and GRIPs in whole genome and exome sequencing data. The core of the method uses paired-end and split

read information by aligning part of a read (pair) against consensus sequences of MEs or transcript sequences, while the remainder is used to anchor the event in the genome.

Using Mobster, we screened a cohort of 231 trios and 19 quartets selected from the Dutch population, who were whole genome sequenced to a median sequencing depth of 14x. In total 12,397 MEIs were identified and an initial screening revealed 5 GRIPs. Per family, 1,588 MEIs (SD 63) were found with the majority being of *Alu* origin (82%). We could validate 92 out of 96 randomly selected MEIs and 6 *de novo* MEIs. A logistic regression model was used for genotyping, with the genotyping error rate estimated to be 3.3 – 6.4%. In addition common and rare exonic insertions in known disease genes were identified. One notable exonic *AluY* insertion found and validated in a single individual was located in *EYS*, a known retinitis pigmentosa (RP) gene, exactly overlapping with a previously described truncating mutation in a family with autosomal recessive RP. When screening an independent, exome sequenced, blindness cohort with Mobster a second individual was found carrying the same insertion in heterozygous state.

Due to developments in NGS it is now possible to perform genome wide identification of both MEI and GRIP events. The discovery of these events in large cohorts provides new insights into the role of genomic variation with positional effects and their implication in disease.

Targeted exome sequencing for the diagnosis of genetically heterogeneous mendelian disorders. Presentation of selected cases.

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Diagnosis of genetically heterogeneous mendelian disorders constitutes a major challenge in everyday clinical practice. The advent of high-throughput sequencing and exome sequencing in particular, has revolutionized the field of genetics, shifting the diagnostic procedure from sequential gene testing to more hypothesis-free approaches such as multi-gene panels, leading to a dramatic increase in the diagnostic yield for such disorders. To date, diagnostic rates of 15% up to 50% have been reported, depending on the investigated pathologies as well as the diagnostic approach utilized. Whole exome sequencing with targeted bioinformatic analysis of disease-specific gene panels is a particularly appealing strategy, allowing the detection of variants in clinically relevant genes and minimizing simultaneously the possibility of unsolicited findings inherent to the test. We present selected cases of genetically heterogeneous mendelian conditions, such as intellectual disability, epilepsies, ciliopathies, hereditary myopathies or Charcot-Marie-Tooth disease, resolved by targeted exome sequencing. Our experience is in line with the current literature,

suggesting that clinical exome sequencing is an efficient and cost-effective approach for the diagnosis of such disorders.

Identification of Novel Deafness Genes in Panels of Patients with a Specific Phenotype

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To date, whole exome sequencing (WES) is the main strategy to identify novel deafness genes in patients with hereditary hearing loss (HHL). Despite major progress in the elucidation of genetic defects using WES, the underlying defects in many cases with HHL remains elusive. This is mainly due to the enormous heterogeneity of HHL and the high frequency of isolated cases. For patients and/or the families, it is of great importance to establish a genetic diagnosis, because it enables counseling on recurrence risk, prognosis and rehabilitation options. Furthermore, knowledge on genetic causes of HHL and phenotype-genotype correlations may contribute to the development of new (gene) therapies or preventive measures.

We assume that identification of novel deafness genes could be more successful when WES or even Whole Genome Sequencing is performed in panels of patients with a similar phenotype. In this way, WES data from (isolated) patients with the same phenotype can be compared, thereby increasing the chance to elucidate the underlying genetic cause.

In the present nation-wide study, genetically undiagnosed patients with HHL from six university medical centers in the Netherlands are being investigated. Patients are grouped in panels with a comparable, specific phenotype, based on history taking, physical examination, audiovestibular symptoms and imaging of the inner ear. WES is being performed in all patients and, where possible, also in an affected family member. Per panel at least 30 patients are included, to enable detection of relatively rare genetic causes of HHL. We expect that this approach will successfully lead to identification of novel deafness genes.

ADCA-DN: A rare condition characterized by the association of Cerebellar Ataxia, Deafness and Narcolepsy - case report.

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Background: Autosomal dominant cerebellar ataxia, deafness, and narcolepsy (ADCA-DN) is a rare adult onset neurologic disorder caused by heterozygous mutations in the *DNMT1* gene on chromosome 19p13. To date, 7 ADCA-DN families have been recognized (Italy, USA, Sweden) with 4 distinct underlying missense mutations in the *DNMT1* gene. We report on a first Czech ADCA-DN family.

Methods: The index patient was screened for mutations in the the *DNMT1* gene by bidirectional Sanger sequencing of exons 20-22.

Results: We found a previously described missense mutation Ala570Val in the *DNMT1* gene. The carrier is a 60 years old man who has been affected with narcolepsy since the age of 41, showed first signs of hearing impairment at the age of 52, first signs of ataxia at the age of 55 and became deaf at the of 56. Similar affection has been documented in other 5 family members from 3 consecutive generations of the family.

Discussion: The Ala570Val has been previously detected in 2 Italian families and 1 family from the USA. This is the first report of a *DNMT1* gene mutation detected as a cause of ADCA-DN in the central European region.

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The effect of intranasal insulin on hippocampal long-term potentiation and activation of the insulin receptor pathway in Shank3-deficient mice: a pilot study.

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Introduction: Patients with Phelan-McDermid (22q13 deletion) syndrome show global developmental delay and autistic-like behavior. This is primarily caused by haploinsufficiency of SHANK3. *Shank3*-deficient mice show deficits in synaptic function and plasticity, social interaction and social communication. Since

intranasal insulin improves development and behavior in a pilot study of six children with Phelan-McDermid syndrome, we investigated the effect of intranasal insulin on long-term potentiation (LTP) and IGF-1/insulin receptor (IR)-pathway activation in *Shank3*-deficient mice.

Methods: We used C57BL/6 mice heterozygous for a deletion of the Shank3 ankyrin repeat domains (n=11) and wildtype littermates (n=11). Intranasal Humuline Regular® or saline was administered to non-anesthetized awake mice over a 2-week period. LTP was measured by extracellular recordings of field excitatory postsynaptic potentials (fEPSPs) from the hippocampal stratum radiatum in area CA1. Activation of the insulin receptor pathway was detected by Western blot.

Results: We observed a 20% increase in the magnitude of LTP at 90 minutes of tetanic stimulation in *Shank3*-heterozygous mice treated with intranasal insulin as compared to heterozygotes treated with saline. We also found a higher expression of the insulin receptor precursor and higher phosphorylation of Akt in *Shank3*-heterozygous mice treated with intranasal insulin as compared to heterozygotes treated with saline.

Conclusion: We observed a trend in improvement of LTP and in activation of the IR-pathway after intranasal insulin treatment in *Shank3*-deficient mice. These experiments are the first attempts in identifying the effects of intranasal insulin at a more fundamental level. An ongoing clinical trial in the UMC Groningen is aimed at investigating the effect of intranasal insulin on development and behavior in children with Phelan-McDermid syndrome.

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