European School of Genetic Medicine

29th Course in

Medical Genetics

Bertinoro, Italy, May 8-12, 2016

University Residential Centre
Via Frangipane, 6 – Bertinoro

Course Directors:

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29th Course in

Medical Genetics

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9.50 – 10.40	Epigenetics and disease **K. Temple**
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11.10 – 12.00 Long range effects
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12.00 – 12.50 Behavioral genetics
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14.30 –16.00 Concurrent Workshops

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16.30-18.00 **J. Burn** - "Dealing with rarity in the clinic"
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9.00 – 9:50 Inherited cancer and prospects for therapy
J. Burn

9.50 – 10.40 SMA: From gene and modifiers to therapy
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14.00 –14.30 Poster Viewing Session

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Thursday, May 12

Morning Session: **The returns of Genomic Medicine**

9.00 – 9.45	Best Posters presentations by students
9.45-10.15	Screening in Iran **S. M. Akrami** (TUMS and Ministry of Health and Medical Education, Iran)
10.15 – 10.45	**Coffee Break**
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12:15 - 12:30	Wrapping up
12:30	Hand in evaluations; hand out certificates
12:45	**Lunch**
14:30	*Discussion groups with high school students and ESGM students (Guglielmo Project - see next page)*

Departure

THE GUGLIELMO PROJECT

LAUNCHED BY THE EUROPEAN SCHOOL OF GENETIC MEDICINE IN BERTINORO, ITALY

FOR HIGH SCHOOL STUDENTS

Since 1988 thousands of young geneticists, especially physicians and biologists, have been trained by the European School of Genetics Medicine (ESGM). They came from every side of Europe, from the southern rim of the Mediterranean sea, from the Middle East and from several other countries.
Taking into consideration only the period 2001-2011 the participants to the ESGM advanced training courses have been 6000. Most of the courses took place at the Centro Universitario di Bertinoro di Romagna. ESGM and Bertinoro have thus become the most important European training center for young graduates in the medical genetic field and for this reason ESGM is recognized and sponsored with scholarships by many Universities across Europe and by the European Society of Human Genetics.In 2016 ESGM and Bertinoro will start a completely new experience for the communication in genetics.
Forty students of the last year of the High School from Forlí (Emilia-Romagna region) in addition to students from Sardinia and students from Iran will be hosted in Bertinoro to attend the last morning lecture of the 29th COURSE IN MEDICAL GENETICS on Thursday May 12th as well as some of the lectures of the previous days. One of the Faculty of the course, the Nobel Prize Prof. Mario Capecchi, will give a talk on the public understanding of genetics directed to the general public on May 9 at 5:00 pm in Forlì.

On May 12th the ESGM students of the European School of Genetics Medicine will become the "instructors" of the High School students for debates, discussions and questions regarding medical genetic research and practice in the contemporary world. In particular in the morning of May 12th the students will attend the lecture of Prof. Dagan Wells from Oxford on preimplantation genetic diagnosis. In the afternoon they will divide into different discussion groups to deal with topics like the thalassemias and others chosen by the students themselves.

Why Sardinian and Iranian students?
During the last 40-30 years several programs for thalassemia prevention have been successfully developed in Sardinia and Iran and it will be of great interest to discover the perception that Sardinian and Iranian students have about questions raised by their national prevention strategies.
Why the Project is named after Guglielmo?
Guglielmo Dall'Ongaro, a native of Rome, was a young brave man who died in August 2015 , at the age of 23, after a long fight against a genetic disease which was diagnosed too late. Early diagnosis, might have saved him.

Which are the goals of the *Guglielmo Project*?
- To increase the knowledge about human genetics and genetic disease among the young
- To test new communication tools between physicians and users of genetic medicine
- To introduce new high-qualification courses for students from secondary school
- To strengthen the cooperation between university and school networks.

The promoters of the *Guglielmo Project* are:
- European School of Genetic Medicine and the Italian Society of Human Genetics
- The Fondazione Cassa di Rispamio di Forlì
- The Centro Residenziale Universitario di Bertinoro and the Bertinoro City Council Administration
- High Schools from Forlì (Italy), from Sardinia, from Teheran and from other regions of the world which intend to participate in the project.

ABSTRACTS OF LECTURES

Sunday, May 8

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.

Ref: Brunner HG, van Driel MA. From syndrome families to functional genomics. Nat Rev Genet. 5:545-551,2004.

Arrays and CNVs

Eva Klopocki
Institute for Human Genetics, Biozentrum, University of Würzburg, 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

Stankiewicz P, Lupski JR. Structural variation in the human genome and its role in disease. Annu Rev Med. 2010;61:437-55.
Watson et al. The genetics of microdeletion and microduplication syndromes: an update. Annu. Rev. Genomics Hum. Genet. 2014.15:215-44
Miller et al. Am J Hum Genet. 2010.86(5)749-64.

Next Generation Sequencing basics

Alexander Hoischen
Department of Human Genetics, Radboud University Medical Center, Nijmegen, 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 and discuss its development and advantages over traditional sequencing technologies.

Recommended reading:

1. Shendure J, Ji H. Next-generation DNA sequencing. Nat Biotechnol 26: 1135-45 (2008).
2. Zhou X, Ren L, Meng Q, Li Y, Yu Y, Yu J. The next-generation sequencing technology and application. Protein Cell 1: 520-36 (2010). Review.
3. Tucker T, Marra M, Friedman JM. Massively parallel sequencing: the next big thing in genetic medicine. Am J Hum Genet 85:142-54 (2009). Review.
4. Ashley EA, Butte AJ, Wheeler MT, Chen R, Klein TE, Dewey FE, Dudley JT, Ormond KE, Pavlovic A, Morgan AA, Pushkarev D, Neff NF, Hudgins L, Gong L, Hodges LM, Berlin DS, Thorn CF, Sangkuhl K, Hebert JM, Woon M, Sagreiya H, Whaley R, Knowles JW, Chou MF, Thakuria JV, Rosenbaum AM, Zaranek AW, Church GM, Greely HT, Quake SR, Altman RB. Clinical assessment incorporating a personal genome. Lancet 375: 1525-35 (2010).

How to deal with next generation sequencing output

Christian Gilissen
Nijmegen Centre for Molecular Life Sciences
Radboud University Nijmegen Medical Centre,
The Netherlands

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]

[1] Unlocking Mendelian disease using exome sequencing. Gilissen C, Hoischen A, Brunner HG, Veltman JA. Genome Biol. 2011 Sep 14;12(9):228. doi: 10.1186/gb-2011-12-9-228. Review. PMID: 21920049
[2] Diagnostic exome sequencing in persons with severe intellectual disability. de Ligt J, Willemsen MH, van Bon BW, Kleefstra T, Yntema HG, Kroes T, Vulto-van Silfhout AT, Koolen DA, de Vries P, Gilissen C, del Rosario M, Hoischen A, Scheffer H, de Vries BB, Brunner HG, Veltman JA, Vissers LE. N Engl J Med. 2012 Nov 15;367(20):1921-9. PMID: 23033978
[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
[4] Disease gene identification strategies for exome sequencing. Gilissen C, Hoischen A, Brunner HG, Veltman JA. Eur J Hum Genet. 2012 May;20(5):490-7. doi: 10.1038/ejhg.2011.258. Epub 2012 Jan 18. Review. PMID: 22258526

Monday, May 9

Complex Disordes

Andrew Read
St Mary's Hospital, Manchester, UK

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.

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

B. Wollnik, MD
Institute of Human Genetics, University of Göttingen, 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.

Tuesday, May 10

Ethics of Medical Genetics

Andrew Read
St Mary's Hospital, Manchester, UK

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, *FAP* type 2; colorectal adenomatous polyposis, autosomal recessive, with pilomatricomas	132600						
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	604772			*RYR2*	180902	AD	KP

ventricular tachycardia							
Arrhythmogenic right-ventricular	609040	20301310	Child/adult	*PKP2*	602861	AD	KP and EP
Cardiomyopathy	604400			*DSP*	125647		
	610476			*DSC2*	125645		
	607450			*TMEM43*	612048		KP
	610193			*DSG2*	125671		KP and EP
Romano–Ward long QT syndrome	192500	20301308	Child/adult	*KCNQ1*	607542	AD	KP and EP
types 1, 2, and 3, Brugada	613688			*KCNH2*	152427		
syndrome	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	145600	20301325	Child/adult	*RYR1*	180901	AD	KP
susceptibility				*CACNA1S*	114208		

Epigenetics and disease – lessons from imprinting disorders

I. K. Temple

Prof of Medical Genetics and Honorary Consultant in Clinical Genetics, Faculty of Medicine, University of Southampton

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. The major overlapping features in most of these conditions (except Angelman) are disordered fetal growth, either excessive or

restricted growth, neurodevelopmental delay (aberrant behaviour) and disordered metabolism. Diagnosis can be difficult as many of the features are non-specific and there are few congenital anomalies. Furthermore it is now well recognised that some patients have involvement of many imprinting loci simultaneously termed MLID (multilocus imprinting disturbance)

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, NLRP2, NLRP5 as a genes essential for DNA methylation maintenance.

www.imprinting-disorders.eu for information on the COSt network for imprinting disorders with many references available.

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 in gene regulation

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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/

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 11

Inherited cancer and prospects for therapy and prevention

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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. The biggest risk factor is age. Somatic analysis can help target key pathways and stratify treatment. For example, Guinney et al (Nature medicine 2015; 21(11): 1350-6) .have distinguished 4 categories of colorectal cancer which have different prognosis and response to intervention.
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. Exciting recent developments include PD1 blockade and PARP inhibitors. 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. PD1 blockers unleash the immune system and are selectively lethal to the CMS1 category of colorectal cancer where mismatch repair deficiency leads to the accumulation of mutations and susceptibility to immune attack.
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.

Thursday, May 12

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

Hypomelanosis of Ito: A case report

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Introduction

Hypomelanosis of Ito (HI) was first described by Ito in 1951. It is a multisystemic neurocutaneous disorder with the distinctive feature of hypopigmented skin areas following the lines of Blaschko, associated with variable phenotypic manifestations.

HI is the phenotypic result of a post-zygotic chromosomal or monogenic event rather than being a distinct disease. HI is usually sporadic, although different modes of inheritance have been reported.

Case presentation

A 2 years old girl was referred to the Genetics Department at the 36th day of life with multiple congenital anomalies. On physical examination she was found to have failure to thrive, unspecific facial dysmorphisms, plagiocephaly, cleft palate, bifid uvula and macular hypopigmented whorls and patches over the trunk and limbs along the Blaschko lines.

In the first months of life she developed supratentorial obstructive hydrocephalus due to Chiari malformation and lambdoid synostosis. An echocardiogram revealed: atrial septal defect, patent foramen ovale with left-to-right shunt and a pulmonary valvular stenosis. Eye examination showed bilateral blepharophimosis, ptosis, telecanthus and epicanthus with microcornea, without papilloedema.

During follow-up a mild developmental delay was documented.

Array-Comparative Genomic Hybridyzation in peripheral blood lymphocytes and in cultured fibroblasts from a skin biopsy detected a clinically significant terminal duplication at 6p25.3-p22. The duplication was found in DNA extracted from fibroblasts, but not from peripheral blood.

Conclusions

A large number of cases of HI remain underdiagnosed in part due to different cytogenetic/molecular etiologies and wide phenotypic variability. In 75% of the cases it involves anomalies of the central nervous, ocular, cardiac, genitourinary and musculoskeletal systems. The prognosis is determined by the associated extracutaneous abnormalities and multidisciplinary follow-up is needed in these patients.

This case emphasizes the value of a cutaneous biopsy in the pediatric population with clinical features suggestive of HI for diagnostic confirmation, avoiding additional cost and time consuming etiological investigations.

South African women's perspective on warfarin and pregnancy

Maureen Conradie

Babies born with potentially preventable birth defects due to teratogens, including chronic medication with teratogenic potential, are regularly seen at genetics clinics and by pediatricians in South Africa. This increases the burden on the already struggling health care system, as well as causing considerable morbidity and mortality for the families involved.

A study was conducted at an academic hospital in Bloemfontein, South Africa, in 2016. The aim of the study was to evaluate the knowledge, attitude and practice of women in the reproductive age group that are taking chronic warfarin therapy (a coumadin derivative). Hundred and one women between the age of 15 and 49 years were interviewed using a research tool in the form of a researcher administered questionnaire. Questions focused on various aspects of teratogenic medication and pregnancy. Preliminary findings of the study will be presented.

"Alice in Founderland" – vignettes demonstrating the usefulness of data on founder mutations to the interpretation of prenatal chromosomal microarrays

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Background:
The availability of chromosomal microarray analysis in the prenatal setting is effectively being used for the detection of copy number changes. Utilizing SNP microarrays provides means for detection of runs of homozygosity (ROH) and identical-by-descent genomic stretches. Upon adding to that the increasing number of founder mutations and the clinical phenotype obtained by prenatal sonography allows the Israeli geneticist to make accurate molecular diagnoses within a limited time-frame.

Cases:
1) A non consanguineous pregnant couple of Ashkenazi Jewish ancestry was referred because of sonographic findings of brain malformations including ventriculomegly and encephalocele. Genome-Wide Human SNP Array 6.0 (Affymetrix) was used for analyzing DNA extracted from amniocytes. No copy number changes were observed, however we utilized the online single nucleotide polymorphism array evaluation tool (**doi:10.1038/gim.2012.136**) for detection of recessive loci associated with the reported clinical findings. As the *FKTN* gene was present within the homozygous regions, a targeted successful detection of the Ashkenazi known c.1167dupA mutation was made leading to the diagnosis of Walker-Warburg Syndrome.
2) A young non consanguineous couple of Bukharian Jewish ancestry was referred due to severe polyhydramnion for amnioreduction and genetic diagnosis. No copy number changes were observed yet the detection of the *BSND* gene within the identical-by-descent stretches lead to focused search in the Israeli National Genetic Database (http://server.goldenhelix.org/israeli/). A common *BSND* mutation found in Jews

originating from Bukhara, c.167ins6[TTTCCC], associated with Bartter syndrome with sensorineural hearing loss was identified.
(3) A non consanguineous couple with sonographic fetal echogenic and enlarged kidneys was referred for prenatal diagnosis. The husband was of Ashkenazi Jewish ancestry and the wife was of Ashkenazi/Yemenite/Iraqi Jewish descent. Coordinates of ROH included 6.7 Mb on chromosome 6p [arr[hg19]6p21.1p12.2(46,104,119-52,837,551)] including the*PKHD1* gene. Thus the founder Ashkenazi mutation c.3761_3762delCCinsG was tested and both parents were found to be carriers and the fetus homozygote.

Discussion:
While most genetic diseases are very rare, in the Israeli population many founder mutations are known to be relatively frequent in definite communities. When clinical findings are characteristic, Searching for the founder mutation is like looking under the spotlight instead of searching all the way. Diagnosis is achievable this way even in the tight schedule of pregnancy, using the elegant way which is also the fast and cheapest. CMA platform can be helpful in "fishing" candidate genes in the ROH.

A novel genetic defect connecting cutis laxa to congenital disorders of glycosylation

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Background and objectives: Cutis laxa syndromes form a heterogenous group of inborn errors of metabolism eventually affecting the formation or function of extracellular matrix proteins leading to sagging, inelastic and wrinkled skin. Several genetic defects in different metabolic pathways have been described, including two disorders of glycosylation: COG7-CDG (wrinkly skin) and ATP6V0A2-CDG.
Case: We present a patient with a unique combination of features in combination with cutis laxa and subtle glycosylation abnormalities. Our male patient presented at birth with severely wrinkled skin and progeroid features, an abnormal fat distribution with almost absent subcutaneous fat and bilateral cataract. Dysmorphic features at birth, including a triangular face, large ears and downslanting palpebral fissures, resembled those seen in ATP6V0A2-CDG. Furthermore, he suffered from severe cardiomyopathy, which improved spontaneously over the years as did his skin phenotype. Psychomotor development was severely delayed. Growth parameters were normal after birth, however, started to deviate from the age of 2 years. Brain MRI revealed white matter abnormalities and widened ventricles. Biochemical analysis showed hypercholesterolemia and a CDG type II pattern on transferrin isofocusing and mass spectrometry.
Results/Conclusion: Exome sequencing revealed a variation in a subunit of the V-ATPase proton pump, from which another subunit (a2) is deficient in ATP6V0A2-CDG, thereby further bridging cutis laxa and CDG.

Revertant mosaicism in a Keratitis-ichthyosis-deafness (KID) syndrome patient prevent gap junction channel formation with connexin 26

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Revertant mosaicism (RM) is a naturally occurring phenomenon in which the pathogenic effect of a germline mutation is corrected by a second somatic event, giving rise to a mosaic appearance. RM has been observed in several inherited conditions, including epidermolysis bullosa. We have previously described a patient diagnosed with Keratitis-ichthyosis-deafness (KID) syndrome, a rare congenital ectodermal disorder. Here, we report on RM in a KID syndrome patient and further investigate the underlying molecular mechanisms. The patient was diagnosed at the age of 10 years old, based on the triad of characteristic skin lesions, hearing deficiency and keratitis. At the age of 20 years old mosaic appearance was observed within the widespread erythrokeratodermic lesions on the inside of the patient's thighs. The reverted patches of skin slowly expanded and increased with age. Later, she also developed squamous cell carcinoma. Investigation of *GJB2*, encoding connexin 26 (Cx26), revealed heterozygosity for the recurrent *de novo* germline mutation, c.148G>A; p.D50N. SMRT sequencing of genomic DNA and cDNA from biopsies of lesional and reverted skin revealed five nonsynonymous somatic mutations. All mutations were present independently *in cis* with the p.D50N mutation. Functional studies in HeLa cells displayed co-expression of Cx26-D50N and wild-type Cx26 in gap junction channel (GJC) plaque. However, Cx26-D50N with second-site mutations displayed no formation of GJC plaque or co-expression with wild-type Cx26. We suggest that the five second-site mutations inhibit Cx26-D50N expression in GJCs and thus, reverts the dominant negative effect of p.D50N. To our knowledge, this is the first time RM is reported in a KID syndrome patient.

Diagnostic clinical resequencing of polyposis-predisposition genes using single molecule Molecular Inversion Probes

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Polyposis, the formation of numerous polyps in the colon and rectum, is strongly associated with a heritable genetic predisposition and colorectal cancer development. Molecular diagnosis of hereditary polyposis is of great importance for the clinical management of patients and their relatives. To date, multiple high-penetrant genes are known that predispose to polyposis. Therefore, a fast, robust and cost-effective assay is warranted that enables resequencing of these genes. By making use of single molecule Molecular Inversion Probes

(smMIPs) resequencing of gene panels is now possible with high sensitivity and in a time- and cost-effective manner[1]. Recently, we have successfully implemented smMIP-based resequencing for the breast cancer-related genes *BRCA1* and *BRCA2* in our routine diagnostic workflow. This has led to minimal amount of rework and a shortened turnaround time. To further implement smMIP-based resequencing we are currently developing more gene panels, among others a polyposis-panel.
We present the principles of smMIP-technology and a smMIP-based resequencing workflow of polyposis-predisposition genes in a diagnostic clinical setting.

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Saethre-Chotzen syndrome: a familial case with *TWIST1* gene mutation

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Introduction:
Saethre-Chotzen syndrome (SCS) is an autosomal dominant craniosynostosis characterized by unilateral or bilateral coronal synostosis, facial asymmetry, ptosis, and characteristic small ears with prominent crus. Cutaneous syndactyly of digits two and three of the hand and duplication of the distal hallux are variably present. Intelligence is usually normal, although those with large genomic deletions are more likely to have developmental delay. *TWIST1* located on chromosome 7p21 is the only gene in which mutations are known to cause SCS. The gene product is a transcription factor containing a basic helix-loop-helix domain important in the development of the head and limbs. Germline point mutations and partial/whole exon deletions in *TWIST1* can be identified respectively in about 50% and 28% of patients. Occasionally, affected individuals have a chromosome translocation involving 7p21 or ring chromosome 7.
Case presentation:
Two brothers were referred to the Genetics Department for familial craniosynostosis. Their mother, who was 33 weeks pregnant at the time, presented turricephaly, blepharophimosis, ptosis and epicanthus inversus. The older brother, 6 years-old, presented with pansynostosis, blepharophimosis, ptosis and epicanthus inversus, hypertelorism, low-set ears with horizontal crus and large hallux bilaterally. The younger brother, 4 years-old, presented right unicoronal synostosis with two parietal foramina, and a similar facial gestalt, although less pronounced. Both have normal intellect. We subsequently observed the newborn who had the same phenotype.
FOXL2 gene sequencing and testing for the *FGFR3* gene P250R mutation in the older brother were negative for pathogenic alterations. *Array*CGH was also performed but no pathogenic CNV was identified. Finally, sequencing of *TWIST1* gene revealed a heterozygous pathogenic mutation, c.395G>C, confirming the diagnosis of SCS. Segregation analysis showed the mutation was also present in the two younger brothers. The three brothers were referred for neurosurgery, ophthalmology and audiologic screening.
Conclusion:
This case further illustrates the variability of the clinical spectrum of craniofacial disorders associated with *TWIST1* abnormalities. Also, there is phenotypic overlap with other craniosynostosis syndromes showing a thorough dysmorphological evaluation is still a valuable tool. Genetic analysis is necessary for diagnostic

confirmation and genetic counseling. Follow-up by a multidisciplinary team is needed to guarantee the best possible outcome.

The power of clinical diagnostic hypothesis in the era of next generation sequencing

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Introduction. Next generation sequencing (NGS) has revolutionized the field of genetic diagnostics with ability to investigate many or all genes at once. It can be debated whether the hypothesis free NGS will decrease the role of clinical phenotyping. In our clinic, besides whole exome sequencing (WES) TruSight One (TSO) NGS panel (Illumina) is used, covering 4813 genes associated with clinical phenotypes. During the interpretation of TSO results, we analyse only the genes listed by doctor on the referral form.

Aim of the study. To compare whether diagnostic yield of NGS panel testing is dependent on the amount of candidate genes listed on referral form by ordering doctor.

Methods. TSO was used for 325 patients in routine clinical diagnostics since April 2015. All class 4 (likely pathogenic) and 5 (pathogenic) variants according to ACMG 2015 classification were included to assess diagnostic yield. Diagnostic WES cohort of 68 patients was used for comparison. Besides standard variant calling, copy number variants were called retrospectively on both cohorts. The TSO cohort was divided into two subgroups based on the amount of candidate genes listed: 10 or less, and more than 10. R software was used for statistical analysis.

Results. The overall diagnostic yield of TSO cohort was 24.9% (81/325) and 25% (17/68) for WES cohort (p-value = 0.99). The diagnostic yield in the TSO subgroup of 10 or less genes analysed was 28.7% (56/194) and in the second subgroup with more than 10 genes analysed 19.8% (p-value = 0.23).

Discussion. Although not all genes are covered on TSO panel the overall diagnostic yield did not differ from WES, which can be explained by clinical difference between those cohorts – WES was used on more difficult cases without clear clinical hypothesis. While not statistically significant, the diagnostic yield was higher in those patients who had fewer candidate genes listed on referral form. This indicates that a clear clinical hypothesis increases the chance of receiving definite diagnosis from NGS testing.

Conclusion. NGS testing is powered by good clinical phenotyping, and the synergy between them is the key for higher overall diagnostic efficiency.

Novel homozygous frameshift variant outside the SAND domain of *DEAF1* resulting in developmental delay, microcephaly and extrapyramidal symptoms

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We evaluated a 3-year-old girl, with developmental delay and microcephaly. Parents (first-degree Pakistani cousins) and siblings are healthy. At 4 months she was assessed for hypotonia, poor head control and reduced eye contact. She exhibited clinical pyramidal tract and extrapyramidal features. She had microcephaly, broad forehead, hypertrichosis with synophrys, inverted nipples and audible expiration. Metabolic investigations were normal. MRI showed delayed myelination which later improved and agenesis of the splenium of corpus callosum.

Whole exome sequencing identified a homozygous deletion in *DEAF1* (NM_021008.2), c.1187del (p.Gly396Alafs*23). Based on literature and clinical presentation, we concluded that the *DEAF1* variant is pathogenic.

Heterozygous *DEAF1* variants were proved to cause Mental Retardation, Autosomal Dominant 24; MRD24 (OMIM 615828) [1] All heterozygous variants were missense, located within the SAND domain of *DEAF1*, and resulted in reduced DEAF1 DNA binding and protein-protein interactions.

Homozygous *DEAF1* variants were also described: two families had a missense variant (c.676C>T, p.R226W) inside the SAND domain [2, 3]. One family had a splice site variant outside the SAND domain (c.997+4A>C), resulting in skipping of exon 7 and nonsense mediated decay [4]. Our patient is the first report of a homozygous frameshift variant in *DEAF1,* predicted to result in nonsense mediated decay.

All patients with heterozygous dominant variants in *DEAF1* were less severely affected than those with homozygous variants. The latter group had features of MRD24 in addition to white matter disease, microcephaly, hypotonia and seizures. Patients with a null variant (c.997+4A>C, [4]) and our patient with a frameshift variant the phenotype also includes extrapyramidal signs.

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A preterm infant with a homozygous variant in *CENPF* showed clinical features of Strømme syndrome

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We report a baby girl, born at 34+3 to a healthy Lithuanian couple. The baby presented "apple peal" type duodenal atresia, hypoplastic kidneys, congenital malformation of the lung and anterior segment of the eye with micropthalmia. She also had microcephaly. Her MRI caput showed lissencephaly, pachygyria, and corpus callosum hypoplasia. The baby was small for gestational age and she died 26 days after birth.
Whole exome sequencing of the baby revealed a *CENPF* (NM_016343.3) homozygous variant c.1068+1G>A, likely resulting in skipping of exon 7 causing frame shift. The variant is not found in an in-house database of 443 exomes, and not in the ExAC database. Sanger sequencing of the family trio verified the segregation of the variant with the disease.

Biallelic variants in *CENPF* cause Strømme syndrome (OMIM 243605)[1], and the six variants so far identified cause frame shift. Because the clinical presentation of our patient is compatible with Strømme syndrome, we concluded that the *CENPF* variant is pathogenic. So far only four families have been identified with Strømme syndrome [1-3]. The phenotypic spectrum ranges from microcephaly with mild to moderate intellectual disability, without additional organ involvement to severe intestinal, renal and cerebral malformations leading to mid gestational lethality [2]. The most consistent features are intestinal atresia, ocular anomalies and microcephaly. Pre term births have also been reported in all live born individuals with Strømme syndrome [2].

CENPF associates with the centromere-kinetochore complex and may be involved in chromosome segregation during mitosis. In addition CENPF has a role in cilia formation and function [3]. *CENPF* null mutations cause Strømme syndrome with features of a ciliopathy.

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Mutation spectrum of *TGM5* gene in a Polish patients with acral peeling skin syndrome (APSS).

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Background:

Acral peeling skin syndrome (APSS) is a genetically inherited genodermatosis. The disease develops when pathogenic mutations in *TGM5* gene are present in both alleles. As a result of a defect in *TGM5* gene, the enzymatic activity of its protein product – transglutaminase 5 (TGM5) is abolished. It further leads to disease manifestations which is characterized by excessive skin peeling, blistering and erythema on hands and feet, exacerbated with heat and humidity. As APSS may resemble phenotype of patients with localized form of epidermolysis bullosa simplex (EBS), currently it is classified as a subtype of EBS. Although the vast majority of APSS cases are confirmed with mutations in *TGM5* gene, cases of APSS patients harboring mutations in *CSTA* gene were also published. Our aim was to identify molecular defect among Polish patients with superficial localized skin peeling on hands and feet.

Patients and methods:

DNA from 42 unrelated patients was analyzed by direct sequencing of the *TGM5* gene. In cases were no mutation was found, we performed sequencing of *CSTA* gene.

Results:

35/42 patients (83%) were confirmed with the molecular diagnosis of APSS and *TGM5* mutations present in both alleles. In one patient we did not identify mutation in second allele of *TGM5* gene. No mutations were found in either *TGM5* nor *CSTA* gene in remaining 6 patients with localized superficial skin peeling manifestations.

The Gly113Cys mutation was detected in 74% (65/88) alleles of affected patients (27 homozygous, 17 heterozygous cases) and in 2 (2%) healthy controls (n=103). We identified 4 novel mutations, one is a previously unreported mutation Leu509Ter identified in one allele and 3 of them (Ala111ProfsTer7, Arg215Trp and Met398Lys) were published together with data summarizing APSS mutation spectrum in Polish, U.K and German populations [Szczecinska W et al., Br J Dermatol. 2014 Nov;171(5):1206-10]. Ala111ProfsTer7 and Met398Lys mutations were specific for Polish population and found in three and two proband's alleles respectively.

Conclusions:

High carrier frequency of Gly113Cys mutation in a European populations (2-3%) suggests founder effect and supports the fact that incidence of APSS is underestimated in Europe, which can result from unspecific clinical phenotype and misdiagnosis. Gly113Cys and Ala111ProfsTer7 are recurrent mutations in a Polish population and should be incorporated to the frequent mutation panel of a first choice during molecular diagnosis of a patients with localized hand and feet skin peeling and blistering.

Clinical manifestations of partial trisomy 12q (12q24.1→qter) and partial monosomy 4p (4p16→qter)

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Abstract - We describe a clinical manifestations and cytogenetic caracterisation of partial trisomy of chromosome 12 long arm and partial monosomy of chromosome 4 short arm in a newborn child with multiple malformations.

Young, non-consanguineous couple with no medical and family history was refered to Genetic counceling after a delivery of their first child. Delivery was uneventful at term, AS were 9 and 10 at the 1st and the 10th minutes, respectively. Birth weight was 2700g which is at the 10th percentile. The lenght was 48cm (P_{25-50}) and head circumference was 33cm (P_{10-25}). Facial feature included: low set, poorly lobulated ears, wide nose bridge and down-slanted palpebral fissure. The neck was short. The nipples were wide set and umbilical cord was inserted low. Anteriorly displaced anus was also observed as well as rectovaginal fistula, mongolian spot and sacral hypertrichosis. She had a simian crease and was hypotonic.

Chromosomal analyses applying G-banding techniques (550 bands) revealed a derivative chromosome 4. Subsequent cytogenetic analyses of parental lymphocytes showed that paternal karyotype was normal, while mother had balanced translocation: 46,XX,t(4;12)(p16;q24.1).

The clinical features of this patient were compared with previously published descriptions for duplication of a segment 12q24→qter. Although, 12p chromosomal aberations are well-defined, duplication of the long arm of chromosome 12 is rare and needs to be better caracterised. Our findings contribute to further clinical delineation of partial trisomy 12q. However, since two chromosomes are involved in chromosomal rearangement observed in this patient precise assesment of each chromosome contribution to patient's phenotype cannot be done.

Key words: trisomy 12q, monosomy 4p, multiple malformations

MOD2, potential SMA modifier and much beyond?

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Previously being known as member of the Neuronal Calcium Sensor (NCS) family, MOD2 was first identified in our lab as a potential Spinal Muscular Atrophy (SMA) disease modifier. SMA a monogenic disorder, is characterized by functional loss of motor neurons in spinal cord, which eventually leads to motor disability in SMA patients. However, in certain individuals who carry the SMA genotype but do not show any SMA phenotype, we found MOD2 to be significantly downregulated. This finding implied the essential role of MOD2 in neuronal cells, which eventually rescues impaired neurons from SMA. Therefore, we are currently searching for the specific physiological role of Mod2 in and out of SMA context using a *Mod2* knockout (KO) mouse model.

We observed that *Mod2* KO mice are hyperactive and show anxiety like behavior, in line with data documented in International Mouse Phenotype Consortium. In order to understand the neurological mechanism behind these behavioral changes we characterized the brains of *Mod2* KO mice at histological, cellular and molecular levels. Nissl staining of Mod2 KO brain sections revealed gross-morphological alterations in CA2, CA1 and Dentate gyrus regions of the hippocampus. These changes were accompanied by the ventriculomegaly and Corpus callosum atrophy. Altogether these phenotypes match various severe neurological conditions, such as Alzheimer's, Schizophrenia and Autism.

In addition to that, at cellular level the primary motor neurons derived from *Mod2* KO/WT and *Mod2* KO/KO mice spinal cord showed significant increase in axon length and axonal branching as compared to wildtype animals at early developmental stage i.e. 4 DIV. This finding supports the rescue of axonal degeneration on MOD2 reduction in SMA patients. However, it also implies that MOD2 has a role in maintaining the balance between neuronal differentiation and neurogenesis.

Moreover, at molecular level we investigated one of the hallmark of neuronal activity, the pERK/ MAP kinase pathway. Western blot of primary motor neurons show significantly upregulated pERK in *Mod2* KO/WT compared to wildtype embryos. As high pERK level has been shown to increase the neuronal complexity these results may suggest a mechanism via which MOD2 affects the axonal length and branching in motor neurons.

Taken together, these results show various phenotypes and mechanisms which are affected by *Mod2* knockout. An in depth analysis of these phenotypes and mechanism can potentially reveal the specific role of *Mod2* in normal physiological condition as well as in SMA.

Mapping population wide genomic variation to protein 3D structures: separating tolerance from pathogenicity

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1. Introduction
The thousands of human genome sequences available to date allow for a good description of population-wide normal genetic variation in healthy individuals and can be used to assess per-gene tolerance to normal variation. This per-gene tolerance has been shown as a successful indicator of disease genes1. We aim to improve on current missense variant pathogenicity prediction methods by combining population wide genetic variation data with the information of protein 3D-structures.

2. Materials & Methods
We designed a software framework that can map coding nucleotide positions for any given protein-coding gene within the Gencode2 Basic set for GRCh37/hg19 to their respective positions in the protein sequence. If a protein 3D-structure is available, we map these nucleotide positions to the positions in a Protein Data Bank (PDB) structure3. This mapping can be used to annotate genomic information to proteins and protein structures, and vice versa. We use the Exome Aggregation Consortium (ExAC) dataset4 to obtain population-wide genetic variation. ExAC contains coding variants from 60,706 unrelated healthy individuals. The Human Gene Mutation Data (HGMD)5 is used to obtain disease causing missense mutations.

3. Results
We applied this framework to 18,185 human proteincoding genes, and were able to map 1997 to a more than 75% complete protein 3D-structure. In our case-study of PTPN11 we found that disease causing missense mutations can be spatially separated on the linear genome, while within the same region of the protein structure and any overlap between population-wide variation and disease causing mutations is almost absent. We will use this framework to study differences between benign and pathogenic variation within the protein 3D-structure and protein sequence.
Protein structure (PDB ID: 4nwg) of gene PTPN11 annotated with non-identical missense variation from HGMD in red, and ExAC in blue. Overlapping positions between the two datasets are in yellow. Mutations spatially separated on the linear genome, but contained within the same region of the structure are marked by the orange dots.

4. Discussion
We hope to show that intolerance to normal genetic variation, combined with information from protein 3Dstructures, provides a complementary source of information for predicting pathogenicity of missense variants.

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A *ZNF711* mutation in a large family with X-linked syndromic intellectual disability

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Exome sequencing was performed in a 4-generation Belgian family with five affected male patients with intellectual disability and additional clinical features. A frameshift mutation in *ZNF711* was identified, resulting in a premature stop codon. The *ZNF711* gene, located on Xq21.1, encodes a zinc finger protein of unknown function. Mutations in this gene have so far only been described in 2 patients in the context of a large-scale resequencing study, in which no further details of the patients were provided.
The patients in our family were originally suspected of fragile X syndrome, but no repeat expansion in the *FMR1* gene was identified. Moreover, linkage analysis limited the candidate region to the pericentromeric region of the X-chromosome at Xp11.4-q21.32 , again excluding the *FMR1* gene. Upon revisit, our patients presented mild to severe intellectual disability, developmental delay with speech and motor problems, childhood hypotonia and autistic-like features comprising diminished eye contact, hand flapping and the need for daily structure and routine. They have slightly dysmorphic facial features including a long face and large ears in adulthood.
In conclusion, we found a *ZNF711* mutation in a family where the affected males showed notable fragile X features.

Chromosomal Imbalances and Screening of Parkin mutation in Parkinson's Disease (PD) patients of Coimbatore population.

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Parkinson's disease (PD) is a chronic neurodegenerative and progressive movement disorder which causes dopaminergic neuronal loss in the nigro striatal pathway. The aim of our research was to perform the cytogenetic and biochemical analyses and to investigate the polymorphism of Parkin (PARK2) gene in PD patients in Coimbatore Population, Tamil Nadu. A total of 16 samples which includes 8 PD patients and equal number of healthy individuals were selected as control subjects matched with age and sex. Chromosomal abnormalities were observed using Trypsin G banding and the higher degree of anomaly was seen in chromosome 6. Mutational analysis was performed using polymerase chain reaction – restriction fragment length polymorphism and revealed the significance between PARK2 in 6 patients with PD. The biochemical parameter dopamine was analyzed and found to be less in PD patients when compared to control subjects. We conclude that by increasing the sample size in future research novel mutations can be examined in PARK2 and epigenetic studies can also be carried out which will be a novel study in Tamil Nadu population.

Keywords: Parkinson's disease, chromosomal abnormalities, PARK2, dopamine

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