Clinical Genomics and NGS

Bertinoro - Italy
April 30 – May 5, 2017

30th Course jointly organized by ESGM, ESHG AND CEUB

University Residential Centre
Via Frangipane, 6 – Bertinoro

Course Directors:

Han Brunner (Nijmegen and Maastricht, the Netherlands); Christian Gilissen (Nijmegen, the Netherlands); Alexander Hoischen (Nijmegen, the Netherlands); Tommaso Pippucci (Bologna, Italy); Giovanni Romeo (Bologna, Italy); Brunhilde Wirth (Cologne, Germany)

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CONTENTS

COURSE PROGRAM

SATURDAY APRIL 29TH Arrival and dinner

SUNDAY APRIL 30TH

Morning Lectures:	**Medical Genetics concepts and principles**
8:30 – 9:00	Participants Registration
9:00 – 9:15	Introduction to the course - **Giovanni Romeo**
9:15 – 10:00	Genomic Medicine - **Dian Donnai**
10:00 – 10:45	Phenotype to genotype - **Han Brunner**
10:45 – 11:00	Coffee break
11:00 – 11:45	Cytogenetics and arrays - **Eva Klopocki**
11:45 – 12:30	Complex disorders and classical gene identification - **Andrew Read**
12:30 – 13:15	Discussion of the morning lectures
13:30 – 14:30	Lunch Break

Afternoon Workshops

Session I (14:30 – 16:00)	Mutation patterns - **Han Brunner**
	Interpreting CNVs for beginners - **Eva Klopocki** Computer room
	Dysmorphology - **Dian Donnai**
16:00 – 16:30	Coffee break
Session II (16:30 – 18:00)	Mutation patterns - **Han Brunner**
	Interpreting CNVs for beginners - **Eva Klopocki** Computer room
	Dysmorphology - **Dian Donnai**

MONDAY MAY 1ST

Morning Lectures:	**Basics of NGS for Mendelian disorders**
09:00 – 09:45	Basics of next generation sequencing technology – **Alexander Hoischen**
09:45 – 10:30	Basics of NGS bioinformatics - **Christian Gilissen**
10:30 – 11:00	Coffee break
11:00 – 11:45	NGS in the clinic - **Anita Rauch**
11:45 – 12:30	Future NGS technologies – **John Tyson**
12:30 – 13:15	Discussion of the morning lectures
13:30 – 14:30	Lunch Break

Afternoon Workshops

Session I (14:30 – 16:00)	NGS Bioinformatics Basics - **Christian Gilissen & Tommaso Pippucci** Computer room
	Targeted NGS approaches - **Alexander Hoischen**
	How to do GWAS - **Carlo Sidore**
16:00 – 16:30	Coffee break
Session II (16:30 – 18:00)	NGS Bioinformatics Basics - **Christian Gilissen & Tommaso Pippucci** Computer room
	Clinical considerations for NGS - **Anita Rauch**
	NGS Technologies of the future – **John Tyson**

TUESDAY MAY 2ND

Morning Lectures:	**Therapy and prenatal diagnostics in the NGS era**
09:00 – 09:45	Therapy and cancer - **John Burn**
09:45 – 10:30	SMA: From gene and modifier to therapy - **Brunhilde Wirth**
10:30 – 11:00	Coffee break
11:00 – 11:45	Non-invasive prenatal testing – **Janneke Weiss**
11:45 – 12:30	Mithocondrial pathologies - **Caterina Garone**
12:30 – 13:15	Discussion of the morning lectures
13:30 – 14:30	Lunch Break

Afternoon Workshops

Session I (14:30 – 16:00)	NGS Bioinformatics, variant interpretation – **C. Gilissen & T. Pippucci** Computer room
	Rarity in the clinic - **John Burn**
	16:00 – 16:30 Coffee break
Session II (16:30 – 18:00)	NGS Bioinformatics, variant interpretation – **C. Gilissen & T. Pippucci** Computer room
	Ethics of medical genetics - **Caterina Garone & Andrew Read**
	From your newly discovered candidate gene to its function – **Brunhilde Wirth**
18:00	Poster viewing session with aperitif (session I)

WEDNESDAY MAY 3RD

Morning Lectures:	**Complex mechanisms of disease**
09:00 – 09:45	Discovering structural variants in cancer using NGS data - **Tobias Rausch**
09.45 – 10:30	Epigenetics and imprinting in human developmental disorders - **Karen Temple**
10:30 – 11:00	Coffee break
11:00 – 11.45	Non-coding mutations/long-range effects - **Eva Klopocki**
11:45 – 12:30	Oligogenic diseases - **Nicholas Katsanis**
12:30 – 13:15	Discussion of the morning lectures
13:30 – 14:30	Lunch Break

Afternoon Excursion

THURSDAY MAY 4TH

Morning Lectures:	**Novel NGS applications**
09:00 – 09:45	Molecular inversion probes and Saturation Genome editing **A. Hoischen**
09:45 – 10:30	Long-read sequencing - **Evan E Eichler**
10:30 – 11:00	Coffee break
11:00 – 11:45	GWAS with NGS - **Carlo Sidore**
11:45 – 12:30	How recycling big data can help to improve to diagnose and treat disease - **Lude Franke**
12:30 – 13:15	Discussion of the morning lectures
13:30 – 14:30	Lunch Break

Afternoon Workshops

Session I (14:30 – 16:00)	NGS diagnostic variant interpretation – **C. Gilissen & T. Pippucci** Computer room
	Genetic Imprinting - **Karen Temple**
	Genomic exotica - **Nicholas Katsanis**
	Mechanisms for non-coding mutations - **Eva Klopocki**
16:00 – 16:30	Coffee break
Session II (16:30 – 18:00)	NGS diagnostic variant interpretation – **C. Gilissen & T. Pippucci** Computer room
	How to set up a NGS lab? - **Alexander Hoischen**
	Copy number variations - **Evan E Eichler**

18:00	Poster viewing session with aperitif (session II)

FRIDAY MAY 5TH

Morning Lectures:	**Large scale NGS**
09:00 – 09:45	Presentations of best poster from students
09:45 – 10:30	The UK's 100,000 Genomes Project - **Augusto Rendon**
10:30 – 11:00	Coffee break
11:00 – 11:45	Phenotype and NGS integration / HPO benefits - **David Fitzpatrick**
11:45 – 12:30	Single cell sequencing and applications to PGD - **Thierry Voet**
12:30 – 13:15	Discussion of the morning lectures
13:30 – 14:30	Lunch Break

Afternoon Workshops

Session I (14:30 – 16:00)	Discovering structural variants in cancer using NGS data - **Tobias Rausch** Computer room
	Duplications and evolution of human gene innovation – **Evan E Eichler**
	How to do RNASeq - **Lude Franke**
16:00 – 16:30	Coffee break
Session II (16:30 – 18:00)	Discovering structural variants in cancer using NGS data - **Tobias Rausch** Computer room
	Large genomics projects - **Augusto Rendon & David Fitzpatrick**
	How to do single cell genomics? - **Thierry Voet**

Social dinner and farewell party

SATURDAY MAY 6TH
Departure

ABSTRACTS OF LECTURES

Sunday, April 30

The 30th edition of the course, the "spirit of Sestri Levante" and the ESHG reforms

Giovanni Romeo, M.D.
University of Bologna

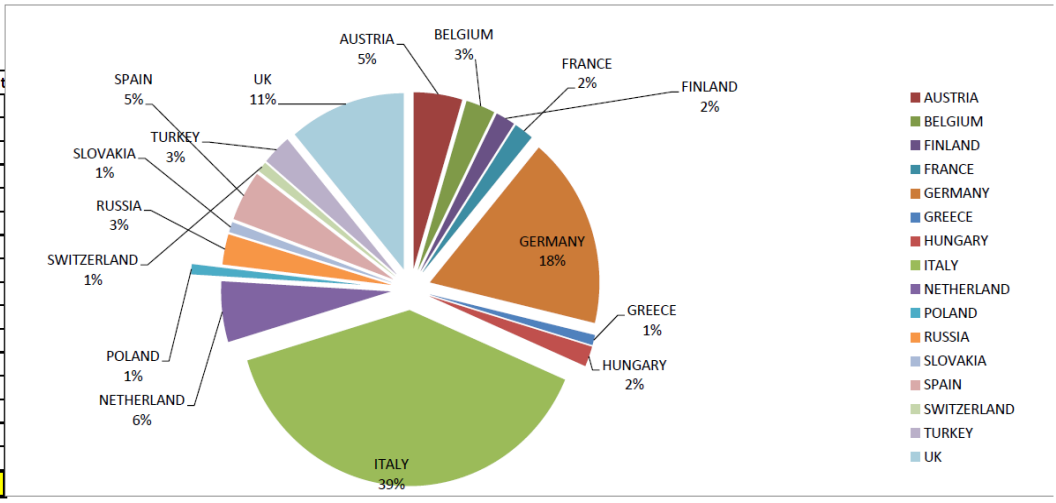
In 1988 an important meeting in Cardiff started the process of reforms of the European Society of Human Genetics (ESHG) which celebrates this year its 50th anniversary in Copenaghen at the end of May. Not by coincidence, 108 young geneticists from 16 European countries travelled to Sestri Levante (Italy) in April 1988 to attend the first week-long course in Medical Genetics, taught by the late Victor McKusick (1921-2008) and by many of the European medical geneticists of the time (see Fig.1 and Fig. 2). From its very beginning this course was made of morning plenary lectures and afternoon workshops, like today, and was quite labor intensive as shown by its tight scientific schedule (Fig. 3). During subsequent years this model was *cloned* into many more specialized courses (Cancer Genetics, Genetic Counselling, Molecular Cytogenetics, Eye Genetics, etc.) which became to be known as the European School of Genetic Medicine (ESGM). The present course which has consistenly been supported by ESHG fellowships takes this year the new denomination of "Clinical Genomics and NGS" and is being attended by 90 students from all over the world (37 countries- Fig. 4). After the first 1988 course some of its faculty became the leaders of the *reformed* ESHG in later years when the new statutes were approved and implemented in 1991 at the Leuven meeting and the European Journal of Human Genetics (EJHG) was started (1992). I insist so much on the link between the ESGM courses and the history of ESHG because I (and others) believe that the reform and expansion of the Society became possible in part through these courses which enabled so many people to become acquainted with each other, breaking down national, regional and linguistic barriers.

All these changes were occurring at a time when Europe was going through big political changes namely the fall of the Berlin's wall on November 9th, 1989, which led to the reunification of East and West Germany, and the signing of major European treaties, starting with "Maastricht" in 1993. It was a time of great enthusiasm and popular consent for the idea of building the European Union (EU) and implementing reforms which for the first time in our history were being accomplished through peace and diplomacy.

In this climate of big changes our small community of scientists was transformed into a democratic society of medical and clinical geneticists. Was this achievement worth the time and efforts invested in it? Among other indicators which can be used to answer this question there is a simple observation based on the breakdown of students attending the main ESGM course in 1988 versus 2017 (Fig. 1 and 4 respectively). This comparison documents the success of ESHG in supporting programs of advanced training in medical and clinical genetics which today are no longer limited to Europe but attract young geneticists from all over the world. This is a tangible result which shows that the *reformed* ESHG is having a tremendous impact on the practice and research in medical genetics far beyond Europe. The spirit that animated the European School of Genetic Medicine since its early days in Sestri Levante probably *imprinted* many young geneticists, among others Brunhilde Wirth (a student in the course of 1988-Fig.2) and Han Brunner (a young faculty since the early '90) who later became the driving force of this ESGM course together with the younger generation of medical geneticists represented by Christian Gilissen, Alexander Hoischen and Tommaso Pippucci.

1988 SESTRI LEVANTE
FIRST COURSE IN MEDICAL GENETICS
COUNTRIES OF PROVENIENCE

nationality	participant
AUSTRIA	5
BELGIUM	3
FINLAND	2
FRANCE	2
GERMANY	19
GREECE	1
HUNGARY	2
ITALY	42
NETHERLAND	6
POLAND	1
RUSSIA	3
SLOVAKIA	1
SPAIN	5
SWITZERLAND	1
TURKEY	3
UK	12
TOT.	108



1988
SESTRI LEVANTE – FIRST COURSE IN MEDICAL GENETICS

Numbering hand-outs

Istituto Scientifico G.Gaslini
International School of
Pediatric Sciences - Genoa

FEBS (Federation of European
Biochemical Societies)

EUROPEAN SCHOOL OF MEDICAL GENETICS
SESTRI LEVANTE (Genoa), April 6-12 1988
PROGRAM

WEDNESDAY 6

8.30-12.30: 1 V.A.McKusick (Baltimore): History of Medical Genetics: an introduction to principles of human genetics
2 A.M. Frischauf (London): Introduction to molecular genetics
3 G.Romeo (Genoa): DNA polymorphisms and disease
4 A.Cao (Cagliari): Beta-thalassemias: molecular basis, phenotype-genotype relationship and detection
5 S.Ottolenghi (Milano): Molecular mechanisms of defects in beta-thalassemias and inherited abnormalities of gamma-globin gene expression.

15-17.30: 6 J.Ott (New York): Introduction to analysis of genetic linkage
-> Workshop 1: pedigree analysis and risk calculation. Use of computerized programs (Linkage, OnLine Mendelian Inheritance in Man, Possum, London Dysmorphology Database): demonstration of these programs will continue throughout the course.

18-18.30: Questions from students

18.30-20: 7 T.Caskey (Houston): Lesch-Nyhan disease: molecular basis, neuropathology and mouse model

THURSDAY 7

8.30-12.30: 8 B.Dallapiccola (Rome): Methods of cytogenetic analysis
9 M.Ferguson-Smith (Cambridge): From chromosomal to molecular mutations
10 C.Junien (Paris): Molecular characterization of constitutional and acquired chromosomal rearrangements
11 M.Rocchi (Genoa): Cytogenetic methods for physical gene mapping

15-17.30: -> Workshop 2: Clinical cytogenetics

18-18.30: Questions from students

18.30-20: 12 A. de la Chapelle (Helsinki) and M.Ferguson-Smith (Cambridge): Clinical, cytogenetic and molecular aspects of sex determination

FRIDAY 8

8.30-12.30: Session on population genetics coordinated by R.Ceppellini
13 A.Piazza (Turin): Principles of population genetics
14 M.Baur (Bonn): Linkage Disequilibrium
15 M.Sarfarazi (Cardiff): Risk calculation for recurrence of mendelian disorders
16 G.Romeo (Genoa): Consanguinity and disease in Italy

15-17.30: -> Workshop 3: problem solving session on population genetics

18-18.30: Questions from students

18.30-20: 17 R.Norio (Helsinki): The Finnish disease heritage

SATURDAY 9

8.30-12.30: Session on linkage coordinated by J.Ott (New York)

18 M.Baur (Bonn): Computerized program for haplotype reconstruction

19 V.A.McKusick (Baltimore): The human genome (status of the map)

20 J.Mohr (Copenhagen): Some highlights of linkage studies in man

21 M.Sarfarazi (Cardiff): Exclusion mapping

22 H.H.Ropers (Nijmegen): Mapping of X-linked genes and research strategies

Afternoon: Trip to Portofino (by boat or bus depending on weather conditions)

SUNDAY 10

8.30-12.30: 23 A.M.Frischauf (London): The methods of reverse genetics

24 M.Bobrow (London): Duchenne muscular dystrophy

25 H.H.Ropers (Nijmegen): Myotonic dystrophy

26 G.Romeo (Genoa), J.Mohr (Copenhagen) and X.Estivill (Barcelona): Review on cystic fibrosis research

15-17.30: → Workshop 4: Molecular genetics (I)

18-18.30: Questions from students

18.30-20: 27 G.Andria (Catanzaro), A.Ballabio (Naples), and M.Ferguson-

28 Smith (Cambridge): Molecular and clinical aspects of steroid sulphatase deficiency in man

MONDAY 11

8.30-12.30: 29 K.Berg (Oslo): Genetics of coronary heart disease and its risk factors

30 G.Utermann (Innsbruck): Genes contributing to the population variance of quantitative lipoprotein traits and multifactorial hyperlipidemia

31 G.Bianchi (Milan): Essential hypertension in man and in animal models

32 R.B.Barber (Milan): Selection and breeding of hypertensive rats

33 A.Sidoli (Milan): Dislipoproteinemias, atherosclerosis and hypertension: molecular analysis of multifactorial diseases

15-17.30: → Workshop 5: Molecular genetics (II)

18-18.30: Questions from students

18.30-20: 34 T.Caskey (Houston): Gene Therapy

TUESDAY 12

8.30-12.30: Session on prenatal diagnosis coordinated by J.C.Kaplan

35 M.Cordone (Genoa): Ob-Gyn techniques

36 B.Dallapiccola (Rome): Cytogenetics

37 P.Durand (Genoa): Biochemical Genetics

38 J.C.Kaplan (Paris): Strategies of prenatal diagnosis by DNA analysis

39 M.Pembrey (London): Practical aspects of prenatal diagnosis by DNA analysis and the associated counselling

Panel discussion on genetic counselling and prenatal diagnosis

15-17.30: 40 R.Norio (Helsinki): Use of a manual data bank as a diagnostic aid in clinical genetics

Workshop 6: Review of problems

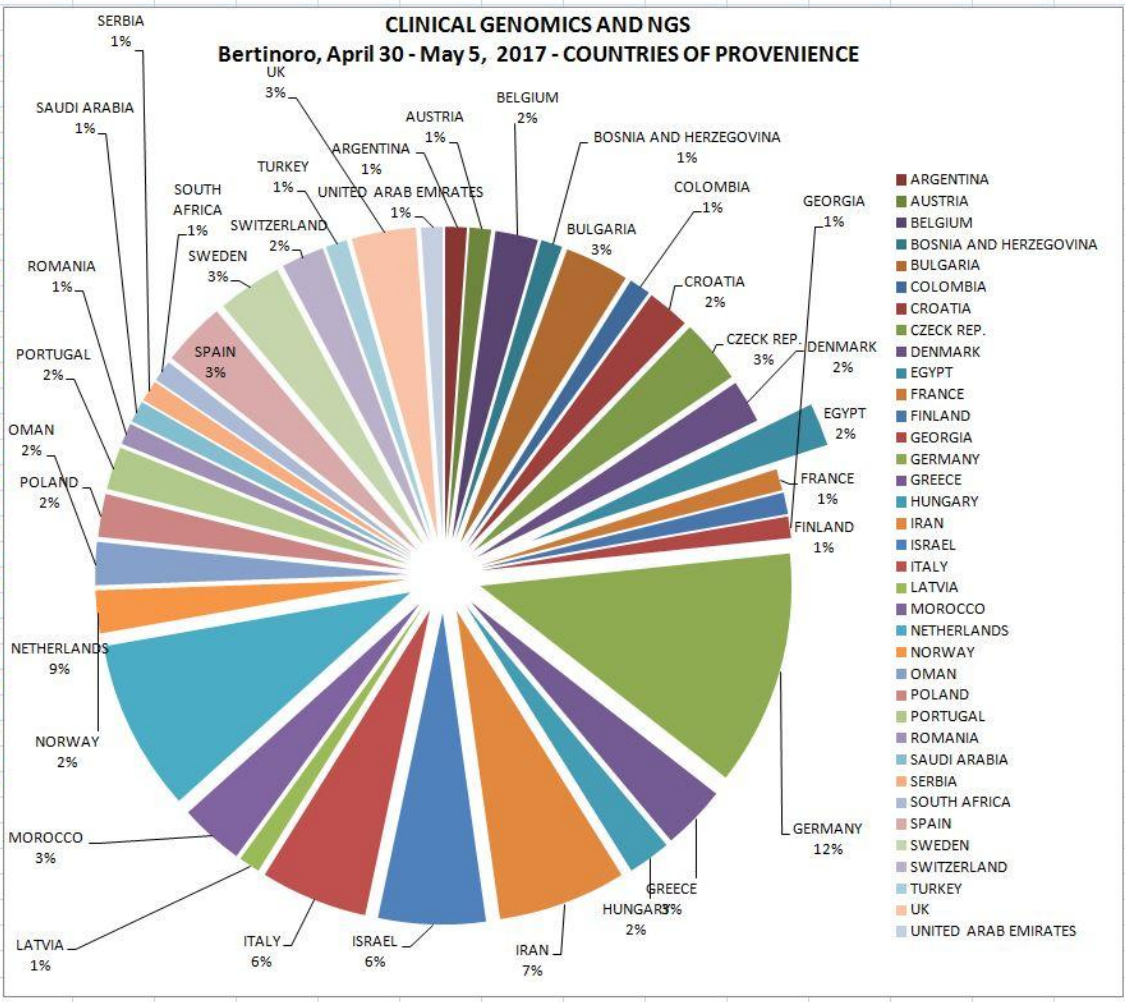
18-18.30: Questions from students

18.30-20: 41 G.Bernardi (Paris): The organization of the human genome

WEDNESDAY 13

Optional guided excursion to the Cinque Terre (by boat or train + walking)

nationality	pax
ARGENTINA	1
AUSTRIA	1
BELGIUM	2
BOSNIA AND HERZ	1
BULGARIA	3
COLOMBIA	1
CROATIA	2
CZECK REP.	3
DENMARK	2
EGYPT	2
FRANCE	1
FINLAND	1
GEORGIA	1
GERMANY	11
GREECE	3
HUNGARY	2
IRAN	6
ISRAEL	5
ITALY	5
LATVIA	1
MOROCCO	3
NETHERLANDS	8
NORWAY	2
OMAN	2
POLAND	2
PORTUGAL	2
ROMANIA	1
SAUDI ARABIA	1
SERBIA	1
SOUTH AFRICA	1
SPAIN	3
SWEDEN	3
SWITZERLAND	2
TURKEY	1
UK	3
UNITED ARAB EMI	1
TOT.	90



Genomic Medicine

Dian Donnai

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Genomic Medicine is changing but is as exciting today as it has always been. Previously the application of medical genetics was limited to diagnosis and risk assessment for patients with a relatively small range of rare diseases; however the vast explosion in knowledge and technologies has allowed genomic medicine today to have a much greater impact on medicine in general. 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.

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. Over the past six to seven 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* 2017) http://www.ddduk.org/intro.html) or targeted testing using large panels of genes for patients with specific disorders such as retinal dystrophy where an 85% diagnostic rate can be achieved. (Taylor et al 2017). Recognising that exome sequencing may miss pathogenic mutations some centres are now introducing whole genome sequencing into diagnostic practice (Gilissen 2014). WGS is also being used for large scale research studies that will have some individual patient benefits as well as generating so-called 'Big Data' www.genomicsengland.co.uk/the-100000-genomes-project. Concerns have been expressed about the non-technical aspects of NGS but as experience deepens most centres are finding ways of addressing these in conjunction with patient groups. Several organisations including the American College of Genetics and Genomics have published recommendations about integrating NGS into clinical practice in a consistent way (Bowdin et al 2016) and reporting incidental findings (Kalia 2017). Other working groups especially from the Nuffield Council on Bioethics have examined and published excellent reviews of technologies and associated ethical dimensions including on Gene Editing, NIPT, Medical profiling and online medicine. http://nuffieldbioethics.org/publications

As gene mutations have been associated with specific disorders, 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. Many hypotheses formulated many years ago have now been proven by our ability to investigate them with more powerful techniques

- 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. Also mosaicism is found in many other disorders.

- 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).
- Interestingly results of diagnostic applications of NGS such as the DDD study 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

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) and the application of so-called 'liquid biopsies' to cancer diagnostics. www.phgfoundation.org/project/ctDNA

Treatment is now an option for an increasing number of disorders based on diagnosis and/or genotype

- For decades some genetic disorders such as PKU have been managed with special diets
- Enzyme replacement has been used for disorders like type 1 Gaucher disease since 1991
- Drug treatments have targeted the effects of tyrosinemia type 1 (nitisinone), the specific mutant protein in F508del- associated CF (lumacafor and ivacaftor) and some drugs have been 'repurposed' such as mTOR inhibitors for certain symptoms of Tuberous sclerosis and trialled in Proteus syndrome www.proteus-syndrome.org/proteus-syndrome-phase-1-trail-patient-enrollment/ (NB I know there is a typo (trail) in this address but it is in the actual link)
- Anti-sense therapies are being trialled in spinal muscular atrophy and in boys with Duchenne muscular dystrophy
- Gene therapy where a normal copy of a gene is delivered has been used for some time in severe combined immunodeficiency and is being trialled in CF and in Leber's congenital amaurosis.
- Stem cell therapies and transplants are used in Type 1 diabetes and being trialled in Multiple Sclerosis
- Gene editing using initially the TALEN system (Qasim et al 2017) and now CRISPR Cas9 is the great hope for treatment of genetic disorders and was recently reported in treatment of Sickle cell disease (Ribeil et al 2017)

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.

The roles of clinicians and scientists in Medical Genetics will change and training needs to change as well. Certainly we will be called upon to educate our colleagues in other specialties. Clinically we should ensure our expertise in deep phenotyping is recognised and we need to ensure consistency in our reports and use appropriate classifications such as www.human-phenotype-ontology.org and other digital systems to capture gestalt such as FDNA www.fdna.com

Clinicians should work with clinical scientists and bioinformaticians to interpret sequencing data in the light of the phenotype and they should be part of Multidisciplinary teams with other specialties in planning investigation, care and treatment of patients with a wide variety of medical conditions.

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Phenotype to genotype

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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.

Cytogenetics and arrays

Eva Klopocki
Universität Würzburg - Institut für Humangenetik
Biozentrum Am Hubland,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.

Recommended 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

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Complex disorders and classical gene identification

Andrew Read
Centre for Genomic Medicine, St Mary's Hospital, Manchester, UK

Research to identify the determinants of human phenotypes has moved through three main phases. During 1985-2000 the main tool was linkage analysis. A candidate linkage interval might contain a dozen or so genes, so exons of those would be sequenced in a panel of unrelated affected people. For loss-of-function conditions, demonstrating deletions, frameshifts or splice mutations in a few of the subjects would identify the correct gene. Missense variants found in the panel would also usually be assumed to be pathogenic, even in the absence of functional data. This led to many variants that are in fact benign being listed in the databases as pathogenic, as eventually demonstrated by EXAC data – but at least the correct gene had usually been identified. McArthur and colleagues (1) provide useful guidelines for avoiding these mistakes.

Gain-of-function conditions typically have much less allelic heterogeneity, and here functional data was much more critical in identifying the correct gene.

From around 2000 the emphasis moved to complex disorders. Attempts to use the linkage methods that had worked so well with Mendelian conditions were generally unsuccessful. Affected sib pair analysis is robust but lacks the necessary statistical power. Analysis of affected individuals across extended pedigrees had a few successes, but the seminal calculations of Risch & Merikangas (2) showed that association studies would be better than linkage for identifying susceptibility factors.

After various false starts and underpowered studies, the Wellcome Trust Case-Control Consortium (3) set the pattern for successful genomewide association studies (GWAS). Over the next decade susceptibility variants for every imaginable complex character were identified (see www.ebi.ac.uk/fgpt/gwas/). However, in almost every case all the known susceptibility factors account for only a small part of the heritability as estimated from family data. This gave rise to the 'missing heritability' problem (4). I will discuss how far the various theories that have been suggested to account for this missing heritability have solved the problem. Most variants identified by GWAS are actually non-pathogenic, but associated with the true causal variant through linkage disequilibrium. The major challenge with GWAS data is moving from these associated variants to the actual causal variants.

More recently the focus has moved to large-scale sequencing, plus analysis of structural variants. An influential model (5) suggested that pathogenic variants could be grouped into three classes: rare highly penetrant variants (responsible for Mendelian conditions and identifiable by linkage analysis); common low-penetrance variants (susceptibility factors for common conditions and identifiable through GWAS); and a third group of variants with intermediate frequency and penetrance, identifiable only by sequencing. It has been controversial how far this third class exists. Such variants may be significant at the individual, but not the population level. Underlying all this work is the question, how far will all this knowledge benefit patients, rather than just being interesting science? In this context the thought-provoking paper by Roberts and colleagues (6) is worth a careful reading.

1. MacArthur DG *et al* Guidelines for investigating causality of sequence variants in human disease. *Nature* 508, 469-476; 2014.

2. Risch N & Merikangas K. The future of genetic studies of complex human diseases. *Science* 273: 1516-7; 1996.

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4. Eichler EE *et al* Missing heritability and strategies for finding the underlying causes of complex disease. *Nat Rev Genet* 11: 446-450 ; 2010.

5. McCarthy MI *et* al Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nature Reviews Genetics* 9: 367; 2008.

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AFTERNOON WORKSHOPS:

Mutation patterns

Han Brunner

Mutations can be viewed in different ways.

Structurally:
Nonsense, frameshift, missense, splice site
Functionally
Loss of function, gain of function, and change of function

This workshop discusses the relationship that exists between these 2 classifications, and how we can make predictions

Interpreting CNVs for beginners

Eva Klopocki

The aim of this workshop is to focus on various aspects of copy number variant (CNV) interpretation and classification in a diagnostic setting. Following a short introduction on the analysis and use of genome browsers and databases i.e. DECIPHER, DGV, etc. the students work on illustrative cases from diagnostic laboratories as well as research cases.
We will discuss the more challenging findings, including low-penetrant, recurrent CNVs and structurally rearranged chromosomal imbalances as well as patients with compound heterozygous variants in a recessive disease gene. Besides the interpretation of CNVs we will talk about appropriate follow-up testing strategies i.e. which methods to be used and relevance of family testing.

Dysmorphology

Dian Donnai

1) The workshop will begin with a brief overview of Dysmorphology for those new to the topic followed by a short quiz
2) We will look at terms in use to describe individual features and how they may suggest particular diagnoses. We will also look at standardised ways of describing features.
3) This will be followed by a review of 'smart' systems being devised for syndrome identification
4) We will then work through a number of scenarios to explore the investigation, diagnosis, family aspects and anticipatory care needs based on actual patients

Monday, May 1

Basics of Next generation Sequencing technology

Alexander Hoischen
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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:

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2. Metzker ML. Sequencing technologies - the next generation. Nat Rev Genet. Jan;11(1):31-46 (2010).
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4. Ashley EA, et al. Clinical assessment incorporating a personal genome. Lancet 375: 1525-35 (2010).
5. Cooper GM, Shendure J. Needles in stacks of needles: finding disease-causal variants in a wealth of genomic data. Nat Rev Genet. Aug 18;12(9):628-40 (2011).

Basics of NGS bioinformatics

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.
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NGS in the clinic

Anita Rauch
University of Zurich, Institute of Medical Genetics, Schlieren-Zurich, Switzerland

Future NGS technologies

John Tyson
QUANTUMDx GROUP LIMITED, Newcastle, U.K

The emergence of massively parallel sequencing technologies was driven by ambitious technology development and has revolutionised fundamental, as well as clinical, genetics. Sequencing platforms were developed from the ground up around new sensors and unique biochemistry. Some of the early NGS systems are already obsolete, such as the Roche 454 and Helicos platforms. These technologies could not meet the incremental improvements in throughput cost, performance and usability of the systems still on market today. The future looks set to hold further significant advances in DNA sequencing fuelled by advancements in technology.

Since acquiring the sequencing by synthesis technology of Solexsa in 2007, Illumna has emerged as leaders in the NGS field with Ion Torrent and PacBio taking more niche positions. But now these existing platforms continue to be improved upon, and new sequencing technologies are still being developed. Oxford Nanopore Tenhnologies hopes to compete against Illumina with its long read single molecule platform. Its USB sized nanopore sequencer, the MinION, is powered from a laptop whilst its PromethION system promises Tb of output per run. Other companies are developing nanopore technology, such as Genia, which is now part of the pharmaceutical giant Roche. New platforms have potential to directly read DNA modifications such as methylation and DNA damage adducts. Long read sequencing technology may one day allow de novo whole genome sequencing. As the time for WGS or WES workflows continues to fall, NGS is becoming more important in clinical decision making. The future also looks likely to include disputes over intellectual property between rival companies, as it has done in the past.

Targeted point of care tests for disease diagnostics, disease monitoring, drug sensitivity and treatment stratification are emerging across many areas of medicine. Companies such as QuantuMDx are developing low cost point of care systems that will bring DNA testing, and eventually sequencing, out of centralised labs providing results whilst patients wait. Such advances will bring complex genetic testing to resource limited countries who currently lack adequate centralised laboratory infrastructure. Such advances even have the potential to allow self-testing for disease status, monitoring or predisposition which would bring its own ethical and regulatory challenges.

Finally, NGS already takes advantage of the digital cloud for storage and data analysis. Sequencing will align further with the digital world in the future to connect digital health records. Furthermore, IBM, Microsoft and Google are all investing in artificial intelligence systems for analysis of genomics datasets and health records. Whilst networks of connected diagnostic related devices could lead to national and international disease monitoring networks.

This talk will cover emerging sequencing technologies, developments of existing NGS systems from a technical perspective, and future applications of DNA sequencing.

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AFTERNOON WORKSHOPS:

Session I	NGS Bioinformatics Basics - **Christian Gilissen & Tommaso Pippucci**
	Targeted NGS approaches - **Alexander Hoischen**
	How to do GWAS - **Carlo Sidore**
Session II	NGS Bioinformatics Basics - **Christian Gilissen & Tommaso Pippucci**
	Clinical considerations for NGS - **Anita Rauch**
	NGS Technologies of the future – **John Tyson**

Tuesday, May 2

Therapy and cancer

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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.

SMA: From gene and modifier 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 a 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]. Based on SMA discordant families, we identified two SMA protective modifiers, plastin 3 (PLS3) and neurocalcin delta (NCALD). Both helped us to identify endocytosis as the main cellular pathway impaired in SMA and restored by either overexpression of PLS3 or knockdown of

NCALD levels [7,10-12]. Moreover, both SMA modifiers are able to rescue SMA phenotype across species (worm, zebrafish, mice)

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. Dec 2016 the first SMN-ASOs (SPINRAZA) has been FDA-approved for SMA therapy [13].

Since the amount of SMN produced from two SMN2 genes may not be sufficient in type 1 SMA patients, additional combinatorial therapies will be mandatory [14].

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Non-invasive prenatal testing (NIPT)

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Key words: NIPT, cfDNA, bioinformatics, population screening

This presentation will be separated in three parts.

I General introduction

This part of the presentation will provide you with background information on the biology underlying NIPT. It will explain what cell-free DNA is, the source of cfDNA will be discussed, and the consequence for the sensitivity and specificity of cfDNA testing. The basics of the different available technologies will be discussed briefly. Finally, a short introduction will be given on the most commonly used bioinformatics tools, based on z-score analysis.

II Tools, pitfalls and tricks: causes of false positive and false negative results

Although NIPT analysis for the detection of Trisomy 21, 13 and 18 is rather straightforward, there are several biological factors that might cause false positive and false negative effects. Although most of them are rare, testing of large numbers of pregnant women will assure the fact that all of these causes might be encountered. The most important causes are:

False negative results
- Low foetal fraction
- True foetal mosaics
- Twin pregnancies

False positive results
- Confined Placental Mosaicism (CPM)
- Maternal CNVs
- Maternal malignancies
- Maternal mosaics

- Vanishing twin

Most of these causes will be discussed based on examples from daily practice. Furthermore, we will discuss how to discern between true negative/positive and false negative/positive results based on several different bioinformatics tools that were developed at VUmc Amsterdam, such as WISECONDOR and Defrag. WISECONDOR detects smaller chromosomal deletions and duplications without increasing the need for NIPT/NGS data. It is now widely used in many countries for routine diagnostic NIPT analysis, including the Netherlands, Denmark, France and South Korea. Defrag has not yet been published, but is a tool to determine foetal fraction. Defrag is based on Y-chromosome fraction. All tools are freely available for non-commercial use (https://github.com/rstraver). WISECONDOR can also be used for the detection of tumour profiles in cfDNA from cancer patients.

III The introduction of NIPT in the Netherlands.

The Netherlands are the first country where NIPT is incorporated into a governmentally supported and health care funded prenatal Down syndrome screening program. In many countries, NIPT has been introduced commercially, without governmental guidance. In the Netherlands the Population Screening Act regulates the introduction of screening programs for untreatable diseases such as Down syndrome. The Dutch NIPT consortium, consisting of all relevant stakeholders (midwifes, gynaecologists, clinical geneticists and clinical laboratory geneticists), obtained a license for a nationwide NIPT implementation study called TRIDENT-1, which started April 1st 2014. Inclusion criteria are an increased risk (>1:200) for trisomy (T) 21, 18 or 13 based on the first trimester combined test, or because of medical history. Data of the first year will be presented, including full clinical follow up of the first five months, and information on findings other than trisomy 21, 13 or 18. On April 1st 2017 we started the TRIDENT-2 study, which offers NIPT to all pregnant women. In TRIDENT-2, women have the choice to either receive information on Trisomies 21, 13 and 18 alone, or to learn about other chromosomal abnormalities as well (with the exclusion of the sex chromosomes). A brief overview of the results of the first weeks will be presented.

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Mithocondrial pathologies

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Mitochondria are eukaryotic intracellular organelles that play a central role in cellular metabolism. They are responsible for the conversion of energy in nutrients into adenosine triphosphate (ATP) through the oxidative phosphorylation (OXPHOS) pathway and participate to other cellular processes, including thermogenesis, amino acid metabolism, lipid metabolism, biosynthesis of heme and iron–sulfur clusters, calcium homeostasis and apoptosis. Mitochondria are dynamic, communicating and highly regulated organelle under the dual control of nuclear DNA and his own mitochondria DNA, a 16.6 kb circular DNA molecule (mtDNA), encoding 22 tRNA, 2 rRNA and 13 subunits of the OXPHOS system. Those unique features are responsible for the complexity of mitochondria biology in health and disease.

Mitochondrial disorders are the most common cause of childhood and adult neurometabolic disease, with a minimum estimated prevalence of 1 in 5000 live births. Genetically, mitochondrial disorders can be caused by mutations in mtDNA, either sporadic or maternally inherited, or in ≈1200 nuclear genes encoding mitochondrial proteins, as autosomal recessive, dominant or X-linked traits. Biochemically, mitochondrial disorders are characterized by defects in respiratory chain activities in the affected tissue. Clinically, they usually present as multi-system disease, although muscle, brain and liver are the most commonly affected tissues. The genetics of mtDNA mutations and his biochemical and clinical readout is complicated by the "bottleneck" segregation mechanism that determines the percentage of heteroplasmy (mutated *vs* wild type mtDNA molecules), the threshold effect for manifesting a clinical phenotype and the mitotic segregation responsible for changes in the level of heteroplasmy during lifetime.

The heterogeneity of mitochondrial disorders challenges the genetic diagnosis that in 60% of the cases is still unknown. Next generation sequencing with targeted mitochondrial library or whole exome sequencing library has been successfully applied for identifying the genetic cause of mitochondrial diseases. Algorithm for rare variant filtering in mitochondrial NGS includes prediction tools for mitochondrial localisation, protein phylogenesis and metabolic pathways in which the defective protein may operate. However, several new mutations have been identified in nuclear genes encoding proteins not previously assigned to mitochondria and with unknown function. Mitochondrial translational science is currently focused on the elucidation of the underlying pathogenetic pathways and to shed light on the complexity and multiplicity of several processes of cell life and death where mitochondria play role. This is a fundamental step for identifying curative treatment for mitochondrial disease.

Currently, there are no effective treatments for the vast majority of mitochondrial diseases except for supportive therapy. The development of in vivo and in vitro models for mitochondrial disorders have enabled the design of new treatment strategies tailored for a specific gene defect or targeting broader mechanism such as mitochondrial biogenesis, lipid milieu remodelling, authophagy with a pharmacological or gene therapy approach. Pre-clinical studies in vitro and in vivo model have recently provided positive results in term of efficacy and safety bringing the promise to translate them into clinical trials.

In conclusion, mitochondrial pathologies are highly heterogeneous and complex genetic disorders. Advances in sequencing technologies and disease modelling have provided important insights into the diagnosis and treatments of mitochondrial disorders but the great effort of highly motivated and enthusiastic scientific community is still needed for solving the paradigm: recognize, understand and treat mitochondrial diseases.

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AFTERNOON WORKSHOPS:

Session I

NGS Bioinformatics, variant interpretation – **C. Gilissen & T. Pippucci**

Rarity in the clinic - **John Burn**

Session II

NGS Bioinformatics, variant interpretation – **C. Gilissen & T. Pippucci**

Ethics of medical genetics - **Caterina Garone & Andrew Read**

From your newly discovered candidate gene to its function – **Brunhilde Wirth**

Ethics of medical genetics

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(2) Centre for Genomic Medicine, St Mary's Hospital, Manchester, UK

The workshop aims to explore several ethical aspects of medical genetics such us informed consent, data analysis and release, incidental findings, therapy in an interactive and original fashion manner. We will present a number of real or imaginary clinical scenarios that raise ethical issues. Student are invited to present their potential experience as well. Given the different background of the two workshop leaders, an intense discussion on ethical principles will be opened and the attendants will help to provide additional view on ethics in order to define potential guidelines in clinical genomics.

Wednesday, May 3

Discovering structural variants in cancer using NGS data

Tobias Rausch
EMBL Heidelberg, Germany

Many cancers harbor a plethora of somatic point mutations, small insertions & deletions and structural variants and a subset of those may confer a growth advantage to the cell. Predisposing germline variants can influence the somatic mutation landscape and varying degrees of tumor purity, heterogeneity and ploidy complicate the discovery and genotyping of somatic variants. Large catalogues of both germline variants (e.g., 1000 Genomes Project [1]) and somatic variants (e.g., International Cancer Genome Consortium [2], Cosmic [3]) have been accumulated but for structural variants these catalogues are often sparse or of inferior quality compared to single-nucleotide variants and small insertions and deletions.

After a brief introduction to crucial parameters in cancer genomics such as purity, ploidy and heterogeneity [4] and a discussion on how these can be estimated from next-generation sequencing data, this lecture will focus on recent progress in the discovery, genotyping and visualization of somatic structural variants. We will cover somatic mutation calling pipelines, somatic signatures, complex genomic rearrangements such as Chromothripsis [5] and give a general introduction to analytical methods applicable to whole-genome Cancer Genomics data sets. The associated workshop will cover these topics in more depth and for a real cancer genomics data set we will apply Delly [6] to discover, genotype and visualize somatic structural variants.

[1] A global reference for human genetic variation. 1000 Genomes Project Consortium. Nature. 2015 Oct 1;526(7571):68-74.

[2] International network of cancer genome projects. International Cancer Genome Consortium. Nature. 2010 Apr 15;464(7291):993-8.

[3] COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer.
Forbes et al. Nucleic Acids Res. 2011 Jan;39(Database issue):D945-50.

[4] Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. Gerlinger et al. N Engl J Med. 2012 Mar 8;366(10):883-92.

[5] Genome sequencing of pediatric medulloblastoma links catastrophic DNA rearrangements with TP53 mutations. Rausch et al. Cell. 2012 Jan 20;148(1-2):59-71.

[6] DELLY: structural variant discovery by integrated paired-end and split-read analysis. Rausch et al. Bioinformatics. 2012 Sep 15;28(18):i333-i339.

Epigenetics and Imprinting in human developmental disorders [including 'Multilocus imprinting disorders' and new imprinting phenotypes]

Karen Temple
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University of Southampton and Wessex Clinical Genetics Service, Univ. Hospital Southampton , UK

Epigenetics

Different tissues are characterised by different functions and different patterns of gene expression despite each cell 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 the 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 epigenetic patterns within the male and female germ line that resist post fertilisation reprogramming of the zygote. Imprinted genes play an important role in fetal growth and their carefully regulated expression is vital for normal cellular metabolism and human behaviour.

Human imprinting disorders are congenital disorders of growth, development and metabolism, associated with disturbance of gene dosage at imprinted loci across the genome. There are eight well recognised imprinting developmental phenotypes that can be diagnosed clinically in childhood associated with specific imprinted loci:- Prader Willi, Angelman, Silver Russell, Beckwith Wiedemann, Temple, Kagami Ogata, Transient Neonatal Diabetes and Pseudohypoparathyroidism type 1B syndromes but imprinting errors may also cause nonspecific growth phenotypes and altered timing of puberty. Several molecular mechanisms are reported in patients including uniparental disomy, copy number errors and gene mutations with a parent of origin effect on the phenotype, but include epigenetic aberrations. These may be the result of genetic and environmental effects at different times during the development of the oocyte, the sperm or the zygote.

Some patients with imprinting disorders have multi-locus imprinting disturbance (MLID). Causative trans-acting mutations in MLID patients have been demonstrated in a number of genes including *ZFP57*: *(*Mackay,

Nat Gen, 2008) in the affected individual and *NLRP2* (Meyer, Plos Gen, 2009 and *NLRP5* (Docherty, Nat Comms, 2015) in the mother. Maternal-effect mutations of *NLRP7* and *KHDC3L* cause familial biparental hydatidiform mole and *PADI6* (Xu Y AJHGm 2016), another protein in the maternal subcortical complex, causes recurrent miscarriages. The clinical phenotypes of these MLID imprinting disorders therefore range from miscarriage to a classical imprinting disorder and include less classical non-specific developmental issues. For example, offspring of mothers with *NLRP5* mutations have heterogeneous clinical and epigenetic features, and cases include a discordant monozygotic twin pair, individuals with idiopathic developmental delay and autism, and families affected by infertility and reproductive wastage.

It is likely that patients with imprinting disorders are not being diagnosed because:-

1) phenotypes do not fit neatly into the classic well-recognised imprinting disorders
2) epigenetic testing is not part of routine screening for patients with an unknown diagnosis
3) exome analysis focuses on patients and not their parents

4) 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.)

http://www.imprinting-disorders.eu/

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Mackay DJG, et al. Hypomethylation at multiple imprinted loci in individuals with transient neonatal diabetes is associated with ZFP57 mutations Nature Genetics 2008 Aug;40(8):949-51

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Non-coding mutations/long-range effects

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Institut für Humangenetik
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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.

Recommended literature:
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Klopocki E, Mundlos S. Copy-number variations, noncoding sequences, and human phenotypes. Annu Rev Genomics Hum Genet. 2011;12:53-72.
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ENCODE project - https://www.encodeproject.org/

Oligogenic diseases

Nicholas Katsanis
Duke University, USA

Thursday, May 4

Molecular inversion probes and Saturation Genome editing

A. Hoischen

Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands

Here I will describe latest high-throughput methods for variant identification and variant interpretation.

Novel targeted re-sequencing approaches [1-3] allow a systematic discovery of (de novo) genetic mutations associated with complex diseases such as intellectual disability or autism spectrum disorder. These are progressing to a point where a "genotype-first" approach is followed up be “reverse phenotying” i.e. changing the classical phenotype-first paradigm [4].

One of the very efficient high-throughput targeted re-sequencing approaches is discussed here in greater detail: molecular inversion probes [2,3]. This approach also allows very accurate detection of mosaic mutations [3].

While variant detection becomes increasingly easy, the interpretation of variants at a high-throughput scale is only very recently getting within reach. To this end CRSISPR/Cas9 based tools like genome saturation editing [5]hold great promises. A first example has shown that all possible missense variants of an entire exon of disease genes can now be tested for their functional consequence [5]. Highlights of this novel technology will be presented here.

Recommended reading:

1. Mamanova L et al. Target-enrichment strategies for next-generation sequencing. Nat Methods. 2010 Feb;7(2):111-8.
2. O'Roak BJ et al. Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. Science. 2012 Dec 21;338(6114):1619-22.
3. Hiatt JB et al. Single molecule molecular inversion probes for targeted, high-accuracy detection of low-frequency variation. Genome Res. 2013 May;23(5):843-54.
4. Stessman HA, Bernier R, Eichler EE. A genotype-first approach to defining the subtypes of a complex disease. Cell. 2014 Feb 27;156(5):872-7.
5. Findlay GM et al. Saturation editing of genomic regions by multiplex homology-directed repair. Nature. 2014 Sep 4;513(7516):120-3.

Long read sequencing

Evan E. Eichler
Department of Genome Sciences and Howard Hughes Medical Institute, University of Washington, Seattle, WA

The human genome is arguably the most well-assembled reference assembly yet many gaps remain and aspects of its structural variation remain poorly understood even ten years after its completion. The discovery and resolution of this variation is critical to understanding disease. I will present our most recent work sequencing human and nonhuman primate genomes using single-molecule sequencing technology. We have developed methods to detect indels and structural variants from several bases up to 50 kbp. We have closed or extended ~50% of the remaining interstitial gaps in the human genome and find that 80% of these carry long polypyrimidine/purine tracts multiple kilobases in length. Comparing the single haplotype to the human reference, we resolve >35,000 structural variants and >500,000 indels at the base-pair level with 99.9% sequence accuracy. More than 50% of insertions and deletions <2 kbp in length are novel representing large swaths >10 Mbp of undiscovered genetic variation within human genomes. We find that such sequences vary extensively in copy number and affect functional elements in the genome. In addition, the analysis uncovers other categories of complex variation that have been difficult to assess, including mobile element insertions as well as inversions mapping within more complex and GC-rich regions of the genome. Our results suggest a systematic bias against longer and more complex repetitive DNA that can now be partially resolved with new sequencing technologies. I will discuss the potential of this technology to create accurate *de novo* assemblies of additional human and nonhuman primate genomes that more comprehensively capture the full spectrum of human genetic diversity and its importance to our understanding of genetic variation and disease.

Whole genome sequencing of 3,500 individuals in an isolated population: the Sardinia Sequencing Project

Carlo Sidore
CNR, Institute of Genetics Research, Sassari, Italy

Genome-wide association studies (GWAS) have increasingly furthered our understanding of the molecular basis of many complex traits by finding, through genotyping and imputation, loci associated with many different traits. However, studies based on variants present in common genotyping arrays and imputation panels may not capture the fraction of human genome variation that is rare or geographically restricted and unique to specific populations. To advance our understanding of the genetics of a variety of traits in the Sardinian population, we are studying a sample of 6,602 individuals recruited from the population of a

cluster of 4 small towns in Sardinia. Using whole genome sequencing, we sequenced DNAs from 3,514 Sardinian individuals enrolled either in this project or in a parallel project on autoimmune diseases, at an average depth of coverage of ~4X. We successfully identified and genotyped >23M single nucleotide polymorphisms (30.6% of them novel, not in dbSNP v135) with an error rate of 0.15%. To increase the power to detect association, we are using the haplotypes generated by sequencing of these individuals to impute missing genotypes in the remaining 6,602 already genotyped with an integrated map of 4 GWAS Illumina arrays (ImmunoChip, CardioMetaboChip, ExomeChip and OmniExpress). Strikingly, imputation using our Sardinian reference panel shows greatly increased accuracy when compared to an equal size reference panel of European haplotypes generated by the 1000 Genomes Project (average imputation accuracy, rsqr=0.9 compared to 0.75 for alleles with frequency 1-3%). With a larger reference panel, imputation accuracy of variants with frequency 1-3% reaches 0.94 %, giving us the possibility of analyzing the rare frequency domain in the Sardinian population.

We will present the results of our GWAS in the Sardinian population focusing on lipid levels, inflammatory markers and height. We will give examples of founder effect and highlight how our approach would help in elucidate the mechanism behind complex diseases and phenotypes.

How recycling big data can help to improve to diagnose and treat disease

Lude Franke
University of Groningen, Groningen, The Netherlands

Developing a new drug is now typically costing over 3 billion Euro's. Is it possible to save some of these enormous amounts of money? Within the UMCG we are developing new computational methods to speed up and 'de-risk' drug development. We do this by studying 10,000 genetic risk factors that have been found in the last 10 years for many different diseases, and by ascertaining whether these genetic risk factors show effects on other molecular levels, such as gene expression (so called trans-eQTLs, see Westra et al, Nature Genetics 2013, Zhernakova et al, Nature Genetics 2017), methylation (so called trans-meQTLs, see Bonder et al, Nature Genetics 2017) or protein levels, with the aim to identify the disrupted disease-causing processes for these diseases. By targeting these biological pathways and genes through drugs, it might be possible to treat patients. In this presentation I will describe how we recycle large amounts of publicly available data (Fehrmann et al, Nature Genetics 2015, Deelen et al, Genome Medicine 2015), what computational and statistical methods we develop to do this (Pers et al, Nature Communications 2015), and how we have now implemented this in the genetics department to better diagnose patients. Finally I will outline ongoing strategies that we are currently pursuing to also use this information to better treat patients.

AFTERNOON WORKSHOPS

Session I — NGS diagnostic variant interpretation – **C. Gilissen & T. Pippucci**

Genetic Imprinting - **Karen Temple**

Genomic exotica - **Nicholas Katsanis**

Mechanisms for non-coding mutations - **Eva Klopocki**

Session II — NGS diagnostic variant interpretation – **C. Gilissen & T. Pippucci**

How to set up a NGS lab? - **Alexander Hoischen**

Copy number variations - **Evan E Eichler**

Genetic Imprinting

Karen Temple
Faculty of Medicine,
University of Southampton and Wessex Clinical Genetics Service, Univ. Hospital Southampton , UK

The workshop on imprinting disorders will be:

1) An opportunity to ask questions about imprinting and discuss the cycle of epigenetic establishment, maintenance and removal

2) Interpret pedigrees from families with imprinting disorders and work through examples where the parent of origin impacts on molecular results

3) Challenge your knowledge of the clinical aspects and consequences of this group of conditions. There are 8 well recognised phenotypes which mainly impact on growth, metabolism and behaviour [Beckwith Wiedemann Syndrome, Silver Russell Syndrome, Transient neonatal diabetes, Temple Syndrome, Wang Kagami Ogata Syndrome, Prader Willi Syndrome, Angelman Syndrome, Pseudohypoparathyroidism type 1B.] The synromes can be difficult to diagnose and have long term impacts on health and may provide clues to understanding more about common chronic long term metabolic disorders.

Friday, May 5

The UK's 100,000 Genomes Project

Augusto Rendon PhD
Director of Bioinformatics, Genomics England
Principal Research Associate, Department of Haematology, University of Cambridge

The UK's 100,000 Genomes Project was set out to sequence patients with rare diseases and cancer. It has been operating in its main phase since 2016: it has been rolled out to recruit participants from over 80 hospitals across England, with other UK nations currently joining; it has sequenced the genomes of over 20,000 participants; and returned diagnostic results from over 4,000 genomes. The project aims to act as a catalyst to transform the National Health Services into a health system where genomic medicine informs routine medical practice in the digital era, and where patients also become research participants, thereby enabling basic and translational research. There are many challenges being tackled to fully deliver on this aim. This presentation focuses on challenges related to data and genome interpretation. It will not discuss challenges around consent, sample preparation, sequencing, or alignment and variant calling.

Data:

From a data perspective, the Programme can be understood as a logistics operation to capture clinical, sample and genome data; integrate it with existing knowledge – be it external (say ClinVar) or internal (the results from previous patients); interpret these data together to provide clinicians with diagnostic insights; and finally, capture the decisions and outcomes that follow to further enrich the existing knowledge base. Careful data modelling becomes a pivotal effort. I will describe some of the systems and approaches we have for data modelling, in particular, two approaches to instantiate a database of data models; modelling approaches; and the relationship between the data model and the rules that validate that data adhere to the specified model. This will be a rather technical section.

Interpretation:

The Programme recruits against nearly 200 different conditions in the Rare Diseases programme, with variable family configurations. In the Cancer programme we recruit participants with over 10 different kinds of tumours. This requires interpretation approaches that can maximally exploit the family designs and effectively deal with the plurality of conditions being recruited.

Following the approach from several other exome and whole genome sequencing efforts, we have adopted a virtual gene panel approach to interpretation. To do so, we have created an application called PanelApp – a crowdsourcing panel curation tool. The application has enabled us to map the conditions currently being recruited in the programme to over 150 gene panels. Each of those panels derives its content from diagnostic panels used by other efforts, and importantly, content suggested by the community. After this phase, in house curation reviews the evidence behind the genes in each panel and makes it available for use in interpretation. At its core, a gene panel is a set that associates a set of conditions with genes and modes of inheritance.

To select appropriate panels for a particular family's condition, we have developed an application that uses the phenotypic information (currently encoded as HPO terms) and finds closest panels based on the phenotypic description of the disorders associated with panels. The referring clinicians are then able to select which of the candidate panels they would like applied, or if additional panels need to be considered

In Rare Diseases, we follow an interpretation approach with two parallel strategies. One approach, which internally we call "tiering", seeks to classify variants into tiers after applying filters for variant frequency, variant segregation matching mode of inheritance and panel membership. The algorithm follows different paths if incomplete penetrance is suspected in that family, with a further "affected only" analysis. Further classification is obtained by considering the consequence to transcript. As a second strategy, we use variant prioritisation algorithms that are less dependent on panels to highlight variants that may have been missing due to panel content. Currently this depends on the Exomiser, Phevor and Vaast algorithms.

In Cancer, we have adopted a two part diagnostic report. A first part focuses on variants that are actionable as defined in two categories: variants with actionable information (therapeutic or diagnostic) as present in the mycancergenome.org knowledgebase; and those variants appearing in the cancer gene census. A second part of the report focuses on analyses derived from the whole genome such as signatures of mutation, mutational load, regions of hypermutation, and structural variation.

Using phenotypic data to recognise genotypes causing developmental disorders

David FitzPatrick
University of Edinburgh, UK

Clinically-defined syndrome diagnoses have an excellent record in predicting defined sets of causative genotypes. The Deciphering Developmental Disorders (DDD)[2,3] project is a UK- and Ireland-wide study that aims to develop and use new genetic technology and statistical analyses to make a definitive diagnosis in individuals with severe or extreme developmental disorders. DNA samples are available from ~13,500 affected individuals have been recruited with 10,000 of these also having samples available from both parents.

We have recently reported a significant excess of damaging de novo variants in 94 different genes in a cohort of 4294 probands with previously undiagnosed developmental disorders[2]. The genome wide significance was based on human genetic data alone. In this talk I want to explore the extent to which clustering in phenotypic space reflects the existence of specific diagnostic genotypes, and, conversely, whether probabilistic phenotypic models constructed for disease genes with SNVs have diagnostic potential.

I will present some of the preliminary data on the use of facial imaging, growth z scores, developmental milestones and HPO terms. The optimal approach to analysis of these data is not yet clear. I will present

some results using the Mclust model-based clustering algorithm applied to the DDD growth and developmental data. I will also present results of a strategy whereby naïve Bayes models were constructed for all disease genes on the basis of the aggregated phenotypic characteristics for the given gene using a subset of the cohort. We then assigned the remaining probands to the gene whose model they matched best an compared this to the observed genotypes.

The phenotype is going to become more important component of the computational approach to the analysis of genome wide sequencing data[1] and a consistent approach to the collection and utilitation of such information is a vital part of study design

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2: Deciphering Developmental Disorders Study.. Prevalence and architecture of de novo mutations in developmental disorders. Nature. 2017 Feb 23;542(7642):433-438. doi: 10.1038/nature21062. Epub 2017 Jan 25. PubMed PMID: 28135719.

3: Deciphering Developmental Disorders Study.. Large-scale discovery of novel genetic causes of developmental disorders. Nature. 2015 Mar 12;519(7542):223-8. doi: 10.1038/nature14135. Epub 2014 Dec 24. PubMed PMID: 25533962.

4: Ferry Q, Steinberg J, Webber C, FitzPatrick DR, Ponting CP, Zisserman A, Nellåker C. Diagnostically relevant facial gestalt information from ordinary photos. Elife. 2014 Jun 24;3:e02020. doi: 10.7554/eLife.02020. PubMed PMID: 24963138; PubMed Central PMCID: PMC4067075.

Single cell sequencing and applications to PGD

Thierry Voet

Department of Human Genetics, University of Leuven, Belgium & Sanger Institute-EBI Single-Cell Genomics Centre, Wellcome Trust Sanger Institute, Hinxton, UK.

Single-cell omics enables investigating the extent and nature of genomic and transcriptomic heterogeneity that occurs within a tissue and as such to provide novel understanding of both normal and disease developmental processes. In this respect, we have developed various wet-lab and computational methods that allow analysing a solitary cell at high resolution via microarray, SNP-array and next-generation sequencing platforms [1-5]. In addition, we recently developed methods to sequence both the DNA and the RNA of the same single cell, enabling genotype-phenotype correlations on the single-cell level [1,6,7]. Data on the application of these methods to study the nature, extent and biology of cellular heterogeneity in cleavage stage embryogenesis [2,8,9] and carcinogenesis [10,11] will be presented. Finally, single-cell genomics provides a platform for novel clinical applications. We developed a novel fast method for single-cell genome-wide haplotyping and imputation of genetic variants causing Mendelian disorders, allowing a generic approach for

preimplantation genetic diagnosis (PGD) of human cleavage stage embryos in the clinic [2,12]. This and a visionary on the future will be presented.

WG: Discovering structural variants in cancer using NGS data

Tobias Rausch
EMBL Heidelberg, Germany

This workshop is aimed at researchers who are applying or planning to apply high throughput sequencing technologies in cancer research and wish to familiarize themselves with bioinformatics tools and data analysis methodologies specific to whole-genome cancer data. We will explain key concepts in Cancer Genomics and provide some examples on how one can estimate the ploidy, purity and heterogeneity of a Cancer sample using whole-genome sequencing data. We will then focus on somatic mutation calling, in particular for structural variants and highlight some challenges & caveats for that. Using real data, we will then call somatic structural variants using Delly [1] and explain some basic analysis steps and crucial quality control methods. We will also discuss visualizing complex re-arrangements. If you want to follow the tutorial on the command-line some experience with the Linux shell and R Statistics is highly recommended but you can also attend the workshop if you are just interested in general concepts and an overview of the Bioinformatics techniques used for somatic structural variant calling.

[1] DELLY: structural variant discovery by integrated paired-end and split-read analysis. Rausch et al. Bioinformatics. 2012 Sep 15;28(18):i333-i339.

WG: Single-cell (multi-omics) sequencing

Thierry Voet
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Our knowledge on the nature and rate of genome mutation in a developing human being or any other organism is at best rudimentary. In addition, the degree of cellular selection acting on somatic genetic variants acquired during development, and the contributions of these mosaic somatic variants to phenotype and disease etiology remain largely unknown. Standard methods sequence DNA that has been extracted from a population of cells, such that not only the genetic composition of individual cells is lost, but also de novo mutations in cell(s) are often concealed by the bulk signal. Single-cell genome analyses can overcome these issues. Recent genome-wide studies on single cells have delivered new insights into the nature and frequency of DNA mutations occurring during human gametogenesis, embryogenesis and neurogenesis beyond those

described in current textbooks. We will focus on the different workflows for single-cell genome sequencing and analysis of the data, their pros and cons, and where the field is progressing to.

Also, single-cell epigenome sequencing methods will be discussed. Epigenomic mechanisms enable functional diversity across cells with identical genomes, and its study is fundamental to understanding cellular identity, cellular function and phenotypes that are not predicted by genotype alone.

Finally, we will focus on single-cell multi-omics assays –enabling genome-plus-transcriptome or epigenome-plus-transcriptome sequencing of the same single cell– and their application to understand the biology of cellular heterogeneity in health and disease.

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AFTERNOON WORKSHOPS

Session I	Discovering structural variants in cancer using NGS data - **Tobias Rausch**
	Duplications and evolution of human gene innovation - **Evan E Eichler**
	How to do RNASeq - **Lude Franke**
Session II	Discovering structural variants in cancer using NGS data - **Tobias Rausch**
	Large genomics projects - **Augusto Rendon & David Fitzpatrick**
	How to do single cell genomics? - **Thierry Voet**

THE GUGLIELMO PROJECT LAUNCHED BY THE EUROPEAN SCHOOL OF GENETIC MEDICINE IN BERTINORO, ITALY, FOR HIGH SCHOOL STUDENTS

2016 Edition

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 theMediterranean 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 MBertinoro 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 started 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 were hosted in Bertinoro to attend the last morning lecture of the 29 th 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, gave a talk on the public understanding of medical genetics directed to the general public on May 9 at 5:00 pm in Forlì.

On May 12 th the ESGM students of the European School of Genetics Medicine became 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 12 th the students attended the lecture of Prof. Dagan Wells from Oxford on preimplantation genetic diagnosis. In the afternoon they divided 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.

The promoters of the Guglielmo Project 2016 were: - 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.

Which goals has the Guglielmo Project achieved in 2016?

- It increased the knowledge about human genetics and genetic disease among the young people

- It tested new tools which will facilitate the understanding of genetics for users of genetic medicine and for the general public

- It proved the need of new courses for science teachers from secondary school

- It strengthened the cooperation between University and High School networks.

The picture below shows a moment of the concert played by the High School students of Verona during an intermission of the conference of the 2007 Nobel prize winner Prof. Mario Capecchi at the Teatro Diego Fabbri in Forlì on May 9, 2016. Capecchi is the second from right in the picture, while the image of Guglielmo (who holds a violin he is making) is projected on the screen.

2017 Edition

What the Guglielmo Project will be like in 2017?

During the 2016 Guglielmo project there were concerts of Italian and Persian music played by some of the students participating in the project and highly appreciated by the others. This prompted the addition of music to the 2017 Guglielmo project which will be focused in particular on the exchange of young students between Iran and Italy in the fields of genetics and music. The extension of the Guglielmo project to music reflects the fact that music is a universal language leading to the same conclusion of modern genetics regarding mankind which says: "We are all different and all related. Races do not exist!" (from the inscription at the entrance of the Musée de l'Homme in Paris). In 2017 the Guglielmo project will involve young medical students from Iran and Italy as well as young musicians by launching in collaboration with Accademia di S.Cecilia in Rome, one of the oldest musical Academies in the world and USERN (Universal Scientific Education and Research Network-http://usern.tums.ac.ir) a fund raising campaign for fellowships which will allow exchange programs of young students in both fields of music and genetic medicine. The fellowships in the latter field will aim in particular at developing genomic research which will take advantage of the enormous population resources of Iran and Sardinia, following the model of the ongoing SardiNIA/ProgeNIA sequencing project led by Prof. Francesco Cucca in Cagliari and will focus on research in primary immunodeficiencies. In addition the activities already established in 2016 in Forlì and other Italian cities (Cagliari, Verona, Genova) with the participation of High School science teachers and students will be continued. A special course for science teachers will be started in parallel with the 2017 ESGM course for young geneticists in Bertinoro in May 2017.

Program of the Guglielmo Project in 2017

On May 4 and 5 lectures and workshops for Italian students and science teachers will be held in Bertinoro and Forlì. On Saturday May 6 Dr. Evan Eichler will give a talk in English for High School students and teachers at 10:00 in Forlì, continuing the tradition started last year by Dr. Mario Capecchi

All the participants of the course in Clinical Genomics and NGS are welcome !!!

For further information re: the Guglielmo Project and didactic material for science teachers needed for the preparation of students from High Schools for this project, please contact Prof. Beatrice Zanini (beatrice.zanini@unige.it)

ABSTRACTS OF STUDENTS POSTERS

Identifying Genetic causes of Autosomal Recessive Intellectual Disability.

Al-Maawali A. Al-Murshadi F. Al-Kindi A. Althahli K. Al-Futaisi A. Al-Hashmi N. Scott P. AlSaegh A. Zadjali F.

Keywords: medical genomics; database; Mendelian disorder; DNA sequencing; whole-exome sequencing; mutation; animal models; counseling

Intellectual disability (ID) is a limitation in intellectual functioning and adaptive behavior. Genetic etiologies play a major role in ID, and its prevalence is estimated to be three times more in countries known for consanguineous marriages. The practice of consanguineous marriage has been the culturally preferred in Oman and estimated to be 50-55% of marriages, among which the majority are first cousin marriages. This increases the incidence of autosomal recessive disorders that have both a social and an economical impact on the nation. The contribution of recessive disorders to ID is as high as 25% of genetic causes. Thus far, only minority of these causative autosomal recessive genes have been identified. Nevertheless, the new next generation sequencing technologies made gene discovery more achievable and rapid.

Whole exome sequencing (WES) is defined as sequencing the exons of all known protein-coding genes. When WES is combined with homozygosity mapping, it has the added benefit of being able to offer a rapid method for screening for candidate disease-causing mutations. The aims of this study are, first, to create a database of detailed phenotypes of affected families. This will assist in identifying new syndromes or further delineating and characterizing known phenotypes. Second, to establish a rapid method of identifying candidate mutations by using WES on one or more affected individuals within a family. Third, perform gene expression and functional studies tailored to the identified mutations to provide additional evidence for causation.

To date we have being able to recruit more than fifty families where WES data are being analyzed. The clinical characteristics of these patients was reviewed. Further confirmation by Sanger sequencing of candidate causative variants is being carried out. Further we have recently recruited additional 30 families where WES raw data is being awaited.

Phenotypic Variability in Autosomic Dominant Polycystic Kidney Disease

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Introduction

Autosomic Dominant Polycystic Kidney Disease (ADPKD) is a mendelian disorder characterized by late onset renal cysts and cysts in other organs, which finally leads to end-stage renal disease and other extra-renal complications. However, <1% of the cases exhibit an early onset disease. This phenotypic variability hinders patients' diagnosis and prognosis, especially during the prenatal period. The objective of this study is to describe the clinical variability of ADPKD in patients diagnosed at Fundación Jiménez Díaz Hospital between 2013 and 2016.

Methods

We performed a retrospective study of the cases genetically diagnosed of ADPKD in the Genetic Department of Fundación Jiménez Díaz Hospital in Madrid, Spain between 2013 and 2016.

The genetic diagnosis was achieved by the analysis of the *PKD1* and *PKD2* genes by Sanger sequencing. Due to the presence of 6 highly homologous sequences of PKD1 in chromosome 16p, we used a protocol validated in more than 100 families with ADPKD, based on the specific amplification of the PKD1 gene by long-PCRs followed by several internal PCRs.

Results

We obtained 9 cases which were diagnosed during the period of study; 4 of them were caused by changes in PKD1, and the remaining 5 by changes in PKD2. Between both groups, there was not a significant difference in the age of diagnosis, in mean kidney size, nor in estimated Glomerular Filtration Rate (eGFR). The diagnosis was suspected in most of the cases given the symptoms reported by the patients, which included: haematuria, back pain and recurrent urinary tract infections. However, one case in each group was diagnosed incidentally. One of them was suspected in the prenatal period by ultrasound signs of renal cysts. After birth, the patient turned out to be carrier of a hypomorphic variant of PKD1, inherited from an asymptomatic parent. The other case was diagnosed in an asymptomatic pregnant patient whose ultrasound depicted a foetus with right kidney agenesis, cysts on the left kidney, olygohydramnios and single umbilical artery. We found 5 novel variants, 2 missense changes and one frameshift mutation of PKD1, and one missense and one splicing site mutation of PKD2.

Conclusions

Still in a small sample of cases of ADPKD, is possible to notice the great range of phenotypic variability of this disease, which oscillates between the asymptomatic carriers and those cases diagnosed prenatally, even

within the same family. For this reason, it is relevant to encourage the research on gene modifiers and complex patterns of inheritance, which may explain how a mendelian disease can produce such unpredictable phenotypes. Ultimately, this will help clinicians to provide a better diagnosis and prognosis.

Clinical and molecular findings in a Moroccan family with Jervell and Lange-Nielsen syndrome: A case report

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Jervell and Lange-Nielsen syndrome (JLNS; MIM 220400) is a rare autosomal recessive cardioauditory ion channel disorder that affects 1/200000 to 1/1000000 children. It is characterized by congenital profound bilateral sensorineural hearing loss (SNHL), a long QT interval, ventricular tachyarrhythmias, and episodes of torsade de pointes on the electrocardiogram. Cardiac symptoms arise mostly in the early childhood and consist of syncopal episodes during periods of stress, exercise, or fright and are associated with a high risk of sudden cardiac death. JLNS is caused by homozygous or compound heterozygous mutations in *KCNQ1* on 11p15.5 or *KCNE1* on 1q22.1-q22.2. We report on a 10 year-old boy with congenital hearing loss, a severely prolonged QT interval who presented with multiple episodes of syncope. His parents are first-degree cousins. We performed Sanger sequencing and identified a homozygous variant in *KCNQ1* (c.1343dupC, p.Glu449Argfs*14). The identification of the genetic substrate in this patient confirmed the clinical diagnosis of JLNS and allowed us to provide appropriate management to the patient and genetic counseling to his family. In addition, this finding contributes to our understanding of genetic disease in the Moroccan population.

Keywords: Jervell and Lange-Nielsen syndrome, long QT syndrome, deafness, Moroccan, mutation.

Mutations in the leukemia inhibitory factor receptor (*LIFR*) gene and *Lifr* deficiency cause urinary tract malformations

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Congenital anomalies of the kidneys and urinary tract (CAKUT) are the most common cause of chronic kidney disease in children. As most CAKUT cases are genetically unexplained, we aimed to identify new CAKUT causing genes. Using whole-exome sequencing and trio-based *de novo* analysis, we identified a novel heterozygous *de novo* frameshift variant in the leukemia inhibitory factor receptor (*LIFR*) gene causing instability of the mRNA in a patient presenting with bilateral CAKUT and requiring kidney transplantation at one year of age. *LIFR* encodes a transmembrane receptor utilized by IL-6 family cytokines, mainly by the leukemia inhibitory factor (LIF). Mutational analysis of 121 further patients with severe CAKUT yielded two rare heterozygous *LIFR* missense variants predicted to be pathogenic in three patients. LIFR mutants showed decreased half-life and cell membrane localization resulting in reduced LIF-stimulated STAT3 phosphorylation. *LIFR* showed high expression in human fetal kidney and the human ureter, and was also expressed in the developing murine urogenital system. *Lifr* knockout mice displayed urinary tract malformations including hydronephrosis, hydroureter, ureter ectopia, and, consistently, reduced ureteral lumen and muscular hypertrophy, similar to the phenotypes observed in patients carrying *LIFR* variants. Additionally, a form of cryptorchidism was detected in all $Lifr^{-/-}$ mice and the patient carrying the *LIFR* frameshift mutation. Altogether, we demonstrate heterozygous novel or rare *LIFR* mutations in 3.3% of CAKUT patients, and provide evidence that *Lifr* deficiency and deactivating *LIFR* mutations cause highly similar anomalies of the urogenital tract in mice and humans (supported by Else Kröner-Fresenius-Stiftung grant no. 2014_A234).

No association of common luteinizing hormone receptor variants with ovarian response to Elonva-controlled ovarian hyperstimulation

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Introduction: There are three common polymorphisms with impact on receptor function in the *LHCGR* gene – ins18LQ (rs2293275), Asn291Ser (rs12470652) and Ser312Asn (rs2293275). So far the influence of combined effect of these variants to controlled ovarian hyperstimulation was not studied. The aim of this study was to ascertain the influence of these variants in *LHCGR* and their genotype combinations with the ovarian response to Elonva-controlled ovarian hyperstimulation (COH).

Materials and Methods: DNA samples from 48 "low" , 191 "intermediate" and 34 "high" responders (273 patients in total) were genotyped using fragment analysis for ins18LQ and TaqMan assays for Asn291Ser and Ser312Asn variants.

Results: The genotype frequency was 56,0%-64,7% for wt/wt, 29,4%-31,3% for wt/ins18LQ and 5,9%-6,3% for insLQ/insLQ genotypes in insLQ variant within compared groups according to COH response. It was 81,3%-94,1% for Asn/Asn, 5,9%-18,8% for Asn/Ser and 0%-0,5% for Ser/Ser genotypes in Asn291Ser variant. It was 35,3%-35,6% for Ser/Ser, 39,6%-55,9% for Ser/Asn and 8,8%-25,0% for Asn/Asn genotypes in Ser312Asn. No difference in genotype frequency was found between "low", "intermediate" and "high" responders to COH ($P \geq 0,05$). These variants cannot predict individual response to COH. Mutual genotype combinations of studied variants didn´t disclosed any significant differences, due to their low prevalence in compared categories.

Conclusions: The prevalence of *LHCGR* gene variants– ins18LQ, Asn291Ser and Ser312Asn is not statistically different within low, intermediate and high responders to Elonva-controlled ovarian hyperstimulation. No implementation in individual COH response prediction is possible by their examination.

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Authors disclosure information: None for all authors.

Rare copy number variants in array-based comparative genomic hybridization in early-onset skeletal fragility

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Objectives: Early-onset osteoporosis is characterized by low bone mineral density (BMD) and reduced bone strength since childhood or young adulthood. Although several monogenic forms have already been identified, the spectrum of mutations and genes behind this condition remain inadequately characterized. Furthermore, it is not clear whether genetic factors determine susceptibility to bone fractures in children with normal BMD. In order to further explore the genetic background we screened a cohort of 69 young Finnish patients with mild to severe skeletal fragility for novel pathogenic copy-number variants (CNVs).

Methods: We used a custom-made high-resolution 400K comparative genomic hybridization array (array-CGH) with enriched probe density in over 300 genes important for bone metabolism and over 800 genes involved in ciliary function. Findings were validated with breakpoint PCR or whole genome sequencing.

Results: The study cohort included 15 subjects with primary osteoporosis before age 30 years and 54 subjects with a pathological fracture history before age 16 years but mostly normal BMD. Overall, we identified 3 novel likely pathogenic CNVs: a 4.6 kb deletion involving exons 1-4 of *COL1A2* (NM_000089.3), a 11 kb duplication of exon 3 in *PLS3* (NM_005032.6) and a 1.6 Mb deletion affecting the entire ETV1 gene and in part *DGKB*. Mutations in *COL1A2*, encoding the α2 chain of type I collagen, and *PLS3*, encoding plastin 3, have already been linked to monogenic forms of osteoporosis but deletions in *COL1A2* are rare and duplications have not been described in *PLS3*. Both CNVs were identified in subjects with significant osteoporosis and were present also in other affected members in the two families.

Mutations in the transcription factor ETV1, which plays a role e.g. in Ewing sarcoma, and in the diacylglycerol kinase beta (DGKB), have not yet been associated with skeletal fragility. This third CNV therefore needs to be further investigated.

Conclusion: Our study expands the number of CNVs currently known to cause bone fragility and underscores the validity of this method in finding novel candidate genes for early-onset osteoporosis.

This study has been supported by the Swedish Research Council. The authors declare no conflict of interest.

Accurate quantification of a mosaic FBN1 mutation as a source of familiar Marfan syndrome.

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Marfan syndrome is a systemic disorder of connective tissue with a high degree of clinical variability. It is inherited in an autosomal dominant manner. Approximately 75% of individuals with Marfan syndrome have an affected parent. FBN1 is the only gene in which pathogenic variants are known to cause classic syndrome, whereas other forms have been described in genes such as TGFBR1 and TGFBR2.

We present the case of a child diagnosed of classical Marfan syndrome with the pathogenic mutation FBN1: c.4615C>T p.(Arg1539Ter). His half-brother is suspected to present the same phenotype. Their mother went to consult a clinical geneticist. She presented a normal phenotype. Sanger sequencing revealed no variation but careful revision of the mutation position made us suspicious of a low-level variant.

In this work we compare several approaches to accurately quantify the level of suspected mosaicism. The techniques used were Sanger sequencing, SnapShot sequencing, next-generation sequencing (NGS) and digital droplet PCR (ddPCR). All methods aimed to evaluate the somatic mutation burden in peripheral blood.

All techniques but Sanger sequencing accurately detected the mutation level. NGS arises as the perfect approach to analyze the whole coding sequence and quantify, all in a single assay, whereas SnapShot sequencing is the cheapest and easiest approach for directed studies.

Recent scientific literature demonstrates that the incidence of mosaicism events has been underestimated as the low-grade mosaic ratio may stay unrevealed mainly due to technical limitations. New technologies are now offering lower detection limits and would permit to detect low-level mutations. However somatic mosaicisms may be found in both somatic and germline cells and genetic counseling of such cases still remains extremely complicated.

Homozygous frameshift mutations in PIEZO2 cause a non-progressive muscle atrophy with arthrogryposis, perinatal respiratory distress, scoliosis and sensory neuropathy

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Motor neuron disorders (MNDs) are a clinically and pathologically heterogeneous group of neurological diseases, characterized by the degeneration of motor neurons. They are inherited or sporadic and may affect either or both the upper and the lower motor neurons.

Among the hereditary MNDs, Spinal Muscular Atrophy (SMA) accounts for the majority of cases. However, for the remaining ones, the diagnosis is still challenging due to the highly overlapping phenotype and the genetic heterogeneity, which often requires deep next-generation sequencing studies.

Here we report 10 individuals of 4 independent consanguineous families from Turkey, India, Libya, and Pakistan affected by arthrogryposis, respiratory impairment at birth, spine abnormalities and muscular hypotonia. WES, SNPchip-based linkage analysis, DNA microarray and Sanger sequencing led to the discovery of three independent homozygous frameshift mutations and a homozygous deletion of 2 exons in *PIEZO2*, which segregated in the affected individuals. These four mutations are spread across the gene and cause the nonsense-mediated transcript decay and consequently the lack of the PIEZO2 protein. *PIEZO2* encodes a mechanosensitive ion channel that plays a major role in light-touch mechanosensation and mechanotransduction. While the ubiquitous ablation of murine *Piezo2* is postnatally lethal, individuals lacking PIEZO2 develop a not life-threatening, not progressive disorder. This phenotype differs from the spectrum of disorders caused by heterozygous, gain-of-function missense variants in PIEZO2, which are mainly localized at the C-terminus. These diseases comprise distal arthrogryposis 3 (DA3), distal arthrogryposis 5 (DA5) or Marden-Walker syndrome (MWKS), and are characterized by contractures of hands and feet, in addition to variably showing ophtalmoplegia, ptosis, short stature and scoliosis. We speculate that these different phenotypes are caused by an altered neuromuscular development during the fetal life due to the increased activity or the loss of the channel.

Taken together, these findings demonstrate that homozygous frameshift mutations in *PIEZO2* cause muscular atrophy, therefore broadening the spectrum of genes related to neuromuscular disorders and suggest a novel pathophysiological mechanism of these diseases.

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 2012-305121 "Integrated European –omics research project for diagnosis and therapy in rare neuromuscular and neurodegenerative diseases (NeurOmics)".

Assessment of BRAF, NRAS, KRAS and EGFR mutation status in thyroid nodules

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Background: Discrimination between benign and malignant thyroid nodules is usually attempted by fine needle aspiration biopsy (FNAB) followed by cytological assessment. Despite many advances in the diagnosis and treatment of thyroid tumors, these methods result in an 'indeterminate' or 'suspicious' diagnosis in 10-20% of cases. To minimize unnecessary surgeries, we started to investigate the validity of a set of expression markers in FNABs of thyroid nodules. Part of this project is also the refinement of the biomarker set by revealing the somatic mutation status of BRAF, NRAS, KRAS and EGFR. Here we present the mutation frequency data of 134 thyroid nodule samples.

Material and methods: DNA was extracted from 134 frozen thyroid nodules including benign Nodular Goiter (NG), benign Follicular Thyroid Adenoma (FTA), malignant Follicular Thyroid Carcinoma (FTC), malignant Papillary Thyroid Carcinoma (PTC), malignant Medullary Thyroid Carcinoma (MTC) and Anaplastic Thyroid Carcinoma (ATC) using Qiagen AllPrep and quantified using PicoGreen. Somatic mutation status was assessed by using ViennaLab BRAF 600/601, EGFR XL, KRAS XL and NRAS XL StripAssays®.

Results: 58/134 (43.3%) thyroid nodule samples showed mutations in BRAF, NRAS or KRAS. None of the samples showed EGFR mutations. 45/134 (33.6%) samples were mutated in the BRAF gene. In 44 samples we detected BRAF c.1799T>A (p.V600E) and in one sample BRAF c.1801A>G (p.K601E). BRAF V600E mutations were mainly found in PTCs (35/43; 81.4%), but at low levels also in FTCs (5/28), FTAs (1/28) and NGs (1/22). 13/134 (9.7%) samples (predominantly FTA and FTC) were mutated in NRAS (c.182A>G [p.Q61R]), and 3/134 (2.2%) samples were mutated in KRAS (c.35G>C [p.G12A] and c35G>T [p.G12V]). Two samples showed BRAF V600E plus NRAS Q61R (FTA) and one sample showed BRAF V600E plus KRAS G12A mutations (FTC).

Conclusions: Due to the high sensitivity of ViennaLab StripAssays®, which allow the detection of mutated alleles at a 1% level, we found a substantially higher number of BRAF mutations in PTC compared to the literature (81.4% versus 40-45%). Similarly, BRAF mutations in FTC were found at a higher rate compared to published results (17.8% vs 1.3%). This has important prognostic implications, as BRAF-mutated tumors have a higher risk of recurrence.

Association of Single Nucleotide Polymorphisms of IL10 and TGFB with Intervertebral Disc Degeneration

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Background: As the controversy regarding the role of inflammation and disc homeostasis imbalance in pathophysiology of Intervertebral disc degeneration (IVDD), current study was conducted to investigate the role of IL-10 and TGF-β single nucleotide polymorphisms (SNP) in Iranian IVDD patients.

Method: Seventy-six IVDD patients and 140 healthy controls were enrolled in this study. Genomic DNA from peripheral leukocytes was tested for 5 SNPs in IL10 and TGF-β genes through PCR-SSP method.

Results: The 'T' allele of IL-10 -819C/T and the 'C' allele of IL-10 -592A/C were more prevalent among patients, whereas the 'C' and 'A' alleles of respective SNPs were significantly more frequent in controls. The genotypes including 'CT' of IL-10 -819C/T, 'CA' of IL-10 -592A/C, and 'GA' of IL-10 -1082A/G were more common among patients, while the 'CC' genotype of both IL-10 -819C/T and IL-10 -592A/CSNPs were more frequent in controls. In addition, the IL-10 haplotypes including 'ACC', 'ATA', and 'ACA' were significantly associated with disease. Meanwhile, the 'TC' haplotype of TGF-β was more common among patients.

Conclusion: The gene polymorphisms of IL-10 were significantly associated with IVDD in Iranian population; proposing that genomic alterations of anti-inflammatory cytokines could lead to homeostasis imbalance in intervertebral discs and degenerative changes.

Keywords: intervertebral disc degeneration, single nucleotide polymorphism, interleukin 10, transforming growth factor β

Evidence for PTGER4, PSCA and MBOAT7 as risk genes for gastric cancer on the genome and transcriptome level

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Gastric carcinoma (GC) represents the fifth most common cancer type worldwide and the prognosis for affected patients remains to be poor. Effort on characterizing the somatic genomic landscape of GC revealed deep insides into the tumor biology. However, questions on interindividual differences in the risk for GC development remain to be solved. To elucidate germline risk variants and genes, GWAS in the Asian population were performed with success, but so far studies in patients of European origin are scarce.

To validate the Asian GWAS findings and to provide further functional insights we performed a fine mapping association study in 1,926 GC patients and 2,012 controls of European descent using high dense SNP marker sets on chromosome 5p13 and 8q24. We confirmed and refined the GC associations at both chromosomal regions. On chromosome 5p13 the strongest association was observed at rs6872282 (P = 2.53 x 10-04 (RR = 1.22)) and on chromosome 8q24 at rs2585176 (P = 1.09 x 10-09 (RR = 1.34)). We next performed expression quantitative trait (eQTL) analyses using genomewide genotype and gastric transcriptome data from 143 individuals focusing on the GC associated makers. On chromosome 5p13 we found cis-eQTL effects with an upregulation of *PTGER4* expression in risk allele carriers ($P = 1.89 \times 10^{-03}$). On chromosome 8q24 we observed cis-eQTL effects with an upregulation of *PSCA* expression in risk allele carriers ($P = 6.73 \times 10^{-09}$). In addition, we found trans-eQTL effects for 8q24 with an upregulation of *MBOAT7* expression in risk allele carriers ($P = 1.07 \times 10^{-08}$).

In summary, we confirmed and refined the previously reported GC associations at 5p31 and 8q24. Moreover, our data imply the upregulation of PTGER4 and PSCA expression as risk-conferring GC pathomechanisms. In addition, our findings provide evidence that GC risk variants on 8q24 lead to an upregulation of MBOAT7, which

Investigation of Killer Immunoglobulin-like Receptor (KIR) and HLA genotypes to predict the occurrence of acute allograft rejection after kidney transplantation

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Background: After kidney transplantation, natural killer (NK) cells play a pivotal role in triggering the immune response to the allogeneic grafts primarily by their killer-cell immunoglobulin-like receptors (KIR). This process may be one mechanism that contributes to graft rejection. In this study, we have evaluated whether acute rejection after kidney transplantation was associated with predicted NK cell alloreactivity based on KIR gene and ligand along with KIR/HLA compound genotype analysis.

Material and methods: DNA from 65 patients with biopsy-proven acute kidney allograft rejection (AKAR), 61 clinically well graft function (WGF) recipients and 176 healthy subjects was identified for the presence or absence of 10 variable KIR genes (both activating and inhibitory receptors) and their HLA ligands using polymerase chain reaction-sequence specific primers (PCR-SSP) assay.

Results: Although no significant difference in the frequency of individual KIR genes, the gene content, and the haplotypic distribution between the three categories was detected, the frequency of the KIR3DL1+HLA-Bw4* A allele combination was significantly lower in AKAR patients compared to WGF recipients (p = 0.004, OR = 0.34, CI = 0.16-0.72) and healthy subjects (p = 0.019, OR = 0.47, CI = 0.25-0.89). Kaplan-Meier survival test showed that the KIR3DL1+HLA-Bw4* A allele combination could be considered protective for AKAR (p=0.04 by log-rank).

Conclusion: The results of this study suggest that KIR/HLA polymorphism may be a genetic susceptibility factor to alloreactivity dysfunction in the NK cells of patients with AKAR. It is likely that a KIR/HLA

combinatorial study can be beneficial in predicting AKAR occurrence for the purpose of selecting donors appropriately.

Keywords

KIR, HLA ligand, kidney transplantation, transplant rejection

A novel p.Ser282Pro *CPA1* variant is associated with autosomal dominant hereditary pancreatitis

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Introduction: Misfolding CPA1 (carboxypeptidase A1) variants such as p.Asn256Lys induce endoplasmic reticulum (ER) stress and are associated with sporadic chronic pancreatitis (CP). Association with hereditary CP have not been demonstrated. We have identified a novel p.Ser282Pro *CPA1* variant in two Polish families with hereditary CP.

Aim: To perform co-segregation analysis of the p.Ser282Pro *CPA1* variant with CP and assess its functional effect on CPA1 secretion, activity and ER stress.

Method: In the index patients, all exons of *CPA1, PRSS1, SPINK1, CTRC* and CFTR exons 4, 9-11 were sequenced. In the rest of the individuals *CPA1* exon 8 and *CTRC* exon 3 were examined. Effect of the p.Ser282Pro on CPA1 secretion, intracellular CPA1 levels and ER stress markers were assessed in transfected HEK 293T cells.

Results: In Family 1, the index patient, her affected mother and affected uncle were heterozygous for p.Ser282Pro. Unaffected individuals, with one exception, did not carry the *CPA1* variant. In Family 2, the index patient inherited the *CPA1* variant from the affected mother and the heterozygous p.Gly60= *CTRC* variant from the affected father negative for the CPA1 variant. The p.Ser282Pro variant abolished CPA1 secretion and induced ER stress as judged by elevated mRNA levels of the BiP and increased splicing of the XBP1 mRNA.

Conclusion: The p.Ser282Pro *CPA1* variant causes hereditary CP by inducing ER stress similarly to the p.Asn256Lys *CPA1* variant and the misfolding p.Leu104Pro PRSS1 variant. Misfolding CPA1 and PRSS1 variants are similarly strong risk factors and ER stress is relevant pathological mechanism in CP.

Robustness of next generation sequencing-based preimplantation genetic screening (PGS) of chromosome aneuploidies from various cell types with a very low amount of template DNA.

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Introduction: Reliable chromosome aneuploidy detection in a single cell or from very limited amounts of template DNA is a prerequisite for preimplantation genetic screening (PGS) of chromosome aneuploidies. The aim of the study was to assess the minimum amount of DNA in terms of diagnostic robustness and time to diagnosis in next generation sequencing-based (NGS) PGS.

Materials and Methods: Single oocytes, polar bodies, blastomeres, trophoectoderm, somatic ovarian cells, cultured choriocytes / amniocytes and isolated DNA were used to assess the diagnostic reliability of whole genome amplification (WGA; SurePlex), followed by the VeriSeq NGS assays (Illumina; USA). Single sperm were examined using a modified WGA protocol (PMID: 23565289).

Results: All samples with known karyotype were accurately replicated by our diagnostic approach. Novel findings, previous missed by standard karyotyping, were confirmed by MLPA and/or array CGH. During the course of the study we detected a broad variety of aneuploidies, one chaotic embryo and one error in second meiotic division. The ability to detect mosaicism was assessed by artificially mixing DNA derived from blood or cultured choriocytes / amniocytes with known chromosomal constitution. We are able to detect mosaicism in aberrant cells down to 40%, while lower mosaicism could be due to WGA/NGS artifacts.

Conclusions: The utilised NGS-based assay is robust and reliable for routine detection of aneuploidies in clinical PGS in DNA derived from various single cell types and is able to unambiguously detect eventual mosaicism.

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Mutations in the leukemia inhibitory factor receptor (*LIFR*) gene and *Lifr* deficiency cause urinary tract malformations

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Congenital anomalies of the kidneys and urinary tract (CAKUT) are the most common cause of chronic kidney disease in children. As most CAKUT cases are genetically unexplained, we aimed to identify new CAKUT causing genes. Using whole-exome sequencing and trio-based *de novo* analysis, we identified a novel heterozygous *de novo* frameshift variant in the leukemia inhibitory factor receptor (*LIFR*) gene causing instability of the mRNA in a patient presenting with bilateral CAKUT and requiring kidney transplantation at one year of age. *LIFR* encodes a transmembrane receptor utilized by IL-6 family cytokines, mainly by the leukemia inhibitory factor (LIF). Mutational analysis of 121 further patients with severe CAKUT yielded two rare heterozygous *LIFR* missense variants predicted to be pathogenic in three patients. LIFR mutants showed decreased half-life and cell membrane localization resulting in reduced LIF-stimulated STAT3 phosphorylation. *LIFR* showed high expression in human fetal kidney and the human ureter, and was also expressed in the developing murine urogenital system. *Lifr* knockout mice displayed urinary tract malformations including hydronephrosis, hydroureter, ureter ectopia, and, consistently, reduced ureteral lumen and muscular hypertrophy, similar to the phenotypes observed in patients carrying *LIFR* variants. Additionally, a form of cryptorchidism was detected in all *Lifr-/-* mice and the patient carrying the *LIFR* frameshift mutation. Altogether, we demonstrate heterozygous novel or rare *LIFR* mutations in 3.3% of CAKUT patients, and provide evidence that *Lifr* deficiency and deactivating *LIFR* mutations cause highly similar anomalies of the urogenital tract in mice and humans (supported by Else Kröner-Fresenius-Stiftung grant no. 2014_A234

Potential novel disease mechanism in a ciliopathy like phenotype

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Ciliopathies are a group of clinically and genetically overlapping disorders whose etiologies lie in defective cilia. They can manifest as a constellation of features that include retinal degeneration, renal disease, cerebral anomalies, diabetes, obesity and skeletal dysplasias. Many ciliopathies display marked heterogeneity making it challenging for the clinical geneticists reach a definite diagnosis. The advent and dramatic advances of next-generation sequencing (NGS) technologies rapidly translated to fundamental changes in clinical care of patients with genetic disorders. Targeted NGS panel is currently the preferred first-tier approach. However, the diagnosis rate of targeted panels ranges from only 10% to 40%, which is not surprising given the heterogeneity of different genetic disorders. In such cases, research studies using whole exome sequencing (WES) and whole genome sequencing (WGS) are appropriate.

Here we present a case of male patient 8 years old, who presented to the genetic clinic with multiple congenital anomalies. He is a simplex case for non- consanguineous parents. The patient had dysmorphic facial features, bilateral SNHL, retinitis pigmentosa, congenital heart disease, unilateral multicystic dysplastic kidney, penopubic epispadius, cryptoichordism, short limb dwarfism and skin manifestations in the form of patches of hypo and hyperpigmentation. The provisional clinical diagnosis was suggestive of cranioectodermal dysplasia (CED). CED [MIM 218330], also known as Sensenbrenner syndrome, is a rare multiple anomaly syndrome with distinctive craniofacial appearance, skeletal, ectodermal, connective tissue, renal, and liver anomalies, suggesting this disorder a ciliopathic condition. Autozygosity mapping to the patient DNA using Axiom SNP Chip platform and Auto SNPa software show that all the SNPs call on chromosomes 13 are present as homozygous. Closer examination to the SNPs call of the patient on chromosome 13 with his parent reveals that the patient inherited paternal uniparental disomy of chromosome 13. Targeted NGS panel of ciliopathay related genes did not reveal pathogenic variants, so the case was considered for WES. Since there are no known CED genes on chr13, this patient will likely reveal a novel cause of this disorder and the work is underway to investigate this possibility.

The genetics of Primary Ciliary Dyskinesia: from flagellum to cilia

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Cilia are slender hair-like cell surface membrane protuberances that are present in a variety of eukaryotic cells. Cilia are motile structures mainly involved in airway mucous clearance, although they can be immotile in other cell types. The mammalian flagellum is a single motile similar structure that is mainly observed as part of the male gamete. Both are composed of the same type of microtubule-based core structure called the axoneme. The axoneme is composed by nine peripheral doublet microtubules surrounding two central single microtubules, with associated structures such as dynein arms (DA), radial spokes and nexin links. Cilia and

flagella exert various tissue-specific functions during development, tissue morphogenesis and homoeostasis. For instance, they are responsible for mucociliary clearance, generation of mucus flow and cerebrospinal fluid flow, flagellar motility and transport of the ovarian follicle from the Fallopian tube fimbria to the uterus.

Genetic variants that affect ciliary components and lead to improper functioning of cilia are a cause of spectra of diseases collectively known as ciliopathies. Primary ciliary dyskinesia (PCD, MIM #244400) is a well-known autosomal recessive ciliopathy characterised by chronic bronchitis, chronic sinusitis and hearing impairment. Organ laterality defects (*situs inversus*) occur in 50% of PCD patients, known as Kartagener syndrome (KS). In addition, reproductive issues, such as sperm immotility have been described in these patients. Several genes have been already associated with PCD. However, the knowledge about this disease is still scarce and most of the studies are associated with respiratory tract and focused on respiratory cilia morphology, with few studies focused on sperm flagellum morphology.

In a previous work, we studied a small cohort of infertile male patients with total sperm immotility and anomalies in axoneme structure, namely DA absence. We found a novel homozygous variant in the coiled-coil domain containing-103 (*CCDC103*) gene, and several bioinformatics algorithms predicted that this variant is potentially damage. Currently, we are studying the effect of this missense variant in human samples, and a transgenic zebrafish is being generated by a CRISPR-Cas9 system to provide further experimental evidence concerning the effect of this variant on sperm motility.

To better understand the genetic pattern of this ciliopathy we are also conducting the genetic characterization of three children with PCD/KS by whole-exome sequencing. Until know, four foresee pathogenic variants (three new variants) in *CCDC40* and *DNAH5* genes were identified in two of the children. With this work, we expect to increase the current knowledge about the genetics and the pathophysiology of PCD.

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Quantification of Cell Free Fetal DNA in normal pregnancies in the first trimester of gestation by Digital PCR technology.

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INTRODUCTION: Quantification of cell free fetal DNA (cffDNA) can be performed by many different approaches. The most standardized method in male bearing pregnancies is using Y chromosome-specific sequencing such as *SRY* gene. To extend cffDNA quantification to all pregnancies it has being proposed other methods based on locus-specific DNA methylation differences found between fetal and maternal DNA, like *RASSF1A*. The main objective of this study is to quantify cffDNA by both assays *(SRY/GAPDH & RASSF1A/GAPDH)* in maternal plasma samples of normal pregnant women in the first trimester of gestation and to assess its relationship with parameters like Maternal Age, Nuchal Translucency (NT), pregnancy-associated plasma protein A (PAPP-A) or free beta human chorionic gonadotrophin (ßhCG).

MATERIAL & METHODS: For male pregnancies there have been used *SRY/GAPDH* assay and *RASSF1A/GAPDH* assay in a total of 62 maternal plasma samples. For female pregnancies we have developed *RASSF1A/GAPDH* assay in another 40 maternal plasma samples. Maternal plasma DNA was extracted, digested with methylation-sensitive restriction enzyme BstUI, and the fetal specific DNA was quantified by the QX200 Droplet Digital PCR (ddPCR™, Bio-Rad) system.

RESULTS: Pearson´s correlation analysis showed different significances between cffDNA (calculated by *SRY/GAPDH* or/and *RASSF1A/GAPDH* assay) and each parameter. In case of the maternal age for the *SRY* study the results revealed not significance (r=-0.1055, p= 0.4144, n=62) neither for *RASSF1A* assay (r= 0.0317, p=0.7521, n = 102). For the Nuchal Translucency parameter the correlation obtained for *SRY* was (r= 0.2777, p=0.0288, n=62) and for *RASSF1A* (r= -0.0196, p= 0.8450, n = 102). In case of the biochemical parameters, for PAPP-A there was exactly the same significance for both assays *SRY & RASSF1A* (r= 0.2; p= 0.04, n= 62 & 102) and finally for ßhCG parameter in *SRY* assay it was (r= 0.2005, p=0.1182, n=62) and for *RASSF1A* assay (r= 0.1265, p= 0.2050, n = 102).

CONCLUSIONS: The universal epigenetic marker *RASSF1A* has shown an underestimation of fetal fraction compared to *SRY* gene in male pregnancies during the first trimester of gestation. The relationship assessed between cffDNA and the different epidemiological (maternal age), ecographical (NT) and biochemical parameters (PAPP-A and ßhCG) reveal a not significance correlation between them and cffDNA in normal pregnancies in the first trimester of gestation. Nevertheless, it could be a first approach with one of the most sensitive technologies recently incorporated, the Digital PCR, which reveals a high accurate and precise method for cffDNA quantification.

Karyotyping Optimised Online Learning

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Introduction: Karyotyping is one of the skill geneticists should master in their formation. The current form of developing this ability is time consuming for both the fellow and the coordinator. We created an application to fasten the process.

Materials and Methods: Using images captured by Zeiss Axio Imager.N1 and sorted in karyograms with the Ikaros v5.2.8 software, with the help of HTML, CSS, JavaScript, SQL, MySQL, PHP scripting language we developed an education software for medical genetics fellows.

Results: The internet based application aids in the karyotyping learning of new geneticists. It has a theory section, MCQ tests based on it and hands on learning how to recognise the chromosomes and set them into pairs. Using a simple drag and drop system the program stops you from misplacing the chromosomes. It counts the number of false positioning attempts and the time you spend on each test, giving you a progress evaluation feedback. At the end of each test a pop up message appears containing the particularity of the arranged chromosome set(eg: deletion, inversions, duplications etc.). The application can be found at http://optimx.ro/ool/.

Conclusions: This tool will help the future generation of geneticist develop their skills faster and give them more schedule flexibility.

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Next Generation precision diagnostics for the Maturity-Onset Diabetes of the Young (MODY): identification of a novel single-base deletion in the *Carboxyl Ester Lipase* (*CEL*) gene

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Next Generation Sequencing (NGS) technologies are driving the new-age of precision diagnostics toward individualized care. NGS still needs to reach its full potential and changes in sequencing technology leads to challenges in validation, data management and interpretation of results.

We present a *case-study* in a diabetic patient suspected with a MODY in whom we were able to detect the pathogenic variant in a very short time compared to the conventional Sanger sequencing protocols. We present the NGS results obtained with the *Illumina® TruSightOne Panel* on a *Illumina® MiSeq* machine, allowing the analysis of 4813 clinically relevant genes with a focus on a subset of 32 genes associated with hereditary forms of diabetes. Analysis of sequencing data was performed with a validated bioinformatic pipeline (100% sensitivity, >94% specificity). We detected a single-base deletion (c.1811delC) in the coding region of *CEL*, causing the production of a truncated putative protein (p.Val607CysfsTer100). *CEL* encodes a digestive pancreatic enzyme mainly expressed in the acinar tissue of the pancreas. It participates in the hydrolysis and absorption of cholesterol and lipid-soluble vitamins.

The variant is located within the fifth unit of the intragenic VNTR in exon 11, consisting of 33-bp units (range 7-23). NGS result was confirmed by both direct sequencing on a LR-PCR specific for the active gene, avoiding amplification of the pseudogene, and a repeat-primed fluorescent PCR. We were able to confirm the presence of the new variant and to define the genetic phase with the VNTR repeat as well as their number on each allele.

This is the 3rd pathogenic variant in *CEL* associated with MODY-type VIII, described so far. As previously reported, also in our patient truncation of *CEL* is associated with insulin secretion deficit determining an insulin dependent diabetes with medium age onset and mild sign and symptom of exocrine pancreas function.

The use of Multiplex Ligation Dependent Probe Amplification (MLPA) in the detection of copy number variance of subtelomeric regions in idiopathic intellectual disability

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Genetic causes of intellectual disability (ID) are heterogeneous. The recorded rate of intellectual disability prevalence is 1-3% depending on the population, criteria and sample methods applied. Subtelomeric regions are usually gene rich, and have more susceptibility to aberrant rearrangements than other chromosomal regions. This study aims to detect copy number variance of subtelomeric regions and validation of Multiplex ligation dependent probe amplification (MLPA) in detection of copy number variance in targeted areas. 30 patients were recruited for this study with normal karyotype and intellectual disability (IQ below 70) as major criteria, and minor criteria including facial dysmorphism, nonfacial dysmorphism, congenital anomalies, abnormal growth, behavioural disorder, family history of mental retardation or history of miscarriages or perinatal death. Patients were subjected to full medical history, pedigree analysis and physical examination. 3 patients with mental disability and chromosomal aberrations as detected by karyotype were included as positive controls for the technique. length or whether it is limited to subtelomere or other areas are included.

In this study it was the first time in Egypt to use MLPA for screening MLPA for the 30 patients showed no copy number variance affecting the subtelomeric regions. Meanwhile the 3 positive control cases showed abnormalties by MLPA confirmed by FISH analysis. Case one showed by karyotype 46,XX,der(22) when included as positive case in MLPA analysis 9p subtelomere duplication was revealed. FISH was done using LSI 9p21 and 9p subtelomere probe and showed duplication in 9p. Case tow showed by karyotype 46,XX,add(14)(q). When included as positive case in MLPA analysis 9p subtelomere duplication was revealed. FISH was done using WCP 14 and total subtelomere 9p probe and showed duplication in 9p subtelomere. Case three showed by karyotype 46,XX,add(1)(q). When included as positive case in MLPA analysis both 1q subtelomere deletion and 4q subtelomere duplications were detected. FISH was done using subtelomere 4q and 1q pobes and revealed deletion of 1q subtelomere and duplications 4q subtelomere as detected by MLPA. The length of deletion and duplication needs further investigation including microarray for detection of the exact of subtelomeric regions in intellectual disability patients. We concluded that MLPA is a sensitive method in detection of copy number variance.

We recommend screening of intellectual disability patients using MLPA and to include large sample number of selected idiopathic intellectual disability patients for detection of cryptic subtelomeric copy number variance in further studies .Screening of idiopathic intellectual disability patients using multiplex ligation dependent probe amplification should include screening for subtelomeric areas together with screening for microdeletion syndromes and other targeted sequences using MLPA panels for intellectual disability to detect copy number variances in different areas of the chromosomes. The use of microarray in further studies for the detection of copy number variant in subtelomeric regions.

A novel 53bp duplication in BRCA1: A case report

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Nowadays cancer accounts for 14.6% of human deaths and many families have at least two members, who have had cancer. Some cancer types are common among family members. This is probably because they are exposed to the same risk factors. However, 5% to 10% of all cancers is thought to be heritable. This also applies to breast and ovarian cancer and the *BRCA1* and *BRCA2* genes. Mutations on the *BRCA1* and *BRCA2* genes account for 85% of hereditary breast and epithelial ovarian cancers. According to Breast Cancer Information Core (BIC), there are thousands of mutations in *BRCA1* and *BRCA2*. In the Greek population only 44 unique deleterious mutations have been revealed and more than 1/3 of them (17) are novel mutations, 9 on *BRCA1* and 8 on *BRCA2*. When a novel mutation is detected, there is always challenging to classify it as deleterious or as a variant one.

We report a case of an otherwise healthy 38-year-old woman with no clinical history, who was referred to our centre for *BRCA1* and *BRCA2* mutation analysis due to her family history. Her mother passed away from breast and ovarian cancer at the age of 33, and her sister was diagnosed with breast cancer at the age of 35 and underwent a bilateral mastectomy and chemotherapy. *BRCA1* and *BRCA2* mutation analysis was performed using the BRCA MASTR™ Dx kit by Multiplicom N.V. kit on the 454 GS Junior platform by ROCHE. Amplicon analysis revealed a duplication of 53bp between intron 18 and exon 19 c.5194-10_5236dup in the *BRCA1* gene. The mutation was confirmed by sanger sequencing. Additionally, a fresh blood sample was used for mRNA analysis, which revealed two different transcripts with a 53bp difference. According to protein sequencing prediction systems, this particular nucleotide insertion leads to the premature introduction of a stop codon and thus to a protein that is shorter than normal by 82 amino acids (p. His1746Leufs*37). Finally, the mutation was also found to the ailing sister.

Weblab – an integrated next-generation LIS

Annika Rökman

Weblab is a next-generation laboratory information system currently under development at Mylab Corporation in Tampere, Finland. The guiding principle in the design of the system is to offer a solution providing functionality for all laboratory specialties (clinical clinical chemistry and hematology, clinical microbiology, anatomic pathology and clinical genetics) via an integrated system and in a coherent way.

Technology

Weblab utilizes the InterSystems Caché object database and development environment as a primary platform for implementing core LIS functionality. In addition to Caché, other technologies are also used, e.g., in UI development where JavaScript tool kits enable a modern single page web application design. The InterSystems Ensemble integration platform is utilized for external device and system interconnectivity.

Design

The goal in the design of Weblab is to support modern laboratory workflows where there is increasing convergence and integration between traditional specialties with a high-usability and high-performance informatics solution. In addition, the goal is to provide a coherent user experience throughout the system enabling a shallower learning curve and to identity functionalities, which can be implemented in a specialty-agnostic way reducing unnecessary variety. Much effort has also been put into offering a smooth and gradual upgrade path from legacy LISs.

The system will cover functionality for the entire core laboratory diagnostics workflow from the pre-analytical phase (order entry, phlebotomy, etc.) to the analytical phase (support for different analytical workflows, analyzer and automation system and other device interconnectivity with broad manufacturer coverage, advanced quality control and auto-verification, specimen archiving, etc.) and post-analytical phase (result delivery, etc.). The system is designed to support the integration of data and image analysis tools supporting digital and molecular pathology as well as genetics.

Results

The latest version of the system, Weblab 2017.1, includes analytical phase functionality for clinical chemistry and hematology, clinical microbiology, anatomic pathology and pre-analytical phase functionality for phlebotomy. Weblab is currently used in three large Finnish laboratories. Next versions will include additional functionality for clinical microbiology, anatomic pathology and clinical genetics.

Conclusions

Weblab provides support for modern clinical laboratory workflows with a next-generation informatics solution. Subsequently we want to keep in mind the needs of Clinical Genomics and Next Generation Sequencing that can be met with our system

Results from the Whole Exome Sequencing performed in the Gaslini Institute: a few examples

Marta Rusmini

We have been studying the molecular causes of rare genetic disorders for years. More recently, besides specific sets of patents affected with heterogeneous disorders, we have approached the genetic characterization of phenotypes otherwise unexplained. To this end, we have been applying Whole Exome Sequencing to a number of families, among which two cases of undifferentiated autoinflammatory disorders, one with psoriasis and another one with skeletal dysplasia are being presented.

- A homozygous mutation of the CD70 gene has been identified in a family with consanguineous parents and a child affected with Chronic Active EBV (CAEBV) infection associated with severe lymphoproliferation. The variant c.163-2A>G affects the exon2 AG-acceptor splice site of the CD70 gene thus leading to a deficiency in the CD70 protein, as confirmed by cell surface analysis of PHA-T cell blasts and EBV-transformed lymphoblastoid cell lines, both derived from the patient. CD70 is the ligand of CD27, whose deficiency also presents with EBV driven lymphoproliferation and hypogammaglobulinemia. While our analysis was ongoing, CD70 mutations were reported in a few patients with a similar condition.
- A missense mutation of the CARD14 gene has been detected in all the affected individuals of a four generation family with recurrence of a non-specific dermatitis. In particular, an exon 4 heterozygous nucleotide change, c.446T>G, was found to lead to the aminoacid substitution p.L149R. The genetic characterization of these patients allowed to start the treatment with Ustekinumab resulted to be a powerful therapeutic option for this unusual and refractory form of disease, also in pediatric age. Finally, CARD14 gain of function mutations can give rise to unusual clinical phenotype like diffuse erytrodermic psoriasis that can be associated with arthritis.

- A three generation family with grandmother, mother and a three years old child affected with a form of skeletal dysplasia was studied finding a heterozygous D426A variant of the USFP2 gene to segregate with the disease phenotype. This gene had already been associated with the Beukes Hip Dysplasia in one only family. Consistently, in our family the major skeletal problem resides in the hip, however, affected members also show generalized metaphyseal involvement and mild spine abnormality, with the mother presenting very short stature. We therefore refined and enlarged the phenotypic spectrum associated with USFP2 mutations.

- In a family with healthy parents and two children affected with a form of undifferentiated autoinflammatory disease, we have identified a homozygous mutation of the GRP31 gene. Literature data support the involvement of this gene in the control of the inflammatory pathway through the regulation of NF-kB, therefore we are approaching with functional assays the question of the possible pathogenicity of the mutation and the possible role of this gene in autoinflammation.

<table>
<tr><td colspan="2">ABSTRACT Submission: Human Genetics Course. Bertinoro, Italy Spring 2017 – Case Report</td></tr>
<tr><td colspan="2">Hospital: Birmingham Women's Hospital, Birmingham, UK</td></tr>
<tr><td colspan="2">Patient initials: TM dob: 23.9.13 Hospital no: NA</td></tr>
<tr><td colspan="2">Diagnosis: Aplasia of the Lacrimal Ducts and Salivary Glands</td></tr>
<tr><td colspan="2">Presenter: Dr Ataf Sabir (Clin Gen SpR)[1] Consultant: Lily Islam[1]
Contributors: Dr Maria Koutsogianni[1], Joanna May[2], Sarah McKaig[2] and Thayalan Kandiah[2]

1. Birmingham Women's and Children's NHS FT 2. Birmingham Dental Hospital.</td></tr>
<tr><td>History</td><td>3yr old female with watery right eye, absent right lower eyelid punctum and aplasia of the salivary glands. Patient also had problems with her teeth (caries) leading to dental visit. The dentist felt that brushing was insufficient (despite brushing twice per day) and suggested removal of eight teeth. TM continued to grind her teeth. There was no obvious delay in tooth eruption and she had never cut her hair since birth. Her hearing was normal, with no recurrent infections, normal sweating and no dry mouth. Her developmental milestones were normal.

Past Medical History:
Dental; 8 teeth removed (4 from the top, 4 from the bottom) 2016.
Mucocoele Right eye- wash out (Dec 2016).

Family History: 2 x 2nd degree relatives (siblings) with similar problems: Both had 'dry mouths' affecting speech and eating and leading to caries. Neither child 'ever shed a tear'. The boy had atresia of the punctum of his right eye and a mucocoele of his left eye. The girl had no prominent tear film but tears were seen with fluorescein staining.</td></tr>
<tr><td>Physical signs</td><td>Skin: not particularly dry
Hair: very rough to touch, thin and stiff and is blonde / brown.
Eyes: missing punctum right lower eyelid
Mouth: not dry. Missing teeth x8.
Glands: Unable to feel saliva glands.
Face: Dimples near nose, ?posteriorly rotated ears, simple. ?frontal bossing.
Hands and feet: N.
Nails: N</td></tr>
<tr><td>Relevant Inx</td><td>Ultrasound salivary glands - aplasia</td></tr>
</table>

Management	DENTAL (operation as mentioned above) - Intensive preventive regime: Education on oral hygiene including diet Minimum 1350ppm Flouride toothpaste, Topical fluoride varnish. - Saliva substitute OPTHALOMOGLOCIAL (operation as mentioned above) Advice and guidance to others in the family to seek help from dental and ophthalmological colleagues early.
*Information about the disorder	First reported case of salivary gland aplasia in 1885 Defective salivary glands Sublingual, Submandibular, Parotid dry mouth (Xerostomia often goes unnoticed) rapid tooth decay as saliva is important for clearing decay. Lacrimal gland Watery eye (epiphora) if duct aplasia Dry eye if gland aplasia Leading to recurrent eye infections Inheritance: AD, Variable Expression Genetic Testing: Oxford – 3months. Alteration in FGF10 gene found. Management: Dental team: Education, good oral hygiene, salivary replacement, dietary, non-sugary chewing gum Ophthalmology referral. No specific support group.
Discussion points	Perfect teeth? think twice. Bad teeth; extensive caries, thin enamel – ask about diet? No obvious dietary cause → ?dry mouth ?salivary gl. dysfunction. Examine mucosa for signs of dryness and palpate/ milk salivary glands. Ask about watery / dry eyes. Be careful not to blame parents for a child's bad teeth. Problems with eyes, hair, skin, teeth – think ectodermal structures. ALSG is AD – can help other members get preventative advice.
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Evaluation of the NOD2 & SOCS3 genes methylation in patients with inflammatory bowel diseases

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Usern Tums, Teheran / Iran

Introduction: Growing evidence supports that changes in the expression of the suppressor of cytokine signaling 3 (SOCS3) protein and nucleotide-Binding Oligomerization Domain Containing protein 2 (NOD2) contribute to the pathogenesis of types of inflammatory bowel diseases (IBDs), including Ulcerative colitis (UC) and Crohn disease (CD) . Despite all of the currently known genetic risk map, an increasing number of observations reveal that epigenetic modifications, including DNA methylation, being as or even more important for IBD pathogenesis than genetic predisposition. We investigated the hypothesis that alterations in DNA methylation status at promoter region within SOCS3 gene and at the TSS200 region witin NOD2 gene in colorectal tissue specimens may be involved in the susceptibility to UC and CD.

Method and material: We studied extracted DNA from intestine biopsies of 15 ulcerative colitis cases, 15 crohn diseases and 15 age- and sex-matched healthy controls and performed genotype analyses of the promoter methylation status analysis of SOCS3 gene and TSS200 region in the NOD2 gene using the real-time quantitative multiplex methylation specific PCR (QM-MSP) assay to show evidence of differential methylation between cases of ulcerative colitis, crohn disease and healthy controls.

Results: Based on methylation assay data profiling, the difference in the CpG island methylation frequency at the SOCS3 gene promoter region in mucosa of patients with UC (mean:0.00007 ± 0.0018) and patients with CD (mean :0.00048± 0.0011) compared to healthy controls mucosa (mean : 0.070± 0.142)and , was statically significant . The difference in the CG methylation frequency at the NOD2 gene region TSS200 in mucosa of patients with UC (mean :0.102± 0.055) and patients with CD (mean : 0.128± 0.089) compared to healthy controls mucosa (mean :0.025± 0.016) , was statically significant .

Conclusions: These data provide an important insight into the impact of epigenetic mechanisms in the UC and CD pathogenesis. Inactivation of SOCS3 gene by promoter hypermethylation and activation of NOD2 by hypomethylation might be risk factor for inflamed mucosa of UC and CD, and might fundamentally contribute to the initiation of the inflammatory process .

Key Words: Ulcerative colitis ; Crohn disease ; DNA methylation; Epigenetics; SOCS3; NOD2 ; Immune regulation

A satellited 15q chromosome: clinical, cytogenetic and molecular characterization.

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Nucleolar organizing regions (NORs) and satellites are normally located on the short arms of acrocentric chromosomes. There are several reports of satellite non-acrocentric chromosomes, as a result of cytogenetic rearrangements. Depending on their characteristics, they may be related to phenotypic abnormalities or constitute rare familiar polymorphisms. There are few reported cases involving an acrocentric chromosome. The present report describes the most distal 15q terminal deletion reported to date, and the second translocation involving NORs. We report on a 21 years old female patient, who consulted for short stature and mild intellectual disability. She had a perinatal history of intrauterine growth restriction (IUGR). Cytogenetic analysis showed a terminal deletion at 15q26.3 and NOR staining confirmed the presence of ectopic stalks. MLPA technique confirmed the subtelomeric deletion, and arrayCGH testing revealed that the anomaly involved a 4.6 Mb deletion. Parental karyotypes were normal. It is difficult to establish a phenotype / genotype correlation due to the few reported cases of 15q terminal deletion. Pre and postnatal growth retardation seem to be a regular clinical feature, and could be related to haploinsufficiency of the IGF1R gene. We emphasize the value of the right combination of cytogenetic and molecular techniques for the characterization of NORs ectopic regions.

Identification of mismatch repair deficient tumours using molecular inversion probe based sequencing assay of short mononucleotide repeats

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Recent draft guidelines from the UK's National Institute of Health and Care Excellence recommends microsatellite instability (MSI) testing to be performed in all patients with colorectal cancer to identify Lynch syndrome patients1. It is estimated that 3 or 4 per 1000 people in the population carry a mismatch repair (MMR) gene defect but only 10% of these are known to medical services. This is in part due to the lack of an automatable, robust, reliable and high throughput MSI assay.

We have previously shown a next generation sequencing based MSI assay using 17 short homopolymer repeats (7-12 bp). Short repeats are less prone to technical artefact than the longer repeats used in existing assays, and are sufficient in number to provide sensitivity and specificity equivalent to fragment analysis. We have enhanced the assay performance by selecting from the TCGA database those intragenic repeats that are linked to an informative SNP. This allows simultaneous separation of PCR

artefacts linked to either allele from genuine mutations which display allelic imbalance. Using fragment analysis as the reference technique, we have demonstrated >97% sensitivity and specificity in over 100 residual samples from diagnostic labs in Newcastle, Edinburgh and Pamplona. However, the current laboratory procedure requires individual amplification of each repeat for every sample with subsequent pooling and library preparation, which adds cost and complexity.

We have upgraded the assay to carry out a simple two-step multiplexed target capture and library preparation method which utilises molecular inversion probes (MIPs)2. We demonstrate that MIPs allow multiplexing of the 17 marker panel plus inclusion of *BRAF* V600E analysis for minimal additional cost. The MIP mode of action minimises PCR and sequencing induced error rates. We will present results of the assay sensitivity, robustness and reproducibility against fragment analysis technique using DNA from fresh and FFPE tissues.

Our novel assay has the potential to streamline diagnostic pathology services by providing a quick and automatable assay for MSI detection. This could speed up the diagnosis pathway and improve the current poor rate of diagnosis of MMR-deficient tumours. This could be a major step towards timely personalised care for patients with MMR-deficient cancers, including access to immunotherapy3, and may help find families at high genetic risk.

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Exome sequencing reveals a novel nonsense mutation of *WTN10B* gene in a Moroccan family with split-hand foot malformation

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Split-hand foot malformation (SHFM) is a clinically heterogeneous congenital limb defect affecting predominantly the central rays of hands and/or feet. The clinical expression varies in severity between patients as well between the limbs in the same individual. SHFM might be non-syndromic with limb-confined manifestations or syndromic with extra-limb manifestations. Isolated SHFM is a rare condition with an incidence of about 1 per 18,000 live born infants and accounts for 8-17 % of all limb malformations. To date, seven chromosomal loci associated with isolated SHFM have been described, i.e., SHFM1 to 6. Most of the known SHFM loci are associated with chromosomal rearrangements that involve small deletions or duplications of the human genome. In addition, three genes, i.e., *TP63* (*tumor protein p63*, MIM 603273), *WNT10B* (*awingless-type MMTV integration site family, member 10B*, MIM 601906), and *DLX5* (MIM 600028) are known to carry point mutations in patients affected by SHFM. The autosomal dominant mode of inheritance is typical for SHFM1 (*DLX5* gene on 7q21), SHFM3 on 10q24, SHFM4 (*TP63* gene on 3q27) and SHFM5 on 2q31. Two autosomal recessive forms have been reported, i.e., SHFM2 on 3q27 and SHFM6 (*WNT10B* gene on 12q13.12). In addition, SHFM2 has been assigned to Xq26 by linkage analyses in large Pakistani kindred.

We have investigated a large consanguineous Moroccan family with three affected members showing feet malformations with or without split hand malformation phenotypes. Using an exome sequencing approach, we identified a novel homozygous nonsens variant p.Arg**115*** of *WNT10B* gene retaining thereby the diagnosis of SHFM6.

Keywords: Split-hand foot malformation, Moroccan, family, *WNT10B* gene, mutation.

Association of epilepsy, antiepileptic drugs and cognitive performance in dizygotic twins with Fragile X syndrome

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Fragile X syndrome (FXS) is the most common cause of inherited intellectual disability (ID) and the leading monogenic cause of autism in males, affecting about 1/5000 males and 1/6000 females (1,2). Most of the FXS cases are due to large expansions in the 5' untranslated region of the *FMR1* gene leading to the partial or full absence of the FMRP (3). It has been shown that epilepsy occurs in about 20% of patients (4,5) and that mosaicism is present in approximately 40% of cases (6,7). Also, it is reported that dizygotic twinning is more common in FXS permutation carrier mothers than in general population (8).

Here we present a case of 13 y.o. dizygotic twin boys with FXS. They both showed typical dysmorphic and behavioral features as well as intellectual disability. Molecular genetic testing showed that the first twin has >200 CGG trinucleotide repeats (full mutation) in the *FMR1* gene and the second twin has 90 and >200 repeats (size mosaic). Initially the younger mosaic brother used to have better cognitive performance than his older full mutation brother, however his development regressed as he developed seizures and was treated

with antiepileptic drugs (AEDs) for four years. He has been seizure-free for the last five years. The first full mutation affected twin never had seizures and he has better cognitive and behavioral skills. Noteworthy there are two more dizygotic twin pairs in the relatives from the mother's side.

Despite the fact that mosaicism is usually associated with better cognitive functioning, at present the younger mosaic twin brother functions at a lower level than his older full mutation brother. Epilepsy and/or AEDs could contribute as an additional impact on cognitive performance. Presence of twinning in the mother or close relatives of the child with intellectual disability may provide an additional clue for the diagnosis of Fragile X syndrome.

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lincRNA expression in mammalian inner ear throughout late embryonic development

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The attempt to characterize 'junk' DNA yielded a novel class of RNA molecules that impose an additional level of genomic regulation on mammalian genomes. These species are known as long intergenic non-coding RNAs (lincRNAs). lincRNAs are discriminated from other non-coding RNA species primarily based on their length, transcripts of >200 nucleotides. Compared with mRNAs, they are not translated, are less abundant and are expressed with higher tissue specificity, although they do share some similarities. The functions of lincRNAs are especially diverse, they are highly regulated, and their levels of expression are altered throughout development and in disease states. The genomic regulation of the sensory portions of the inner ear, the cochlea and the vestibule, is extremely complex and determined throughout specific windows during development. This led us to hypothesize that lincRNAs are critical players in the genomic regulation of inner ear development. RNA was isolated from auditory and vestibular sensory epithelia from two stages, E16.5 and P0, and was subjected to high-throughput RNA sequencing (RNA-seq). Using a specifically developed bioinformatic pipeline, the reads were assembled and filtered. Our analysis identified 1920 lincRNAs, of which 403 are novel un-annotated lincRNA genes. To select candidate lincRNAs for further investigation, a set of logical criteria was derived, including profiling in the whole animal, tissue, developmental stage expression and study of specific mode of action. We anticipate that studying specific regulation of lincRNAs in the inner ear will expand our knowledge of auditory and vestibular neurosensory systems and aid in our understanding of deafness and balance disorders.

MAMLD1 deletions in three patients with proximal hypospadias

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Hypospadias is a congenital malformation that has a prevalence of 4-43:10.000. Distal or (sub)glandular hypospadias is far more common than proximal hypospadias. In most cases of isolated distal hypospadias inheritance is multifactorial, while the more proximal anomalies are part of the spectrum of Disorders of Sex Development (DSD) and can have a variety of genetic defects. *MAMLD1* is one of the genes associated with hypospadias, although functional studies in mouse KO models do not support this as the male knockout mice do not show hypospadias and have normal fertility[1,2,3]. Patients with a contiguous syndrome involving the *MTM1* gene and *MAMLD1* have been described as well as patients with deletions encompassing *IDS* and *MAMLD1* not showing hypospadias.

We present the findings in two sibs with proximal hypospadias and a very small deletion of *MAMLD1* confirmed with MAQ assay and a third patient with Hunter syndrome and hypospadias carrying a deletion encompassing the *IDS* gene, which extends to the first exon of transcript 1 of MAMLD1.

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Simultaneous preimplantation genetic testing of monogenic diseases and embryo aneuploidies using whole genome amplification product

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Introduction: Preimplantation genetic testing (PGT) is an alternative to prenatal testing for couples at risk of transmitting a genetic disorder. Development of PGT protocols are challenging and prone to amplification failure, DNA contamination and allele dropout (ADO) thus affecting the reliability of the approach. Significant improvements are now achieved through combining direct and indirect testing, performing whole genome amplification (WGA) for initial material or using platforms like Karyomapping for genome wide linkage analysis. Another step in reaching considerably good results for single gene disorder PGT is embryo aneuploidy exclusion.

The aim of our study was to develop effective, affordable and robust embryo testing protocol, we present our experience for three families with autosomal recessive congenital ichthyosis (ALOX12B), Huntington disease (HTT) and classic late infantile neuronal ceroid lipofuscinosis (TPP1).

Materials and methods: WGA for 5th day embryo trophectodermal cells biopsies were carried out by multiple displacement amplification technology. Microsatellites (STR markers) adjacent to a specific gene were selected using UCSC database, semi-nested primer system for two round PCR was designed for multiplex STR testing. Aneuploidy testing using the same WGA material portion were done by aCGH.

Results: Family member's STR haplotyping revealed 14 out of 15 informative/semi-informative markers for ALOX12B-case, 5 out of 13 for HTT-case and 8 out of 15 in TPP1-case. All embryos analyzed (11 retrieved in two stimulations in HTT-case, 7 in ALOX12B-case, 3 in TPP1-case) resulted in successful WGA. After STR testing in case of ichthyosis two embryos had normal genotype, seven were heterozygotes and three affected, six were mutation-free in case of Huntington disease; one was normal, one heterozygote and one affected in case of lipofuscinosis. Overall ADO rate was 0.04%. Direct HTT gene CAG repeat sizing confirmed results for mutation-free haplotype embryos. Single euploid embryo transfers were done for two cases and resulted in clinical progressive pregnancies, TPP1 gene family is waiting for embryo transfer.

Conclusions: Initial embryo biopsy whole genome amplification ensures possibility of multifactor preimplantation genetic testing without compromising embryo viability and in general chance of achieving healthy pregnancy. Semi-nested testing system minimizes embryo misdiagnosis risk due to ADO, non-specific amplification or contamination. Aneuploidy screening excludes leading failed embryo implantation cause in case of PGD thus saving time and money leading to higher pregnancy rates. These are first single gene PGT cases carried out in Latvia, developed protocol can be further applied for families seeking alternatives for prenatal testing.

Whole Exome sequencing approaches for clinical use in primary immunodeficiency disorders with unknown cause

Samaneh Zoghi and Nima Rezaei

Primary immunodeficiency diseases (PIDs) are rare and usually early onset disorders of the immune system. Many of these disorders can remain genetically undiagnosed for years. Using common genetic methods such as PCR and sequencing of particular genes seems technically infeasible or cost prohibitive, since there are plenty of genes which had been reported to be responsible in PIDs.

Meanwhile, High throughput methods such as next generation sequencing (NGS) is an important tool in the implementation of predictive and individualized medicine and helps to find the genetic cause of the disease. Therefore, Whole Exome Sequencing (WES) is an ideal approach to help understanding allelic variations and their relationship to phenotype. WES consists of different steps such as; DNA extraction, library preparation, cluster generation, massive parallel sequencing and data analysis. By the way, collecting an informative clinical data such as family history and patient phenotype and laboratory data (such as antibody levels and immunophenotyping) with the consent of the patient's family is crucial.

Finally, data analysis of WES is the most important step which needs careful consideration of disease phenotype, family pedigree and literature review.

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