Clinical Genomics and NGS

Bertinoro - Italy
April 29 – May 4, 2018

31th 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 28TH Arrival and dinner

SUNDAY APRIL 29TH

Morning Lectures:	**Medical Genetics concepts and principles**
8:30 – 9:00	Participants Registration
9:00 – 9:15	Introduction to the course – **G. Romeo**
9:15 – 10:00	Genomic Medicine – **D. Donnai**
10:00 – 10:45	Phenotype to genotype – **H. Brunner**
10:45 – 11:00	Coffee break
11:00 – 11:45	Cytogenetics and arrays – **E. Klopocki**
11:45 – 12:30	Complex disorders and classical gene identification – **A. 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 – **H. Brunner**
	Interpreting CNVs for beginners – **E. Klopocki** Computer room
	Dysmorphology – **D. Donnai**
16:00 – 16:30	Coffee break
Session II (16:30 – 18:00)	Mutation patterns – **H. Brunner**
	Interpreting CNVs for beginners – **E. Klopocki** Computer room
	Dysmorphology – **D. Donnai**

MONDAY APRIL 30TH

Morning Lectures:	**Basics of NGS for Mendelian disorders**
09:00 – 09:45	Basics of next generation sequencing technology – **A. Hoischen**
09:45 – 10:30	Basics of NGS bioinformatics – **C. Gilissen**
10:30 – 11:00	Coffee break
11:00 – 11:45	NGS in the clinic – **H. Brunner**
11:45 – 12:30	Long-read sequencing – **E. E. Eichler**
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 – **C. Gilissen & T. Pippucci** Computer room
	Targeted NGS approaches – **A. Hoischen**
	Clinical Considerations for NGS **– H. Brunner**
16:00 – 16:30	Coffee break
Session II (16:30 – 18:00)	NGS Bioinformatics Basics - **C. Gilissen & T. Pippucci** Computer room
	How to set up a NGS lab? – **A. Hoischen**
	Copy number variations **– E. E. Eichler**

TUESDAY MAY 1ST

Morning Lectures: **Therapy and prenatal diagnostics in the NGS era**

09:00 – 09:45	Therapy and cancer – **J. Burn**
09:45 – 10:30	Novel Cancer immunotherapy approach – **G. Germano**
10:30 – 11:00	Coffee break
11:00 – 11:45	SMA: From gene and modifier to therapy – **B. Wirth**
11:45 – 12:30	The therapy for cystic fibrosis as a paradigm for other genetic diseases **– L. Galietta**
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 – **J. Burn**
	NGS and the understanding of disease-causing versus neutral alleles: the cystic fibrosis (CF) paradigm – **C. Castellani & L. Galietta**
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 –**A. Read**
	From your newly discovered candidate gene to its function – **B. Wirth**
	In silico prediction of putative neoantigens: a workflow between tumour DNA alteration and cell mediated immune response – **G. Germano & G. Rospo**
18:00	Poster viewing session with aperitif (session I)

WEDNESDAY MAY 2ND

Morning Lectures:	**Complex mechanisms of disease**
09:00 – 09:45	Molecular inversion probes and mosaicism – **A. Hoischen**
09.45 – 10:30	Epigenetics, imprinting, clinical – **K. Temple**
10:30 – 11:00	Coffee break
11:00 – 11.45	Non-coding mutations/long-range effects – **E. Klopocki**
11:45 – 12:30	Massively parallel functional assays – **L. Starita**
12:30 – 13:15	Discussion of the morning lectures
13:30 – 14:30	Lunch Break

Afternoon Excursion

THURSDAY MAY 3RD

Morning Lectures:	**Novel NGS applications**
09:00 – 09:45	Discovering structural variants in cancer using NGS data - **T. Rausch**
09:45 – 10:30	Phenotype and NGS integration/HPO benefits – **D. Fitzpatrick**
10:30 – 11:00	Coffee break
11:00 – 11:45	GWAS with NGS **- M. Nothnagel**
11:45 – 12:30	Data integration – **L. Franke**
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, par. 1 – **T. Rausch** Computer room
	Genetic Imprinting – **K. Temple**
	Multiplexed functional assays/variant interpretations – **L. Starita**

16:00 – 16:30	Coffee break
Session II (16:30 – 18:00)	Discovering structural variants in cancer using NGS data, par. 2 – **T. Rausch** Computer room
	How to do GWAS – **M. Nothnagel**
	How to do RNASeq – **L. Franke**
18:00	Poster viewing session with aperitif (session II)

FRIDAY MAY 4TH

Morning Lectures:	**Large scale NGS**
09:00 – 09:45	Patterns of rare variation contributing to disease – **K.Samocha**
09:45 – 10:30	Genomics England – **A. Rendon**
10:30 – 11:00	Coffee break
11:00 – 11:45	Non-invasive prenatal testing – **J. Weiss**
11:45 – 12:30	Single cell sequencing and applications to PGD – **T. 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, part. 1 - **T. Rausch** Computer room
	Interpretation of rare variants – **K. Samocha**
	Practical considerations for NIPT – **J. Weiss**
16:00 – 16:30	Coffee break
Session II (16:30 – 18:00)	Discovering structural variants in cancer using NGS data, part. 2 – **T. Rausch** Computer room
	Large genomics projects – **A. Rendon & D. Fitzpatrick**
	How to do single cell genomics? – **T. Voet**
18.00 – 18.30	Best poster presentations

Social dinner and farewell party

SATURDAY MAY 5TH

Departure

ABSTRACTS OF LECTURES

Sunday, April 29

Introduction to the course

Giovanni Romeo, M.D.
University of Bologna & ESGM

This introduction will explain the daily life of the course.

This year is the 30th anniversary of the foundation of the course and of the European School of Genetic Medicine (ESGM) which were started in 1988 in Sestri Levante (Italy).

In order to get a feeling of the origin and past history of the European School of Genetic Medicine you can read the article below which highlights the role of Victor A. Mckusick, one of its main founders. He passed away 10 years ago, on July 22, 2008.

The accompanying picture shows Victor together with his wife Anne when he received the Japan Prize from the Emperor of Japan a few months before his death. Anne, a faculty at Johns Hopkins Hospital and a distinguished rheumatologist, was attending all the courses together with Victor between 1988 and 2007. She passed away on September 17, 2017.

We miss them both.

European Journal of Human Genetics (2017) 25, S6–S12

www.nature.com/ejhg

PERSPECTIVE

The early years of the ESHG leading to the reform of 1988 and the spirit of the Sestri Levante school

Giovanni Romeo*,[1], Eberhard Passarge[2] and Albert de la Chapelle[3]

European Journal of Human Genetics (2017) 25, S6–S12; doi:10.1038/ejhg.2017.142

At the Third International Congress of Human Genetics in Chicago in September 1966 a group of human geneticists from Europe met and agreed that there should be a European Society of Human Genetics (ESHG). This was formally established in 1967, as reviewed elsewhere in this issue by Peter S Harper. As two of us (EP and AdlC) attended the discussion in 1966 and all three were involved subsequently in the early development of the ESHG we would like to add a few comments. Following its first annual meeting in 1967 in Copenhagen, the ESHG held meetings each year in various European cities arranged by different colleagues as local hosts, but not yet organised as a scientific society comparable to the American Society of Human Genetics.

At the 1988 ESHG meeting in Cardiff a process to reform the Society was started as described by Brunner and Harper in this issue of the Journal (EJHG, 2017). In April of the same year 108 young geneticists from 16 European countries travelled to Sestri Levante, Italy, to attend the first week-long course in Medical Genetics, taught by the late Victor A McKusick (1921–2008) and by many of the European medical geneticists of the time (see Figures 1 and 2).

The model for this course was the 'Short Course in Medical and Mammalian Genetics' held in Bar Harbor, Maine, organised each year by Victor A McKusick and attended in 1968 by some young European participants, including the three of us. The support of the Istituto Gaslini (Genoa) and of the Federation of European Societies of Biochemistry (FEBS) made it possible to start the European equivalent of the Bar Harbor course 20 years later; this was quite labour-intensive as shown by its tight scientific schedule (Figure 3), consisting of morning lectures and afternoon practical workshops (but also characterized by long lunch breaks of about 2 h dedicated to the delicacies of Genoese cuisine…).

During subsequent years this model developed further into many more specialised courses (Cancer Genetics, Genetic Counselling, Molecular Cytogenetics, Eye Genetics, etc.) that became to be known as the European School of Genetic Medicine (ESGM). The 30th edition of the main ESGM course took place at the beginning of May 2017 in Bertinoro, Italy, with the new name 'Clinical Genomics and NGS'. It was attended by 89 students from all over the world (37 countries, Figure 4). Most of the ESGM courses have been supported consistently by ESHG fellowships. In what is more relevant for the history of ESHG, some of the highly motivated faculty of the 1988 course became the leaders of the reformed ESHG in later years, after the new statutes

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
THE NETHERLANDS	6
POLAND	1
RUSSIA	3
SLOVAKIA	1
SPAIN	5
SWITZERLAND	1
TURKEY	3
UK	12
TOT.	108

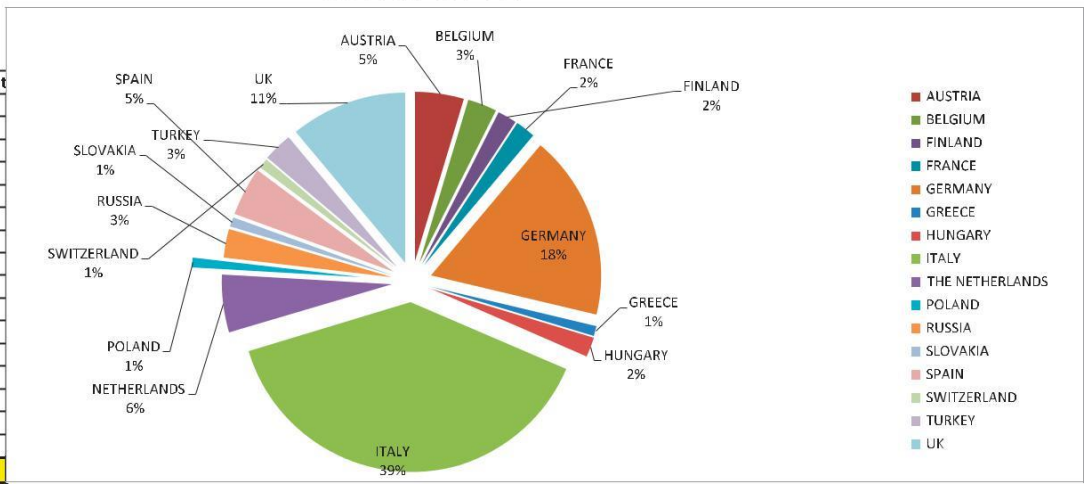

Figure 1

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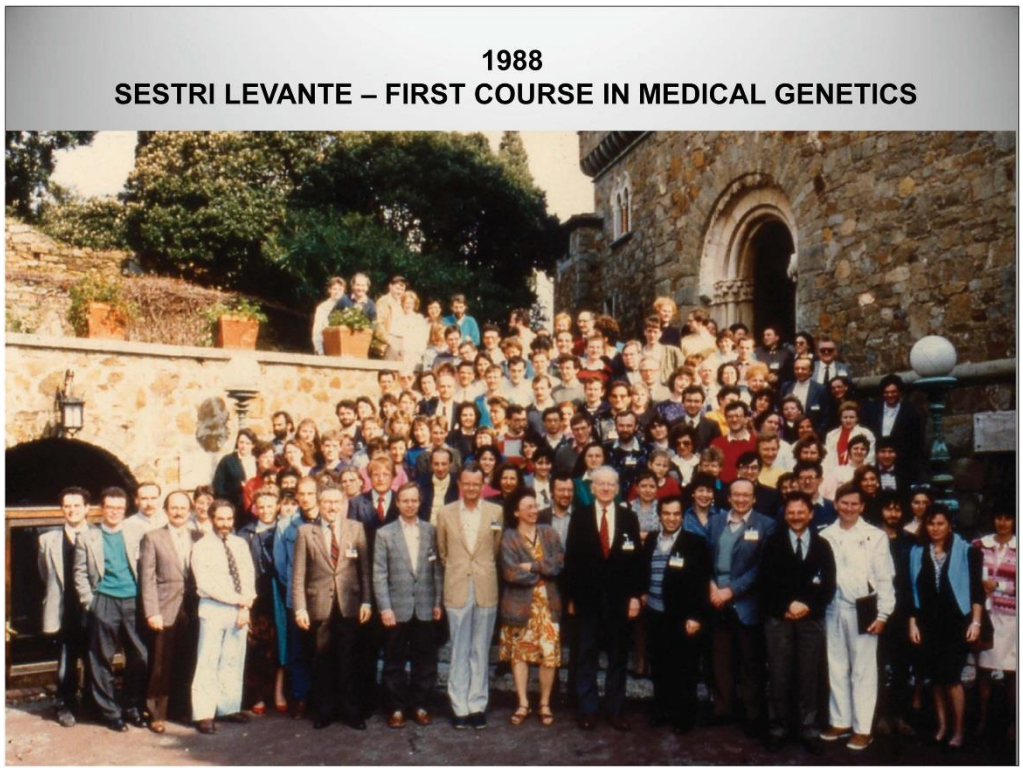


Figure 2

proposed by a committee consisting of Christos Bartsocas, Charles Buys, Marco Fraccaro, Peter Harper, Jan Mohr, Anne de Paepe and Eberhard Passarge) were approved and implemented in 1991 at the Leuven meeting where one of us (GR) took office as the first democratically elected ESHG President and went on to found the EJHG the following year. We have placed so much emphasis on the ESGM courses because we 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.

Initially Jan Mohr, the founding secretary-general, contacted one of us (EP) in 1987 and suggested that he should take over as secretary-general in due time. Such a change appeared to be a good opportunity to make the ESHG more democratic and inclusive by electing a board (president, president-elect, secretary-general) and a programme committee. Sup-ported by Peter Harper at the 1988 Cardiff meeting, the newly elected board took office in 1989 at the meeting in Groningen organised by Charles Buys (once again described by Peter Harper). All this happened at a time when Europe was going through great political changes—namely the fall of the Berlin Wall in November 1989, which led to the unification of East and West Germany in 1990, and the signing of major European treaties, such as Maastricht in 1993. It was a time of great enthusiasm and popular approval 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 changes our small community of scientists was transformed into a democratic society of medical and clinical geneticists. We sometimes ask ourselves: was this achievement worth the time and effort 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 (Figures 1 and 4 respectively). This comparison docu-ments the success of ESHG in supporting programmes of advanced training in medical and clinical genetics that 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 like Brunhilde Wirth (a student in the course of 1988; Figure 2) and Han Brunner (a young faculty member since the early ’90s) who later became the driving force of the main ESGM course.

In conclusion, the reform of ESHG was useful, as evidenced by the universal acceptance and recognition of the Society’s role in medicine and genetics. The success of the ESHG today is also shown by its excellent annual meetings under the guidance of its programme committee, and by its Journal that serves to unite geneticists from all over Europe and to let the rest of the world know what is happening in Europe. The three authors of this review were actively involved in the transformation of the ESHG from a somewhat loosely organized association to a well-organised scientific society in the late 1980s and early 1990s. AdlC and GR served as presidents during that period, EP as secretary-general, all elected by the membership assembly at annual meetings. During his Presidency GR founded the Journal, which he directed until 1995.

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

S8

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)

Figure 3

nationality	pax
ARGENTINA	1
BELGIUM	2
BOSNIA AND HERZE	1
BULGARIA	3
COLOMBIA	1
CROATIA	2
CUBA	1
CZECK REP.	3
DENMARK	2
EGYPT	3
FINLAND	1
GEORGIA	1
GERMANY	4
GREECE	3
HUNGARY	2
IRAN	7
ISRAEL	5
ITALY	5
LATVIA	1
MOROCCO	3
THE NETHERLANDS	8
NORWAY	2
OMAN	2
POLAND	2
PORTUGAL	2
ROMANIA	2
SAUDI ARABIA	1
SERBIA	1
SOUTH AFRICA	1
SPAIN	3
SUDAN	1
SWEDEN	3
SWITZERLAND	2
TUNISIA	1
TURKEY	3
UK	3
UNITED ARAB EMIR	1
TOT.	89

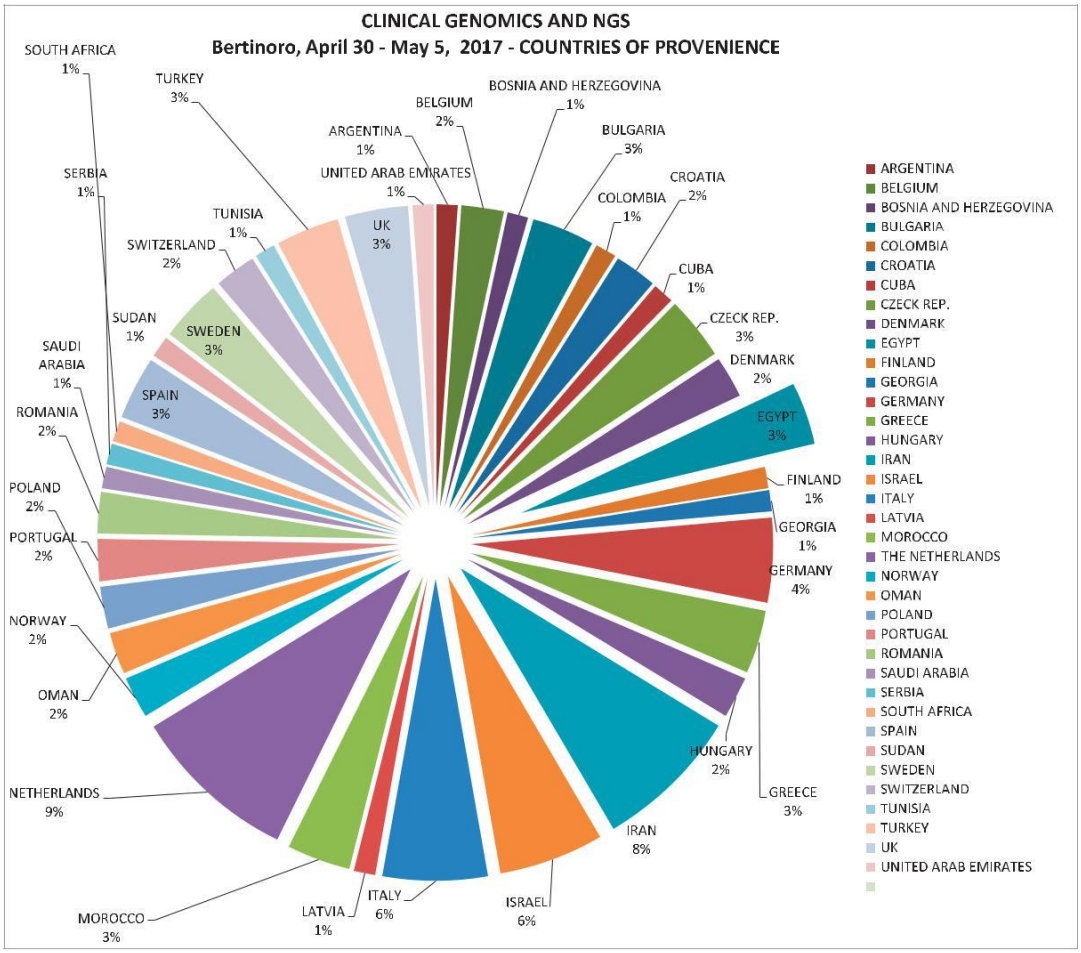


Figure 4

Giovanni Romeo

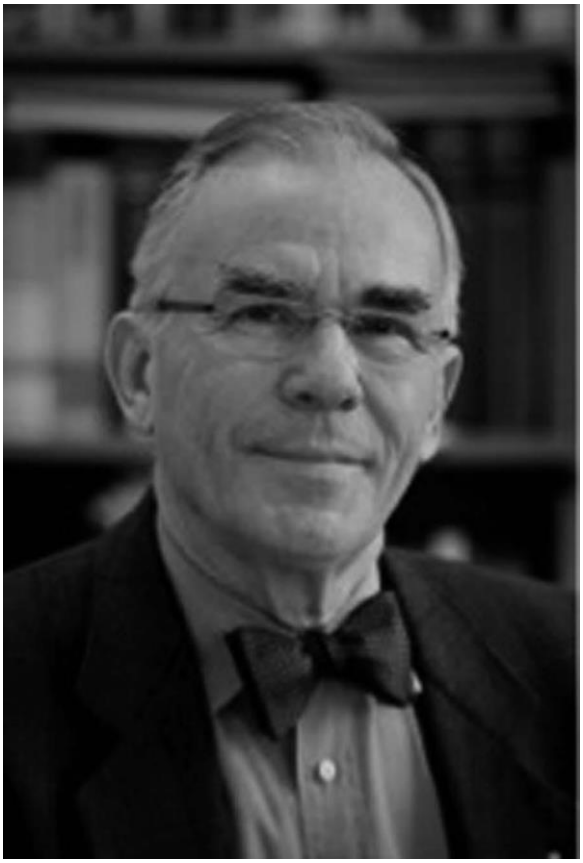
Professor of Medical Genetics at University of Bologna Medical School (2001-2012). Degree in Medicine (1965); Internship & Residency in Paediatrics, University of Bologna Medical School (1965-1967); Research Fellow, Genetics Division, Department. of Pediatrics, Johns Hopkins Medical School, Baltimore, Md. (1968-1971).

Medical geneticist with a wide international research experience documented by his leadership role during the past 30 years in different Institutions: Genova (Istituto G Gaslini), Lyon (International agency for Research on Cancer) and Bologna Medical School. Major research interests: Hirschsprung disease, RET protooncogene, consanguinity studies and genetic epidemiology, mtDNA mutations in cancer. He has published more than 380 papers in peer-reviewed international journals.

In collaboration with the late Prof. Victor McKusick in 1988 he founded the European School of Genetic Medicine, now located in Bertinoro, Italy, attended so far by more than 7000 students and devoted to the advanced training in genetics and genomics of young geneticists and health professionals from Europe and elsewhere in the world.

In 1992 he became the first democratically elected President of the European Society of Human Genetics (ESHG) and founded the European Journal of Human Genetics which he directed up to 1995.

Among other prizes, he was awarded the ESHG Educational Award in 2005 and the Arno Motulsky-Barton Child Award for Excellence in Human Genetics Education in 2011 by the American Society of Human Genetics. During the past 15 years he developed several research and educational projects in Oman.



Eberhard Passarge, MD (1935), is a US-trained German human geneticist at the Medical Faculty Essen of the University of Duisburg-Essen, Germany. He became a founding member of the ESHG after attending the discussion of forming a European Society of Human Genetics at the Third International Congress of Human Genetics 1966 in Chicago. He served as secretary-general of the ESHG 1989-1991. He was host of the annual meeting of the ESHG in Essen in 1984 on 'Cancer and Genetics'.

He graduated as MD at the University of Freiburg (1960), had postgraduate training in Hamburg and Worchester (MA), specialized in Paediatrics and Genetics in Children´s Hospital Cincinnati, Ohio and Cornell Medical Center New York. He was Head of the Division of Cytogenetics and Clinical Genetics at the Department of Human Genetics, University of Hamburg, Germany, 1968-1976, and moved to Essen in 1976. From 2010-2014 he was Intermediary Chairman, Department of Human Genetics, University of Leipzig, Germany.

His main interests are the scientific investigation of hereditary and congenital diseases and the application of this knowledge in genetic diagnosis and counseling. He is author or co-author of about 250 articles in international, peer-reviewed journals. His experience in academic teaching is reflected in his single author book Color Atlas of Genetics, its 5th edition in press, to be published by Thieme Medical Publishers Stuttgart-New York, in 2018.

EP was President of the German Society of Human Genetics 1990-1996, of which he became an honorary member in 2011. He is music coordinator at the University of Duisburg-Essen where he organizes the annual festive university concert. He was elected to be an honorary member of the university senate in 2016. He was awarded prizes from scientific institutions in Germany, Romania, Czech Republic, and India.

(Institute of Human Genetics, Emeritus Director, University Hospital Essen, Hufelandstr. 52, 45122 Essen, Germany)

Albert de la Chapelle is a Distinguished University Professor and Cancer Scholar at The Ohio State University. He received his MD in 1957 and PhD in Human Genetics in 1962 at the University of Helsinki. He received board certification in Internal Medicine but soon left clinical medicine for genetics. He became Finland's first Professor of Medical Genetics in 1974 and remained at that position until 1997 when he moved to The Ohio State University to start a program in Human Cancer Genetics. In the European Society of Human Genetics he served as a Board member 1966-1995, Chairman of the Aims and Statutes Committee 1990-1991, and President 1993-1994. His major honors include: Memberships in the Academy of Finland, the Royal Swedish Academy of Sciences and the US National Academy of Sciences. Dr de la Chapelle started as a cytogeneticist specializing in mechanisms of sex determination. He then turned to molecular genetics, pioneering the mapping and cloning of those Mendelian disorders that are enriched in the Finnish founder population, clarifying the molecular basis of over a dozen of these disorders. He is best known for his role in determining the molecular basis of hereditary cancer, notably the role of the mismatch repair genes in Lynch syndrome. He detected the phenomenon of microsatellite instability in hereditary cancer. He has pioneered the translation of these molecular events into clinical work and cancer prevention. Presently his laboratory is heavily committed to the study of the genetics of thyroid cancer.

Genomic Medicine

Dian Donnai
University of Manchester,
Manchester Centre for Genomic Medicine
St Mary's Hospital, Manchester M13 9WL, UK

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, for example in de Lange syndrome.
- 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/
- 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, clinical trials and treatment of patients with a wide variety of medical conditions.

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Phenotype to genotype

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

I shall discuss the following categories:

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

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

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

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

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

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

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

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

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Cytogenetics and arrays

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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:

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Complex disorders and classical gene identification

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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 GnomAD 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.

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Monday, April 30

Basics of Next Generation Sequencing technology

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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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Basics of NGS bioinformatics

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Next Generation Sequencing (NGS) technologies have revolutionized the field of medical genetics research by generating large numbers of DNA sequences within a matter of days at very low cost. Next generation sequencing is being used extensively to search for Mendelian disease genes in an unbiased manner by sequencing the entire protein-coding sequence, known as the exome, or even the entire human genome.

Increasingly, NGS is also being applied for the diagnosis of patients with genetically heterogeneous disorders, where sequencing of all individual disease genes in infeasible.
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.

Reading material:
Disease gene identification strategies for exome sequencing. Gilissen C, Hoischen A, Brunner HG, Veltman JA. Eur J Hum Genet. 2012 May;20(5):490-7. doi: 10.1038/ejhg.2011.258. Epub 2012 Jan 18. Review. PMID: 22258526

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NGS in the Clinic

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Next generation sequencing can bring a diagnosis when clinical diagnostics, and doctor-driven testing is not successful. Current analysis of our own experience with ~20.000 diagnostic exomes, and that of other large centers around the world suggest the following:

1. There is a limited set of conditions that is so obvious from their clinical phenotype that directed testing (involving one or two tests) is the most effective strategy. Examples are Huntington's disease, Fragile X syndrome, chondroplasia, myotonic dystrophy, Down syndrome, progeria, and others.
2. Clinical conditions that involve repeat expansions such as fragile X, Huntington, myotonic dystrophy and others are not detectable with current standard short read sequencing technologies.
3. When most genes are known, and the number of possible genetic defects is small (up to 50 or so), a diagnostic sequencing panel may be the most cost-effective strategy. This applies to genetic cardiac rhythm disturbances, and familial breast cancer.
4. If the phenotype is not specific for a single (or at most a few) conditions, and the number of possible genetic defects is large, then exome sequencing (and perhaps an array), is the most effective strategy
5. Exome sequencing is cost-effective in clinical setting, once the expected number of genetic test is >3.

6. In pediatric neurology, it is generally cost-effective to do exome testing first, before doing other tests that the neurologist would order such as imaging, metabolic testing, invasive biopsies, and single gene tests.
7. The diagnostic rate of exome sequencing currently varies from 20-60% for a wide range of clinical categories.
8. For intellectual disability trio sequencing is preferred because the rate of new mutation is high.
9. Doctors are great, exomes are often better.
10. Patients with complex phenotypes can have 2 independent genetic diseases (Hickam's dictum). This applies to up to 5% of all patients elected for clinical sequencing with exome.
11. In the clinical arena, and at the current stage of our knowledge, whole genome sequencing does not offer much added benefit over whole exome sequencing and careful array analysis. This may change soon.
12. For Mendelian conditions the vast majority of causative variants is in the coding region of genes.
13. The pick-up of incidental findings is between 1-3%.
14. There are a number of conditions that look convinvingly monogenic but cannot be diagnosed by any genetic test incluing exome sequencing. Examples of these currently unsolvable diseases are Hallermann-Streiff syndrome, Aicardi syndrome, Gomez-Lopez-Hernandez syndrome.
15. With time, the diagnostic rate increases for a given patient cohort, because more disease genes are identified, and bioinformatics algorithms improve. This means that ideally, data are stored so that they can be reanalyzed at a later date, either at the request of the patient, or as part of a planned reanalysis update.
16. Interpreting missense variants is often tricky. This applies especially to variants in X-chromosomal genes inherited from the mother.
17. If NGS is negative and your clinical diagnosis is secure, go back to the lab, and convince them to reanalyze.
18. Sometimes a variant is missed on NGS because the exon is not well-covered.

Reading material:

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

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Resolution of Disease Phenotypes Resulting from Multilocus Genomic Variation. Posey JE, Harel T, Liu P, Rosenfeld JA, James RA, Coban Akdemir ZH, Walkiewicz M, Bi W, Xiao R, Ding Y, Xia F, Beaudet AL, Muzny DM, Gibbs RA, Boerwinkle E, Eng CM, Sutton VR, Shaw CA, Plon SE, Yang Y, Lupski JR. N Engl J Med. 2017 Jan 5;376(1):21-31

Detection of clinically relevant copy-number variants by exome sequencing in a large cohort of genetic disorders. Pfundt R, Del Rosario M, Vissers LELM, Kwint MP, Janssen IM, de Leeuw N, Yntema HG, Nelen MR, Lugtenberg D, Kamsteeg EJ, Wieskamp N, Stegmann APA, Stevens SJC, Rodenburg RJT, Simons A, Mensenkamp AR, Rinne T, Gilissen C, Scheffer H, Veltman JA, Hehir-Kwa JY.
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Detection of clinically relevant copy number variants with whole-exome sequencing.
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N Engl J Med. 2012 Nov 15;367(20):1921-9

Long-read sequencing of complex genomes

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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 a diversity panel of more than 10 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 >20,000 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.

Tuesday, May 1

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. Hanahan and Weinberg (*Cell 2011;144: 646-74*) provide a summary of the functional changes which might result from mutations that confer a selective advantage and promote malignant change. They include sustaining replicative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis and activating invasion and metastasis. Underlying these features are genomic instability which generates genetic diversity and inflammation which fosters multiple hallmark functions. Genomic analysis is allowing the driver mutations in individual cancers to be identified leading to reclassification of cancers; 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 monogenic germline susceptibility, typically resulting from an autosomal dominant loss of function in a tumour suppressor gene. Just over 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 PDL-1 blockade and PARP inhibitors;

PARP inhibitors block single strand DNA repair forcing cells to rely on homologous recombination (HR). This pathway requires functional BRCA1 and 2. Therefore, in BRCA gene carriers, cells that have lost the second gene copy and are becoming a cancer have compromised HR is and PARP inhibitors become lethal. The first drugs in this class, olaparib and rucaparib, are reaching the clinic (Underhill et al Ann Oncol 2011;22:268-79)

PDL-1 blockers block the inhibition of white blood cells and unleash the immune system to attack cells which look different (Le DT et al NEJM 2015;372:2509). They are selectively lethal to the CMS1 category of colorectal cancer where mismatch repair deficiency leads to the accumulation of mutations, production of frameshift peptides 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 is testing different doses of aspirin in 2000 MMR gene defect carriers beginning 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.

Novel Cancer immunotherapy approach

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The immune system, a complex network of cells and antibodies, has been designed by evolution to protect us not only from virus and bacterial infections, but also from cancer. In order to escape this protective barrier, tumours evolve continuously, thereby altering the mechanism that makes them detectable or blocking the activity of specific effector cells of the immune system (cytotoxic T cells). Any therapeutic approach, that aims to spark cancer immunity, is therefore potentially relevant to increasing the survival rate of cancer patients.

In the last decade a therapeutic revolution occurred in the field of cancer treatment and the immune-genomic came to occupy a leading role. The hypothesis, created by visionaries conducting research in cancer immunology during the late 1980s and the 1990s, stated that cancer cells presented novel, tumor-specific (i.e., mutated) peptides on the surface exposed by the patient's HLA molecules(Schumacher and Schreiber, 2015).

The T cell transfer was the first cellular immunotherapeutic strategy developed in the 1980s. This approach entails the isolation and activation of tumor-infiltrating lymphocytes, the in vitro expansion and therefore re-infusion in the patient. An example of this procedure is a recent case-report that reveals an objective regression of lung metastasis after expansion of CD8 T cells against mutant KRASG12D(Tran et al., 2016) colorectal cancer.

Another form of cellular immunotherapy is the genetic engineering of the chimeric antigen receptor (CAR) on T cells, against specific epitope. Notably, the receptor is modified in order to have an external **target-binding domain** that is designed to recognize a specific tumour antigen and an internal **activation domain** responsible for activating the T cell when the CAR-T binds its target. This productive strategy has ensured a high response rate after the infusion of the CAR T cells that have been modified against CD19 in the case of B-cell cancer (Schuster et al., 2017).

Several therapeutic strategies rely on the onset of non-self-molecules in order to foster immune response against exogenous stimuli such as DNA, RNA and peptides, which work as vaccines. The latest strategy can both trigger new antigen-specific T cell activation and amplify the existing responses against tumour cells.

Additionally, the oncolytic virus frontier is acquiring relevance in cancer immunotherapy whereby engineered virus selectively replicates and kills cancer cells. The efficiency of tumour response is caused by the acute tumor shrinkage owing not only to deleterious viral infection but also to the immunogenic activation of dying cells. Thus, the viral lysis of tumor cells sustains the antigen release in tumor microenvironment, mounting a CD8+ T cell-mediated anti-tumor response(Farkona et al., 2016).

Particularly relevant in the case of cancer immunotherapy is the contribution of checkpoint blockade through which specific antibodies are administered in order to tackle cancer-mediated T cell blockade and restore the immune control.

The treatment of melanoma, lung and colorectal cancer patients with immune checkpoint modulators (anti PD-1 and anti-CTLA-4 antibodies) and their positive outcome has radically changed the therapeutic options in

oncology (Garon et al., 2015; Le et al., 2015; Wolchok et al., 2013). A seminal observation that inspired the field was that response to checkpoint inhibitors correlated with mutational burden across tumor types(Snyder et al., 2014). In colorectal cancer, approximately 15% of patients carry alterations in genes involved in mismatch repair (MMR) and, accordingly, are classified as microsatellite instable (MSI). The genomes of MSI tumours bear hundreds of thousands of somatic mutations, a feature that fosters cancer progression and the rapid evolution of resistance to targeted therapies. Yet, it has been known for a long time that CRC carrying defects in DNA mismatch repair often display favourable prognosis and indolent progression (Vilar and Gruber, 2010) . Recent evidence suggesting that MSI tumors respond prominently to anti-immune checkpoint blockade (based on the anti PD-1 antibody) led to the seminal hypothesis that elevated mutation loads (high mutation burdens) are required for immunotherapy to be effective (Gubin et al., 2014; Rizvi et al., 2015). This assumption is supported by the observation that tumors with high environmental exposure-related mutational burdens (such as melanoma and lung cancers) also preferentially respond to immunotherapy (Gros et al., 2016; Schumacher and Schreiber, 2015; Sharma and Allison, 2015). Significantly, the clinical follow-up of MSI-CRC treated with immune-modulators reveals that responses are often long lasting, and this is in sharp contradiction with the observation made during treatment with targeted therapies such as kinase inhibitors (Arena et al., 2015; Misale et al., 2014). Most notably, immune check point blockade also displays long-lasting responses in melanomas, and also in that case, a substantial fraction of the patients display survival curves that are unprecedented and outstandingly different from the observation made for the same clinical setting with kinase (BRAF) inhibitors (Misale et al., 2012).

The other side of the coin constitutes the adverse events due to Immuno-checkpoint blockade. The acquired resistance to checkpoint inhibitors has been well characterized by the loss of sensitivity to IFN-γ, either through mutations or epigenetic silencing of mediators of the IFN-γ-JAK/STAT/IRF1 signaling pathways (Zaretsky et al., 2016). In addition, around 9% of patients treated with anti PD-1/PD-L1 have a hyper-progressive disease (> two fold change in tumor growth rate after a single anti PD-1/PD-L1 dose)(Kato et al., 2017).

Tumors gather thousands of alterations that have been considered to be the added value of neoplastic in comparison with normal cells. This value allows tumors to not only evade the control of our security system and gain proliferative advantages but also acquire resistance to therapies. Here, we describe the way in which these genetic alterations are potential targets to make tumours visible, restoring the control against what is a potential pathogen by the immune system.

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Spinal muscular atrophy: from gene to therapy

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

SMA is caused by homozygous absence (or rarely subtle mutation) of SMN1, whereas disease severity is influenced by the number of SMN2 copies and other SMA modifying genes [5-7]. Since SMN2 mRNA is mainly alternatively spliced lacking exon 7 due to a single translationally silent variant, 90% of SMN protein is truncated and unstable. The remaining 10% of transcripts, however, are full-length and produce 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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PHARMACOLOGICAL CORRECTION OF CYSTIC FIBROSIS BASIC DEFECT AS A PARADIGM OF PRECISION MEDICINE FOR GENETIC DISEASES

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Cystic fibrosis (CF) is caused by mutations that impair the function of CFTR, a plasma membrane chloride channel expressed in different types of epithelial cells. Importantly, it has been demonstrated that mutant CFTR expression and/or function can be rescued in vitro with small molecules, a finding that paves the way for a pharmacological approach to correct the basic defect in CF. Pharmacotherapy of CF basic defect has to be tailored to the various mutations present in CF patients. Some mutations, particularly Phe508del, the most frequent among CF patients, cause a severe impairment of CFTR protein folding and stability. In this case, small molecules such as VX-809 (a.k.a. lumacaftor) may in part solve the problem by acting as "correctors" to rescue Phe508del-CFTR from premature degradation. A combination of VX-809 and a "potentiator", VX-770 (ivacaftor), to boost channel activity, has been approved for the treatment of patients with two copies of Phe508del mutation. However, more effective treatments are needed to treat Phe508del/Phe508del patients and, particularly, those with a single copy of the mutation. Other types of CF mutations, such as the archetypal Gly551Asp, do not affect CFTR protein maturation but strongly impair the opening of the CFTR channel pore. In such cases, treatment with the potentiator VX-770 is enough to restore mutant CFTR function to therapeutic levels. The increasing availability of drugs targeting the different defects of mutant CFTR offers the possibility for a precision medicine approach to CF. The goal is to find the best treatment for each single patient based on the CFTR genotype. This is particularly important but also problematic for all those patients who carry one or two of the hundreds of rare mutations whose associated molecular defect is still to be defined. The evolving trend to address this problem is to use airway and/or intestinal epithelial cells from the patient to test the best single drug or combination of drugs to maximize mutant CFTR rescue. This can be done with 2D or recently developed 3D epithelial models. In conclusion, the development of drugs with different mechanisms of action and of in vitro tests to evaluate their potential therapeutic benefit allows the definition of a personalized treatment for CF patients.

Wednesday, May 2

Molecular inversion probes and mosaicism

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

The latter is only possible due to the digital nature of NGS, and detection is further improved using unique molecular identifiers (UMIs) [3,6]. We only start to understand the importance of mutations in mosaic state for human biology [5-9] and disease [7].

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Epigenetics, Imprinting, clinical

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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 (Begemann et al JMG 2018). 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 mothers

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, poor feeding Early puberty, Short stature	Developmental delay Cleft palate Scoliosis Obesity	uncertain	Ioannides, Y
WKO syndrome (Paternal UPD 14 associated syndrome)	unknown	Bell shaped chest Hypotonia Developmental delay	Umbilical defects Larger birth weight	uncertain	(Kagami et al.)
Pseudohypoparathyroidism 1B	unknown	Hypocalcaemia due to Parathryoid resistance (tetany/parasthesia)	Obesity	>90%+	(Bastepe et al.)

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Non-coding mutations/long-range effects

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Complex developmental processes require tightly controlled regulatory networks which ensure correct temporal and spatial gene expression during development. Gene expression programs are guided by cis-regulatory elements including promoters, enhancers, repressors and insulators. Some of these elements are located at large distances from the target gene itself and are therefore termed "long distance" or "long-range" regulatory elements. Disruption of long-range gene regulation can cause tissue- and stage-specific effects some of which have become recognized as a significant cause of developmental defects and 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 of topological chromatin domains (TADs) 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. Examples will be presented in this lecture.

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

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

Developing multiplexed assays for variant effect to interrogate the impact of noncoding and coding genetic variants.

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Interpreting the effects of genetic variation is the central challenge for the progress and prospects of precision medicine. Variant-effect prediction algorithms can classify any possible genetic variation but despite substantial efforts, they do not have the accuracy to support their use in a clinical setting. On the other hand, experimentally testing the effect of each individual variant on protein function or gene expression yields high-quality information, but is low-throughput. To resolve this, we are developing multiplexed assays for variant effect (MAVE) to determine the impact of thousands of genetic variants on protein function and/or gene expression in a single experiment. We have developed massively parallel reporter assays to interrogate the effects of thousands of enhancer or promoter variants in a single experiment. We also developed multiplexed functional assays for coding variants that can be reused for multiple proteins. Using these approaches, we comprehensively evaluated the effects of >4,000 single nucleotide variants on both mRNA splicing and protein function of *BRCA1*. And determined the effect of 7,801 amino acid substitutions on the stability and function of two proteins important for the etiology and treatment of cancer, PTEN and TPMT. The resulting large-scale functional data can be combined with machine learning and clinical knowledge to develop “lookup tables” of accurate pathogenicity predictions. A coordinated effort to produce, analyze and disseminate large-scale

functional data generated by multiplex assays may be essential to address the variant interpretation crisis caused by the increased use of DNA sequencing in the clinic.

Thursday, May 3

Discovering Structural Variants in Cancer using NGS data

Tobias Rausch
EMBL Heidelberg

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. The lecture covers analytical methods to call somatic mutations, determine somatic signatures, and identify complex genomic rearrangements such as Chromothripsis [5]. The associated workshop will cover these topics in more depth and for an example 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.

Using phenotypic data to recognise genotypes causing developmental disorders

D. Fitzpatrick

MRC Human Genetics Unit
IGMM, 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 disorders2. 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 data1 and a consistent approach to the collection and utilitation of such information is a vital part of study design

References:

1: FitzPatrick DR. Resequencing at scale in neurodevelopmental disorders. Nat Genet. 2017 Mar 30;49(4):488-489. doi: 10.1038/ng.3827. PubMed PMID: 28358133.

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.

GWAS with NGS

M. Nothnagel
Cologne Center of Genomics Cologne, Germany

This lecture will introduce genome-wide association studies (GWAS) based on next-generation sequencing (NGS) or on genome-wide chip genotyping. It will focus on single-marker analysis but will also provide some coverage of rare-variant collapsing tests. A particular emphasis will be put on the severe issues that valid GWAS are facing and that have to be addressed, including data quality control, confounding, statistical power and variant interpretation. Examples of successful GWAS will also be presented.

Data integration

Lude Franke
Univ. of 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.

Friday, May 4

Patterns of rare variation contributing to disease

K. Samocha
Sanger Institute, UK

A primary challenge of human genetics to distinguish disease-causing variation from the multitude of neutral variants found in any genome. The release of increasingly large collections of human sequencing data—such as the Genome Aggregation Database (gnomAD), which contains sequence data from over 138,000 individuals—has improved our ability to interpret genetic variation. These databases can be used to determine the allele frequency of a given sequence variant in a reference population and can therefore aid in filtering out variants that occur far too frequently to be compatible with rare and severe disease.

Additionally, the patterns of variation observed in these databases have been leveraged to identify genetic sequences that are significantly depleted of nonsynonymous variation, which indicates selective constraint against such protein-altering mutations. Gene-level metrics of constraint have proven to be a useful way of highlighting a subset of variants with a larger impact on disease risk. For example, the previously established excess de novo loss-of-function variation found in patients with neurodevelopmental disorders manifests primarily in genes identified as highly loss-of-function constrained. Overall, it is critical to study patterns of variation in reference populations when evaluating potentially pathogenic variation.

Genomics England and the UK's 100,000 Genomes Project

A. Rendon
Queen Mary University of London, UK

In this session I will present an update on the UK's 100,000 genomes, describe solutions to some of the challenges that we have confronted getting the project implemented, and provide some early investigations into new things we are putting in place.

At the time of writing we have sequenced just over 50,000 genomes and returned results to over 7,500 families with rare diseases and 1,800 patients with cancer. We steadily recruit over 500 new participants per week across a network of over 85 hospitals in the UK. The project processes around 20 terabytes of data every day and is beginning to steadily return results for about 500 families or cancer patients per week.

I would like to present our efforts in selecting alignment and variant calling pipelines that can scale to the size of the project while maintaining analytical performance. I will describe methodology to benchmark small variant calling pipelines and results validating copy number calls. I will also describe some of the challenges of whole genome sequencing cancer samples, the impact of tissue preservation and the benefits of fresh frozen

and PCR-free sample preparations. I will then talk about how we do interpretation with both the cancer and rare diseases programme and present results in terms of our actionability and diagnostic rates.
Finally, I will discuss some of the work we are doing to include Short Tandem Repeats into the interpretation process and early explorations of the utility of returning pharmacogenetic findings.

Suggested readings:
Best Practices for Benchmarking Germline Small Variant Calls in Human Genomes: https://www.biorxiv.org/content/early/2018/03/15/270157
Clinical Pharmacogenomics Implementation Consortium: https://cpicpgx.org/
Genomics England's PanelApp application: https://panelapp.genomicsengland.co.uk/

Non-invasive prenatal testing

J. Weiss
VU University Medical Center Amsterdam, the Netherlands

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.

Single-cell multi-omics to study the biology of cellular heterogeneity in health and disease

Thierry Voet

1Department of Human Genetics, University of Leuven, Leuven, Belgium.
2Sanger Institute-EBI Single-Cell Genomics Centre, Wellcome 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. To this end, we have developed various wet-lab and computational methods that allow analysing single cells at high resolution via microarray, SNP-array and next-generation sequencing platforms [1-5]. In addition, we developed methods to sequence both the DNA and 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], the (ageing) brain 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.

ABSTRACTS OF WORKSHOPS

Sunday, April 29

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. UCSC, 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 then go through several groups of paralogous genes and examine the syndromes associated with loss and gain of function of the group members exploring overlaps in phenotypes. We will cover the phenotypes associated with mutations in NFI, AFF and ASXL groups of genes.

Monday, April 30

NGS Bionfirmatics Basics

C.Gilissen & T. Pippucci

In this workshop you will familiarize yourself with the file formats and contents of standard NGS formats such as FASTQ, BAM and VCF files. You will learn about the advantages/disadvantages of different sequencing technologies

Targeted NGS approaches

A. Hoischen

This workshop allows to discuss targeted re-sequencing approaches in greater detail.
One of the very efficient high-throughput targeted re-sequencing approaches is discussed here in greater detail: molecular inversion probes [1,2]. This approach also allows very accurate detection of mosaic mutations [2].
The purpose is however to have as much interaction as possible, so I would like to encourage all participants to bring forward their experience and faced issues that can be discussed as part of this workshop.

Recommended reading:

1. Mamanova L et al. Target-enrichment strategies for next-generation sequencing. Nat Methods. 2010 Feb;7(2):111-8.
2. 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.

How to set up a NGS lab?

A. Hoischen

This workshop allows to discuss all requirements to start your own NGS lab. But it is also suited to discuss outsourcing vs. in-house sequencing possibilities.
The purpose is really to have an open discussion about all issue you face in your own lab, so please bring examples/questions from your own experience.

Clinical Considerations for NGS

Han G. Brunner

This workshop aims to discuss clinical diagnoses and whether one would use NGS, or another test, or no test at all. We shall also look at diagnostic reports and discuss their uses and shortcomings.

Copy number variation, disease and evolution

E.E. Eichler

Copy number variation is an important source of human genetic variation. Copy number variants (CNVs), however, are typically more difficult to discover and genotype. This workshop will focus on the development of both experimental and computational methods to characterize such variation. We will explore different classes of CNVs, their population genetic properties, and their impact with respect to disease. I will highlight examples of novel genes that have evolved within the human lineage as a result of CNVs and how these appear to have contributed to adaptive aspects both within our species as well as adaptations unique to the human lineage. Paradoxically, the high background rate of copy number variation mutations associated with neurodevelopmental disease in the human species is inextricably linked to the emergence of these novel human-specific genes.

Tuesday, May 1

NGS Bionfirmatics, variant interpretation

C.Gilissen & T. Pippucci

In this workshop you will learn how to interpret variants identified through NGS and how to prioritize likely pathogenic variants for Mendelian disorders.

Rarity in the clinic

J. Burn

Rare diseases are a major collective burden in healthcare. This workshop will explore the common features which challenge the care of families in the clinic while also addressing the wider societal issues like consanguinity and termination of pregnancy. The session will include some role play and mental arithmetic but don't let that deter you! Guidance on how to cheat at Blackjack is also included at no extra cost.

NGS and the understanding of disease-causing versus neutral alleles: the cystic fibrosis (CF) paradigm

Carlo Castellani and Luis J.V. Galietta

CFTR analysis is widely employed for diagnosis in symptomatic individuals, for carrier testing in the general population, as part of newborn screening and most recently for the identification of patients treatable with variant-specific molecular compounds. Limitations in the understanding of the disease-causing capability of most *CFTR* mutations affect in different ways sensitivity and specificity of carrier testing, newborn screening, and in general CF diagnosis.

Genetic testing of *CFTR* is mostly performed using commercial kits that rarely approach 100% sensitivity. Next generation sequencing increases sensitivity at the cost of poorer specificity, as it may identify a *CFTR* variant that is uncharacterized or one that is known to be associated with partial penetrance, and in some cases contribute more to worsen than to clarify the diagnostic uncertainty.

In fact, not all the mutations impair synthesis or function of the CFTR protein to the extent of causing CF: some, known as CF-causing, result in CF, some produce mild and/or mono-organ manifestations (the so-called "CFTR-related disorders"), and others have no clinical consequences at all. Since assessing disease liability of a CFTR mutation may be challenging, a database named "Clinical and Functional Translation of CFTR" (CFTR2) has been developed (www.cftr2.org). On the grounds of clinical, functional and epidemiological data, CFTR2 chooses among three possible definitions for every mutation observed: a) CF-causing mutation, always expected to produce CF when in trans with another CF-causing mutations; b) mutations of varying

clinical consequence, expected to produce CF when in trans with a CF-causing mutations in some but not in all patients; c) non CF-causing mutations, not expected to cause CF. So far, the disease liability of only a few hundreds has been ascertained. These cover most of the allelic pool in the CF population but a minority of the total number of CFTR mutations so far detected. Most rare mutations remain clinically unlabeled. Their clinical impact can be roughly estimated weighing available clinical, functional and population evidence.
In standard diagnostic practice, applications that allow reporting only mutations with well-established disease liability may help to offset the potential side effects of NGS. A conservative approach like this would retain the ability to "go back"and reveal variants of uncertain disease liability if the clinical scenario warrants it.

Ethics of Medical Genetics

A. Read

The public tend to see medical genetics as a particularly ethically challenging branch of medicine. Whether or not this is really true is debatable, but it is true that the use of exome and genome sequencing in diagnostic laboratories had given increased prominence to some pre-existing ethical issues. The distinction between research and service has become blurred, and questions about consent and incidental findings have become more important. This Workshop will use some fictional scenarios to focus discussion of some of the issues.

From your newly discovered candidate gene to its function

Brunhilde Wirth

You identified families with a so far unknown disease gene. You performed WES/WGS analysis and even found some promising potential pathogenic variants. But how to decide, which is the best candidate to follow functionally and to prove your hypothesis is correct?
In this interactive workshop, which will take place in the amphitheater outside in the garden, we will discuss how you decide, which is the best candidate gene(s) to proceed for functional analyses, which strategies to use, which animal models, which techniques to provide solid data biochemical and cellular data for your next top publication.

In silico prediction of putative neoantigens: a workflow between tumour DNA alteration and cell mediated immune response.

G. Germano & G. Rospo

In the era of big data Next Generation Sequencing (NGS) and bioinformatics impact the knowledge of the genetic landscape of tumour suggesting novel clinical approaches or predicting the therapeutic response in patients. The knowledge between tumours and immune system improve and the High-throughput technologies may overcome existing challenges in the field.

Computational methodologies are unveiling novel features allowing to characterize genetic mechanisms involved in cell-mediated immune responses such as human leukocyte antigens (HLA) typing, neoantigens prediction and characterization of tumor immunogenicity. Notably tumour-specific antigens may, in some cases, trigger immuno-response and different computational approaches are available for their prediction in silico: standard workflow that includes the use of different tool freely available or custom; bioinformatic tools ready-to-use; outsource analysis. We present a bioinformatic standard workflow to perform calling of somatic variations and to predict tumor-specific antigens according to a patient specific HLA. The pipeline is composed by multiple steps that involve parameter settings for calling mutations, an intermediate steps such as filtering annotations data and the final instructions allowing predicting the immunogenicity of filtered variants. During the workshop the variant annotation file will be provided to participants that can proceed to filter alterations on the basis of allelic frequency, strand bias, SNP databases and RNA gene expression values. The resulting variants will be arranged on their sequence context and then provided to netMHC 4.0 server in order to calculate the immunogenicity of predicted neoantigens.

Suggestion readings:

Hundal et al., Genome Medicine (2016), doi:10.1186/s13073-016-0264-5

Yarchoan, Johnson III et al., Nature Reviews Cancer (2017), doi:10.1038/nrc.2016.154

Bjerregaard et al., Cancer Immunology Immunotherapy (2017), doi:10.1007/s00262-017-2001-3

Giannakis et al., Cell Reports (2016), doi:10.1016/j.celrep.2016.03.075

Germano et al., Nature (2017), doi:10.1038/nature24673

Thursday, May 3

Discovering Structural Variants in Cancer using NGS data

T. Rausch

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. We will apply Delly [1] to call somatic structural variants in a cancer sample showing patterns of Chromothripsis [2]. Basic analytical methods in cancer genomics and crucial quality control methods will be reviewed in detail. For a subset of candidate variants we will also design PCR primers and analyze Sanger traces using GEAR [3] as one method of genomic variant validation. 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 primarily interested in general cancer genomics analytical approaches 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.
[2] Genome sequencing of pediatric medulloblastoma links catastrophic DNA rearrangements with TP53 mutations. Rausch et al. Cell. 2012 Jan 20;148(1-2):59-71.
[3] https://gear.embl.de/

Genetic Imprinting

K. Temple

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 syndromes 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.

Multiplexed assays for variant effect workshop

L. Starita

Multiplexed assays for variant effect (MAVE) determine the impact of thousands of genetic variants on protein function and/or gene expression in a single experiment. In this workshop we will discuss the rules for developing these assays, molecular protocols for making and testing all of the variants as well as basic data analysis.

How to do GWAS

M. Nothnagel

This workshop extends the introducing morning lecture and provides a more detailed and more technical view of genome-wide association studies (GWAS). In a seminar-style format, the workshop will demonstrate the basic steps of a GWAS. Each step will start with a more in-depth introduction, followed by a practical how-to demonstration and a discussion of issues. The format will leave plenty of time for interactive questions & answers.

How to do RNASeq

L. Franke

To better understand why certain mutations cause disease gaining knowledge on their downstream molecular consequences is very valuable. One of the ways to gain that insight is by using gene expression data. For instance through allele specific expression analysis it is possible to determine whether certain rare mutation alleles have a detrimental effect on gene expression. For common variants it is also possible to conduct eQTL analysis, to identify downstream pathways. In this workshop I will discuss these strategies, and will also highlight how GeneNetwork can be used to identify the causal genes when using exome-sequencing data.

Friday, May 4

Interpretation of rare variants

K. Samocha

As exome and genome sequencing have become increasingly commonplace, there is an ever-pressing need to be able to interpret sequence variants. This is particularly important in a clinical context, where there is a suspected genetic diagnosis. In this workshop, we will discuss multiple lines of evidence that can be considered when determining the potential pathogenicity of a sequence variant identified in a patient. Specifically, we will be using the DECIPHER website (https://decipher.sanger.ac.uk) as a platform to evaluate evidence of pathogenicity for a set of variants in an example patient. DECIPHER, established in 2004, is a major global platform for the visualization of phenotypic and genomic relationships. It currently contains over 25,000 patient records with open-access consent, which have >75,000 phenotype observations and >33,000 open-access copy number and sequence variants. This workshop will showcase many of the key features of a workflow to interpret variation using DECIPHER, including exploring the genome and protein browsers, the annotation interface, and finding patients with similar phenotypes.

Reporting NIPT results, and ethical concerns

J. Weiss

Reporting NIPT results:

NIPT analysis is a relatively new branch on the tree of genetic testing. As with other genetic tests, reporting test results is complex, even when the results seem straightforward. There are no best practice guidelines available yet. However, the report must be written to provide a clear result and conclusion which is not open to misinterpretation. An important difference with many other genetic tests is the fact that NIPT is a screening test, not a diagnostic test.

Issues specific for NIPT reporting that will be discussed during this workshop are:

Testing information

In order to interpret the results, it is important that a laboratory reports the basis of the test. This is not only limited to the technical basis (for instance Massively Parallel Sequencing or array), but also the bioinformatical tools. Is the software able to distinguish between a trisomy and a maternal CNV. Is analysis limited to trisomies alone, or are other anomalies found as well. It is also important that the limitations of the test are discussed, especially when a normal result is reported. One of the major issues is whether fetal fraction should be reported. Several tools are available to determine fetal fraction, especially in the case of a male fetus (bases on the Y-

chromosome). However, it is very difficult to properly validate these tools as no real golden standard exists. Furthermore, it is unclear how to use knowledge of fetal fraction. Although a lower limit of 4% has been suggested, other reports describe reliable detection of trisomy 21 at lower fetal fractions. During a recent workshop at the ISPD in berlin, no consensus could be reached on whether or not fetal fraction should be reported.

Reporting the result

The terminology used to report the result is very important. It must be easy to find within the report, clear and not open to any misinterpretation. As this is a screening test, results cannot be reported to confirm or rule out a diagnosis. Terms such 'high risk/low risk of aneuploidy' are more appropriate. Furthermore, when reporting a high risk result, the report should also include a recommendation to confirm the result with an invasive test. During the workshop students will write NIPT reports based on several NIPT cases. Reports will be discussed in the group.

Ethical issues:

During the last part of the workshop we will discuss several ethical issues concerning NIPT. This will be done based on the following propositions:

NIPT should not be used for trisomy 21, 13 and 18 alone, but results on other chromosomes should be reported as well

Down syndrome is not a severe burden anymore, therefore parents should be offered a choice for NIPT analysis with an opt-out for chromosome 21 test results

Including sex chromosome testing in NIPT will allow sex based termination of pregnancies and should be forbidden

Large Genomic Projects Workshop

D. Fitzpatrick & A. Rendon

This workshop will mostly take the form of an informal question and answer session. The focus will be on how to implement and review a large-scale clinical genomic study. There will be with particular emphasis on defining the genetic architecture of the target disorders as this will influence the informatics pipelines and variant reporting algorithms used. The workshop's aim is to facilitate the participants to establish such studies and will make reference to the various presentations throughout the course.

How to perform single-cell (multi-)omics?

Thierry Voet

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.

Furthermore, 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.

Finally, depending on the interest of the students in preimplantation genetic diagnosis (PGD), the procedure for single-cell genome-wide haplotyping and imputation of genetic variants causing Mendelian disorders will be detailed and exercised.

References

1 Macaulay, I. C. *et al.* G&T-seq: parallel sequencing of single-cell genomes and transcriptomes. *Nature methods* **12**, 519-522, doi:10.1038/nmeth.3370 (2015).

2 Zamani Esteki, M. *et al.* Concurrent Whole-Genome Haplotyping and Copy-Number Profiling of Single Cells. *Am J Hum Genet* **96**, 894-912, doi:10.1016/j.ajhg.2015.04.011 (2015).

3 Van der Aa, N. *et al.* Genome-wide copy number profiling of single cells in S-phase reveals DNA-replication domains. *Nucleic Acids Res* **41**, e66, doi:10.1093/nar/gks1352 (2013).

4 Voet, T. *et al.* Single-cell paired-end genome sequencing reveals structural variation per cell cycle. *Nucleic Acids Res* **41**, 6119-6138, doi:10.1093/nar/gkt345 (2013).

5 Konings, P. *et al.* Microarray analysis of copy number variation in single cells. *Nature protocols* **7**, 281-310, doi:10.1038/nprot.2011.426 (2012).

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THE GUGLIELMO PROJECT

Background of the Project: the European School of Genetic Medicine

Since 1988 thousands of young geneticists, especially physicians and biologists, have been trained by the European School of Genetic Medicine (ESGM). Every year they come to the Rocca (castle) of Bertinoro (a small town of Romagna) from every side of Europe, from the Southern rim of the Mediterranean sea, from the Middle East and from several other countries.

Taking into consideration only the period 2000-2011 the participants to these ESGM advanced training courses have been 6205, including approximately 200 faculty and 6000 postgraduate students (Ph.D. candidates, post-doc and MD). Today ESGM and the Centro Universitario di Bertinoro (CeUB) (http://www.ceub.it/), which is part of the Alma Mater Studiorum-Università di Bologna, represent the most well known and active European center for the advanced training of young graduates in the field of Genetic Medicine. For this reason ESGM is strongly supported and sponsored with scholarships by the European Society of Human Genetics (ESHG).

The newly added feature of ESGM: Genetics and Society

In 2016 ESGM and Bertinoro started a completely new experience aimed at the public understanding of genetics. One of the Faculty of the course in Medical Genetics, **Dr. Mario Capecchi, Nobel Prize winner for Medicine in 2007, gave a talk in English for the public understanding of genetics to about 300 High School students on May 9, 2016 in Forlì** (12 km away from Bertinoro). In addition a selected group of High Schools students (40 from Forlí, 7 from Cagliari, 12 from Verona, 2 from Genova) with their respective science teachers were hosted in Bertinoro to attend the last lectures of the 29th COURSE IN MEDICAL GENETICS on Thursday May 12. On the same day the 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 concerning the prevention of genetic disorders). All the talks and debates were in English and many questions were asked in English by the Italian students. This first experience of public understanding of human/medical genetics made possible by the resources offered by ESGM (faculty, venue, etc.) was highly successful and showed the usefulness of a program regarding "Genetics and Society" specifically tailored for High School students thus initiating the "Guglielmo Project".

The social meaning of the Guglielmo Project

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 (a primary immunodeficiency) diagnosed too late in Bologna. Early diagnosis might have saved him. He was also a student of music and a lute-maker. His death at such an early age because of an undiagnosed genetic disorder represents a strong message for young people and teaches them the need and usefulness of awareness of human/medical genetics for themselves and for their future progeny.

Which goals did the Guglielmo Project achieve in 2016?

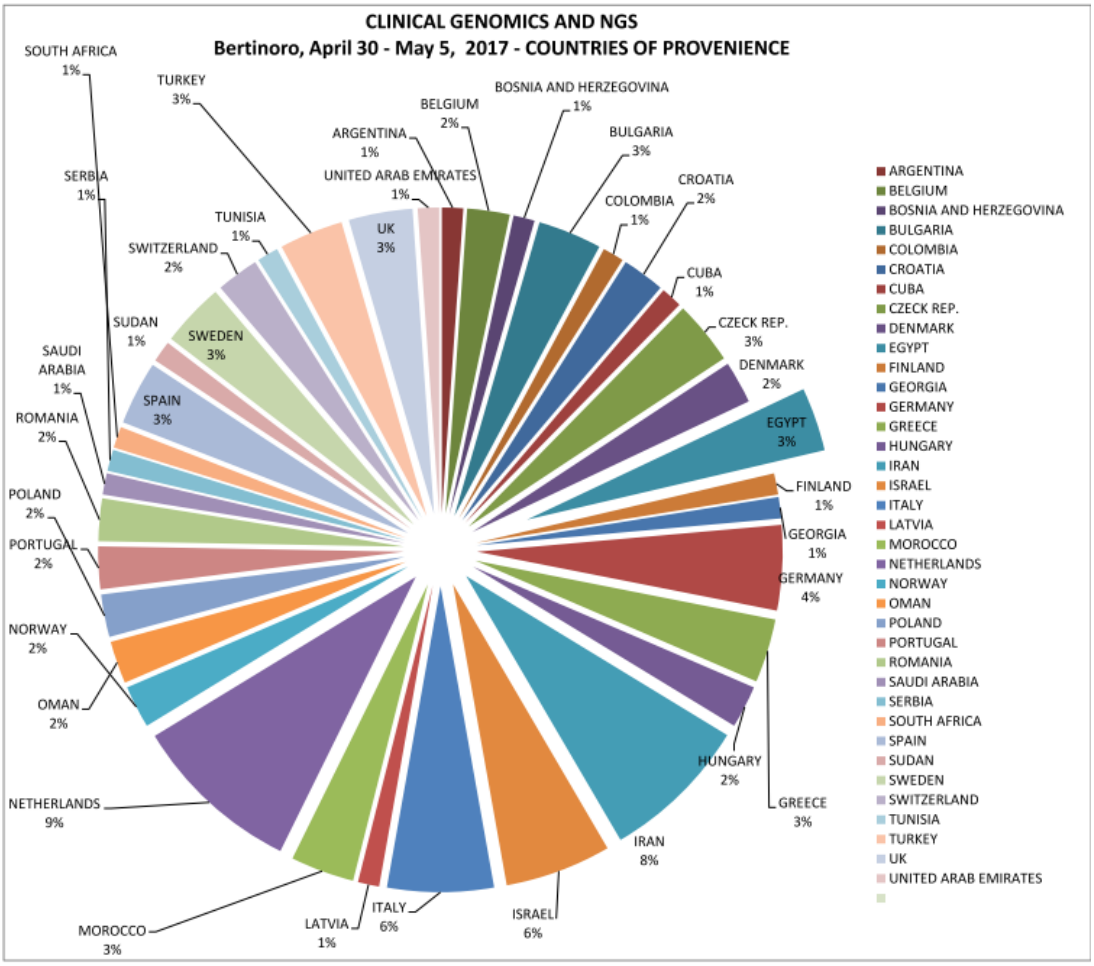
- It increased the knowledge about human genetics and genetic disease among High School students.
- It tested new tools of communication to facilitate the understanding of genetics for users of genetic medicine and for the general public.
- It proved the need of new courses for science teachers from secondary school
- It strengthened the cooperation between Universities and networks of High School students and science teachers.

The Guglielmo Project in 2017

On May 4 and 5, 2017, lectures and workshops for a selected number of Italian High School students and science teachers were held in Bertinoro while the 30th edition of the main ESGM course was taking place. It is worth noticing that this course (which in 2017 changed its denomination from "Medical Genetics" into "Clinical Genomics and NGS") was attended by 89 students from 37 different countries from all over the world, in contrast to the 108 students from 16 countries (only European) who attended the first edition of the Medical Genetics course in 1988 (see below).

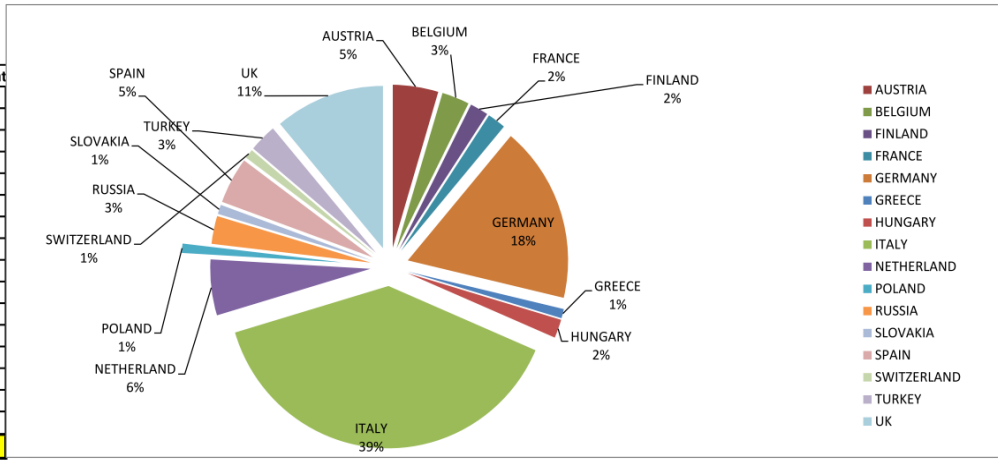
Breakdown of students attending the main ESHG-ESGM course (2017 vs 1988)

nationality	pax
ARGENTINA	1
BELGIUM	2
BOSNIA AND HERZ	1
BULGARIA	3
COLOMBIA	1
CROATIA	2
CUBA	1
CZECK REP.	3
DENMARK	2
EGYPT	3
FINLAND	1
GEORGIA	1
GERMANY	4
GREECE	3
HUNGARY	2
IRAN	7
ISRAEL	5
ITALY	5
LATVIA	1
MOROCCO	3
NETHERLANDS	8
NORWAY	2
OMAN	2
POLAND	2
PORTUGAL	2
ROMANIA	2
SAUDI ARABIA	1
SERBIA	1
SOUTH AFRICA	1
SPAIN	3
SUDAN	1
SWEDEN	3
SWITZERLAND	2
TUNISIA	1
TURKEY	3
UK	3
UNITED ARAB EMIF	1
TOT.	89



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



This simple comparison shows the tremendous impact on the practice and research in human/medical genetics that this and other ESHG-ESMG courses are having far beyond Europe.

A similar achievement can be yielded by the Guglielmo project if it becomes a common endeavor of many different countries (see below).

Continuing the tradition of the Guglielmo Day started by Dr. Mario Capecchi, in 2016, on Saturday May 6, 2017 a faculty of the course "Clinical Genomics and NGS", **Dr. Evan Eichler from Seattle, WA, gave again a talk on autism (always in English) to about 300 High School students and teachers in Forlì**. During both Guglielmo Days (in 2016 and 2017) musical performances were given by groups of students participating in the project and highly appreciated by the others (see picture at the end of this text). The inclusion of music in the Guglielmo project reflects the fact that music is a universal language leading to the same conclusion of modern genetics regarding mankind which, following from the inscription at the entrance of the Musée de l'Homme in Paris, states: "Tous parents, tous different. Les races n'existent pas" (We are all different and all related. Races do not exist"). It should be also underlined that the joy which is always associated with music is the ideal vehicle to spread among young people the message of the Guglielmo Project.

The preparation and distribution of didactic material needed for science teachers and their High Schools students has been coordinated by Prof. Beatrice Zanini from Genoa (beatrice.zanini@unige.it) who is also the coordinator of the DNA Day organized each year by the European Society of Human Genetics (ESHG) during its annual meeting and, again, is aimed at High School students.

The continuation of the Guglielmo Project in 2018

High Schools students from Bologna and other cities of the Emilia-Romagna Region will convene in Forlì on April 28th 2018 for the Guglielmo Day which will be characterized again genetic talks in English (see enclosed program in Italian) and by musical pieces played by the students of the Music Lyceum of Forlì in addition to several talks on human evolution which will be given by molecular geneticists and anthropologists.

For further information on the Guglielmo Project please contact:

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The **picture below** shows a moment of the concert played by the High School students of Verona during an intermission of the conference of Dr. 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 the violin he is making) is projected on the screen.

ABSTRACTS OF STUDENTS POSTERS

The influence of genetic variants involved in thrombophilia pathogenesis and folic acid metabolism on embryo implantation and pregnancy outcome

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Introduction: Thrombophilia is a hypercoagulable blood state. This abnormality of a hemostasis can lead to pathological blood clot forming. Congenital thrombophilia is caused by changes in different genes. The most frequent genetic variations altering coagulation are those in genes encoding coagulation factors: *F5* – c.1601G>A, also called FV Leiden mutation (rs6025), c.4070A>G (rs1800595), c.5279A>G (rs118203907); *F2* – c.*97G>A (rs1799963); *SERPINE1* 4G homozygous deletion c.-820_- 817G (4_5) (rs1799889). Also *MTHFR* variants c.665C>T (rs1801133) and c.1298A>C (rs1801131) are associated with hyperhomocystenemia and following blood hypercoagulation. All these genetic variants increase the risk of thrombosis and might be the cause of infertility and adverse pregnancy outcomes (failed IVF cycles, missed abortion, fetal retardation) [1., 2.]. All the researches are performed in general populations, but wider studies are still needed, how these genetic variants affect exactly pregnant women.

Aim, materials and methods. The main purpose of this study was to find out whether there are statistically significant differences of thrombophilia pathogenesis and hiperhomocysteinemia related genetic variants between female patients with different factors of infertility. 500 patients were included in this study, aged 35 – 45 years, with previously diagnosed infertility and single nucleotide polymorphisms in genes *F5*, *F2*, *MTHFR* and *SERPINE1*. The patients were clustered into different groups, each group representing only one genetic variant or determined number of genetic variants. The genetic association study between genetic variants and missed pregnancies or homocysteine levels was performed. The following parameters were included in statistical analysis – thrombophilia prevalence in the research population, allelic frequencies of polymorphisms and ratio between patients with and without investigated event in anamnesis (missed abortion or elevated homocysteine levels). The data were processed with PLINK software, the threshold of statistical credibility $p<0.05$.

Results: Hyperhomocystenemia was found in 232 patients' biochemical screening data (46.4%). Missed pregnancy was found in 146 patients' anamnesis (29.2%). iVF cycle was carried out for 100 patients, from which 65 cycles ended with failure. Analysing the occurence of allelic variants, it appeared that the most frequent polymorphism in population is *MTHFR* c.1286A>C heterozygous genotype, which is followed by

c.665C>T heterozygous genotype (23%), and also *MTFHR* c.1286A>C / c.665C>T compund heterozygous genotype (23%). Homozygous *MTHFR* c.1286A>C and c.665C>T genotypes were found in 11% and 9% of the investigated population. *F5* and *F2* variants compose very small proportion of investigated population (4%), in which the most frequent is *F5* c.3980A>G heterozygous genotype. *MTHFR* c.665C>T relevance to hyperhomocystenemia is very high odds ratio (OR=3.37, p<0.001) when compared to c.1286A>C (OR=1.03, p=1). *MTHFR* c.665C>T and missed pregnancy assocation results indicates increased risk (OR=1.7, p<0.001). *F2* c.1601G>A SNP has the highest risk of missed pregnancy because of thrombotic events - OR=8.24 (p<0.001). Risk for missed pregnancy increases, if there is raising number of changes (>1) in those genes involved in coagulation pathway.

Conclusion: *MTHFR* polymorphisms are not related to missed pregnancy, but hyperhomocystenemia might be an additional factor of increased risk of thrombophilia during the pregnancy. Increased risk could be explained with the presence of *F5* c.1601G>A or *F2* c.*97G>A genetic variants which usually are found together with *MTHFR* variants. Genetic changes of *MTHFR* are very often (~50% frequency), and, even if they do not have significant influence on spontaneous stopped pregnancy, they might increase the risk of inherited thrombophilia, if found together with *F5* c.1601G>A or *F2* c.*97G>A.

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Single-cell Gene Expression Profiles of the Developing Human Heart

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The heart is the first organ that becomes functional in the human embryo, beginning to beat and pump blood three weeks after fertilization. Although functional at this early stage, the developmental process that the nascent heart must follow is complex. We currently lack a complete understanding of how mature cardiomyocytes develop and there is no defined stem cell that can give rise to all components of the adult heart. Many studies have been using uniform pieces of heart tissue with bulk techniques, but this approach produce only an average expression pattern, masking gene activity of individual tissue parts. Therefore, a spatial view of the global gene expression in distinctive areas of the developing heart, together with complete gene profiles

of progenitors committed to the cardiomyocyte linage, would provide new insights in the field of human embryology and cardiovascular medicine. In this study, we used spatial transcriptomics (ST) technology[1]; a method that allows spatial gene expression analysis within the context of whole tissue sections, together with single-cell RNA sequencing. Thin tissue sections were placed on top of ST microarrays printed with clusters of poly-T capture probes, and the mRNA population above each cluster could then be hybridized and reverse transcribed. All capture probes within each cluster contain individual barcode sequences, giving us information about the X and Y position of the array. This allows information about the spatial distribution to be retained and the sequencing data can then be superimposed back onto the histological image of the tissue. Combining ST and single-cell technologies demonstrate functional clusters related to the architecture of the heart, as well as the spatial location of cells with stem cell signatures. Furthermore, spatial resolution of all gene activity that gives rise to the human heart has been, to our knowledge, studied for the first time.

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Ending an Odyssey: a diagnosis of Kagami Ogata syndrome due to UPD (14) pat in a 30 years old patient.

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Kagami Ogata syndrome (KOS) is an imprinting disorder caused by loss of the maternal contribution at 14q32.2.[1] It is most frequently caused by UPD(14)pat, although several other pathogenic mechanisms have been described.[2] In this study, we report the case of a 30 year old man with a longstanding history of multiple congenital anomalies, intellectual disability, and dysmorphic features who was recently diagnosed with KOS due to UPD(14) pat after a lifelong series of genetics clinic assessments and investigations. Many aspects of his presentation were similar to those reported in the literature[3] including complex a prenatal history involving polyhydramnios, omphalocele, and premature delivery. At birth, he was noted to have a small, bell-shaped chest and severe respiratory distress. Over time, he was noted to have significant dysmorphism, which included a broad midface, full cheeks, medial sparseness of the eyebrows, a protruding philtrum, thin lips with a widened cupid's bow, and a prominent chin. He had a high arched palate, kyphoscoliosis, inguinal hernia, and hypoplastic laterally displaced scapulae. Other features have not been previously described, including extreme macrocephaly, neonatal edema, skin laxity, a deep furrow in his tongue, and pronounced joint laxity of his MCP, PIP, and DIP joints. The diagnosis was made following the finding of complete absence of heterozygosity of chromosome 14 on SNP array, which was later confirmed to be due to paternal isodisomy with microsatellite analysis.

This patient represents the oldest reported case of KOS, and presents with a number of unique features that have not been previously described. Also, his genetic diagnosis occurred following the non-specific application of a genomic technology. While methylation studies of the 14q32.2 locus remain the gold standard for detecting KOS in suspected cases,[1] many genome-wide technologies commonly applied in the clinic, including SNP array and whole exome sequencing, may (or may not) also detect this condition. When performing these studies in a patient where KOS is on the differential, it is important to understand the potential and limitations of each of these techniques, and a strong clinical suspicion may warrant further investigation.

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Germline translocation disrupting ETV6 predisposes to childhood acute lymphoblastic leukemia (ALL)

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Introduction:

Studying familial childhood B-cell precursor acute lymphoblastic leukemia (BCP ALL) provides a unique opportunity to understand the pathogenic genetic mechanisms behind the disease. Recently, rare pathogenic germline variants in ETV6 have been reported in families with thrombocytopenia and predisposition to hematological malignancies, predominantly

childhood BCP ALL.1–6 In addition, a strong overrepresentation of high hyperdiploid (HeH) subtype (80%) and higher age at onset (13.3 vs 6.8) was observed in children with BCP ALL and pathogenic germline variants in ETV6.5,7

Methods and Results:

We studied a family with nine carriers of a balanced germline reciprocal translocation, t(12;14)(p13.2;q23.1), disrupting ETV6. Two female carriers developed childhood HeH BCP ALL at age 8 and 12 years respectively, but no thrombocytopenia was reported in any of the carriers. The translocation was discovered by karyotyping and confirmed by whole genome sequencing of the two affected and one unaffected carrier. Genomic analysis enabled detailed analysis of the translocation, creating two fusion genes, ETV6–RTN1 and RTN1–ETV6, with breakpoints in intron one of each gene. Both fusion genes were actively transcribed but predicted non-functional due to loss of functional domains. Subsequently, a theory of ETV6-haploinsufficiency as the mechanism of predisposition was stipulated. Therefore, expression of ETV6 mRNA was analyzed in germline and diagnostic samples, but results were inconclusive. In addition, genomic analysis of both females' leukemia excluded second hits in *ETV6*.

Conclusion:

Here we report a unique family with predisposition to childhood BCP ALL carrying a germline translocation, t(12;14)(p13.2;q23.1), disrupting *ETV6.* In line with previous reports, our affected carriers developed HeH subtype of BCP ALL and had a higher age at onset. In contrast, no thrombocytopenia was observed. To our knowledge, this is the first report of a germline structural variant in *ETV6* causing predisposition to childhood ALL. Although the mechanism remains unknown, our finding further supports the role of ETV6 in predisposition to childhood ALL.

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Structure of the 5q13.2 segmental duplication as a modifier of the phenotype of spinal muscular atrophy and amyotrophic lateral sclerosis

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Background: Spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS) are devastating motor neurone diseases leading to muscle wasting and, ultimately, death. SMA is the most frequent monogenic cause of infant mortality caused by homozygous absence of *SMN1* located in the telomeric part of the 5q13.2 segmental duplication, while in its centromeric part resides a nearly identical copy named *SMN2* producing only ~10% of full-length SMN RNA/protein. Clinical presentation of SMA is extremely variable ranging from a severe infantile form (type I) to mild adult-onset form (type IV). Homogeneity of the disease-causing mutation and extensive phenotypic variability clearly indicate the existence of genetic, epigenetic and environmental factors modulating disease progression. 5q13.2 segmental duplication is extremely unstable and harbours not only the two *SMN* genes, but is also enriched in other genes, i.e. *SERF1* and *NAIP*. Its inherent instability leads to a high rate of unequal crossing over resulting in complex structural rearrangements and in copy number variations (CNVs) of encompassed genes, which might modulate disease severity. Sporadic ALS (SALS) comprises ~90% of all ALS cases and is an incurable and the most common late-onset motor neurone disease with an inexorably progressive course and unknown aetiology. Even though aetiology, age at onset and progression may differ between the diseases, due to the involvement of SMN and some ALS-associated proteins in common molecular pathways, *SMN1* and *SMN2* genes were repeatedly studied as SALS susceptibility genes.

Aim: We aimed to reconstruct genomic structure and the nature of rearrangements of the 5q.13.2 telomeric part and to examine the main and joint effect of *SMN2*, *SERF1* and *NAIP* CNVs on phenotypic variability of early-onset SMA. Furthermore, we aimed to expand previous association studies of the *SMN1* and *SMN2* genes and sporadic ALS (SALS) to other genes residing in the same genomic region, i.e. *SERF1* and *NAIP*, and to assess whether their CNVs are associated with susceptibility to SALS and its severity in patients from Serbia.

Materials and methods: Multiplex ligation-dependent probe amplification (MLPA) was used to determine *SMN1*, *SMN2*, *SERF1* and *NAIP* CNVs in 99 Serbian patients with homozygous absence of *SMN1* (23 with severe type I, 37 with intermediate type II and 39 with mild type III), and 122 patients' parents, as well as in a

clinically well-characterised group of 133 Serbian SALS patients and 133 controls. We tested main and joint effect of *SMN2*, *SERF1A* and *NAIP* CNVs on disease severity indicated by the type of SMA. Association of *SMN1*, *SMN2*, *SERF1* and *NAIP* CNVs with SALS susceptibility was also examined, and Cox regression analysis was used to determine independent prognostic factors.

Results: The inherent instability of the 5q13.2 segmental duplication was witnessed by the existence of 20 different telomeric alleles among 442 analysed chromosomes. Inverse correlation was found between CNVs of each individual gene and SMA type (Spearman rank test, *SMN2* p=2.2e-16, *SERF1* p=4.264e-10, *NAIP* p=2.722e-8). Generalised linear model and backward selection, starting with a full model including the *SMN2*, *SERF1* and *NAIP* copy number and their interactions, revealed that the best minimal model explaining phenotypic variation in childhood-onset SMA with the smallest set of variables included the *SMN2* (p<2e-16) and *SERF1* (p<2e-16) copy number and their interaction (p=0.02628).

Individual association between copy number of the *SMN1*, *SMN2*, *SERF1* or *NAIP* genes and SALS susceptibility or survival was not found. Adjusted survival curves based on the Cox model obtained by the multivariable Cox regression analysis and encompassing genetic, demographic (gender) and clinical variables showed that three *SMN1* copies (HR=1.934, 95% CI 0.391-9.561) and faster decline of forced vital capacity (FVC) (HR=1.342, 95% CI 0.898-2.006) and revised ALS functional rating scale (ALSFRS-R) score (HR=2.745, 95% CI 1.301-5.792) result in shorter survival of Serbian SALS patients.

Conclusions: Large-scale deletions were mostly found in severe SMA patients, while gene conversion of *SMN1* to *SMN2* was mainly a characteristic of milder SMA phenotypes. *SMN2* and *SERF1* CNVs, as a consequence of complex rearrangements in the 5q13.2 region, modify the early-onset SMA clinical outcome among Serbian patients as independent variables and through their joint effect.

Change of FVC and ALSFRS-R score over time might be complemented with *SMN1* copy number in prediction of survival in Serbian SALS patients.

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Identification of multiple single gene disorders in one family by WES – a challenge from clinical and diagnostic perspective

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Background

Whole exome sequencing (WES) using next generation sequencing has proven to be a powerful tool in determining the underlying genetic cause of rare disorders. Here, we show, that clinical follow-up and diagnostic re-evaluation can be crucial for uncovering further disease-causing mutations.

Clinical report and genetic findings

We report follow-up data of a previously published consanguineous family with two children, a boy and a girl, suffering from severe encephalopathy, hypotonia, microcephaly and retinal dystrophy. WES had shown a homozygous intronic splice variant in *PGAP1* (c.1090-2A>G; p.?) causative for the symptoms. Both parents were heterozygous carrier for the *PGAP1* variant (Granzow, Paramasivam et al, Mol Cell Probes 2015). In the next pregnancy, the unborn child presented hydrops fetalis, omphalocele, short tubular bones and cystic kidneys. Chorionic villus sampling showed the fetus to be homozygous for the *PGAP1* variant. However, neither of these symptoms fit with a *PGAP1*-associated disorder. Additional WES of fetal DNA and re-evaluation in the family showed a homozygous nonsense variant in *IFT140* (c.G3577T;p.E1193*) consistent with a diagnosis of Mainzer-Saldino syndrome (MSS) which is characterised by the association of renal disease, retinal pigmentary dystrophy, cerebellar ataxia and skeletal dysplasia, as a second diagnosis in the fetus. Again, both parents were shown to be a heterozygous carrier for the *IFT140* variant. Yet, as omphalocele was not accounted for by any of the identified conditions, a third genetic cause cannot entirely be excluded. Alternatively, omphalocele may be a rare manifestation of MSS, or be the result of a combination of both disorders. The couple opted for induced abortion.

Discussion

It is estimated that an individual carries multiple heterozygous variants for autosomal recessive disorders in his or her genome. Especially in consanguineous families, this results in an elevated risk for children with more than one disorder. In recent publications of clinical exomes, double diagnoses have been reported in 0 to 12% of investigated subjects. Thus, the possibility of more than one causative gene should be carefully explored when working with WES and re-evaluation in case of additional clinical symptoms within a family should be considered. Also, follow-up of families with rare genetic disorders may lead the clinical geneticist beyond the assumed single cause to multiple single gene disorders in the same family.

Conclusion

Using WES, we have identified two independent single gene disorders in a consanguineous family demonstrating that clinical follow-up and diagnostic re-evaluation can be crucial for uncovering multiple disease-causing mutations in one family.

DISECTING OSTEOARTHRITIC'PHENOTYPES TO FIND NEW VARIANTS: SELECTION OF SUBJECTS WITH OSTEOARTHRITIS FOR NEXT GENERATION SEQUENCING APPROACHES

Martha C. Castano-Betancourt

Background. Osteoarthritis (OA) is one of the most common causes of joint pain and disability in elderly. Approximately half of the variation in OA-susceptibility depends on genetic factors. Recently, rare variants associated with complex traits as OA have been identified performing whole-exome (WS)- next generation sequencing (NGS). Using that approach, family-based studies can be powerful to identify those rare variants segregating with the phenotype. However, selection of the correct phenotype to be studied and families affected with a clear genetic component is challenging for OA. Objective: To investigate which phenotypes are associated with familiar history of OA for subjects and families selection for NGS. **Methods.** This is a Hospital based- cohort study of patients that received or were waiting for total joint arthroplasty (TJA). In total, 230 patients were interviewed. Questionnaires included: OA-family history, joints affected, pain score, age at which OA-symptoms started (onset) and diabetes, between others. Height and weight were measured and BMI calculated. Factors were selected in their relation with family history using logistic regression. A cluster analysis (silhouette method) was performed to see the how well the variables group according to OA-family history. **Results:** Positive family history was associated with female gender, being overweight or obese, early onset of the disease and knee- more than hand-OA, being stronger the association in subjects with both: knee and hand-OA ($P<0.05$). Based on these factors, cluster analyses revealed 3 clusters associated with family history of osteoarthritis. They might help in identification of subjects and families suitable for NGS. **Conclusions:** Most successful studies using NGS approaches have used what is called extreme ends of the phenotypic spectrum" of common traits to increase the power and finding true variants. It seems that for osteoarthritis in our population, an extreme phenotype is the convergence of female gender with family history of OA (more than 2 relatives), with knee and hand affected by OA (or at least knee OA) and with early onset of the disease (before 55 years).

A DESCRIPTIVE REPORT OF WILLIAMS-BEUREN SYNDROME IN A SOUTH AFRICAN POPULATION

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Introduction:

Williams-Beuren syndrome (WBS) is a well described genetic disorder that includes typical cranio-facial features, a specific behavioral profile, congenital heart defects (CHD) and hypercalcemia. Diagnosis is based on the detection of a 7q11.23 contiguous gene deletion that includes the elastin gene (*ELN*). Gene deletion can be detected by fluorescent in situ hybridization (FISH), and by molecular microdeletion/duplication analysis including Multiplex Ligation-dependant probe Amplification (MLPA) and Chromosomal Microarray. Early diagnosis has been shown to significantly reduce morbidity and mortality.

Cases from Sub-Saharan Africa have seldom been reported in literature. Recent case reports on patients from Cameroon recommend that clinical suspicion should be based on cardiac defects and behavioral phenotype as the craniofacial phenotype was found to be less distinctive when compared to the normal population features than that described in cohorts with predominantly European ancestry.

Recognition that population differences influence the phenotypic expression of common genetic syndromes has led to projects such as the NIH Atlas of Human Malformation Syndromes in Diverse Populations in which photographic resources from worldwide collaborators aim to aid the early diagnosis of genetic disease in patients of non-European ancestry. Here we report our experience of diagnosing WBS in the South African setting.

Material and Methods:

Retrospective analysis of a cohort of 19 patients diagnosed with Williams Beuren syndrome that presented to genetic clinic at Red Cross War Memorial Children's Hospital, Cape Town between 2001 and 2017. Data collected included gender, ethnolinguistic ancestry, age of referral to the genetic clinic, referral system followed, age at molecular diagnosis and method of molecular diagnosis used. Description of the cranio-facial features and CHD if present and notes on behavior were also documented.

Results:

Fifteen (79%) patients were of mixed-race ancestry and 4 (21%) of indigenous Black African ancestry. The cohort included 11 male patients and 8 female patients.

The majority of children (58%) were referred from cardiology. The average age at molecular diagnosis was 4,8 years, ranging from 1 to 10 years. Two patients were referred with a confirmed diagnosis. 15 patients

received diagnostic confirmation after their first visit and in 2 patients the diagnosis was not initially suspected. Most of the patients had some cranio-facial features commonly associated with WBS. Eleven patients (58%) had CHD, the most common type being supravalvular aortic stenosis which was present in 6 of the 19 patients (32%). Ten patients presented with typical behavioural phenotype.

Diagnostic confirmation was attained through FISH in 17 cases. The two remaining cases, those in whom the diagnosis was not initially suspected, were diagnosed by MLPA (1) and chromosomal microarray (1).

Conclusion

These results demonstrate that the use of available international diagnostic criteria can be effective in the diagnosis of most South African children affected with WBS. Despite that the diagnosis remains delayed and the diagnosis often not recognized by other health care professionals. We believe that training of more clinicians in Dysmorphology could potentially lead to an earlier diagnosis of WBS and reduce the number of undiagnosed patients in our environment. This emphasises the importance of a multi-disciplinary approach in resource limited settings.

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The prevalence of mutations in genes related to congenital myopathies and muscular dystrophies in patients with “floppy child syndrome”.

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Objective: The term „floppy child syndrome” (FCS) is used to describe the specific phenotype associated with generalized hypotonia and decreased muscle tone in newborns or in early infancy. One of the most common causes of FCS is the genetic background, especially alterations in genes related to the neuromuscular disorders (NMD) such as: spinal muscular atrophy, congenital myopathies and muscular dystrophies and others. Till now, about 100 different genes related to NMD were identified and their mutations might be causative for neuromuscular pathogenesis observed in FCS patients.

Materials and methods: One hundred seven (137) patients with dominant peripheral hypotonia and decreased muscle tone were qualified to the project. Whole exome sequencing (WES) was performed for 78 probands using QXT Sure Select Human All Exome v.6. *In-silico* analysis and co-segregation analysis was carried out to verify selected likely pathogenic variants.

Results: First-line analysis aimed to identify pathogenic variants in NMD genes. Together, 853 variants rare in in-house population (1-3 alleles) have been identified including 134 and 190 variants in congenital myopathies and muscular dystrophies genes, respectively. Further filtration using frequencies from gnomAD database, allowed to identify 35/134 (26%) and 45/190 (24%) likely pathogenic variants in our patients. Several variants were identified in the *RYR1* gene (7 heterozygous variants: c.14567G>T, p.Arg4856Leu; c.14663G>A, p.Arg4888Gln in 2 cases; c.10205G>C, p.Cys3402Ser; c.5314A>G, p.Arg1772Gly; c.3016T>G, p.Tyr1006Asp; c.9579C>G, p.Cys3193Trp) and *LMNA* (5 heterozygous variants: c.827G>C, p.Arg276Pro, 4 *de novo* variants: c.824T>C, p.Leu275Pro; c.409C>T, p.Arg137Trp; c.1072G>A, p.Glu358Lys; c.824T>C, p.Leu275Pro). In single patients, potentially pathogenic variants in *BICD2, LAMA2, PIEZO2, SEPN1, PMP22, SGCA, PIGA, DNM2, PPP2R1A, COL12A1* or *COL6A1* genes were found, analyzed for the inheritance in families and confirmed to be related to the patient specific phenotype. **Conclusions:** Analysis for the presence of mutations in known neuromuscular genes seems to be a good starting point for further studies of new genes related to the etiology of FCS. Our results also will be used to assess the possible role of next generation sequencing in the diagnostic approaches used for patients with "floppy child syndrome".

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Prevalence of Multilocus Inherited Neoplasia Alleles Syndrome among Lynch syndrome patients.

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Introduction

Multilocus Inherited Neoplasia Alleles Syndrome (MINAS) has been recently described as the presence of two or more inherited cancer predisposition alleles in the same individual1. This condition could explain a part of the large phenotypic variability in Lynch syndrome (LS) 2,3. We aimed to study the prevalence of MINAS in genetically diagnosed LS patients.

Material and Methods

Eighty-four LS probands with characterized pathogenic variants in MMR genes were included in this study. All patients were treated through the Hereditary Cancer Program, Comunidad Valenciana (Spain). DNA from PBL was sequenced by NGS for 94 hereditary cancer related genes (MiSeq and TrueSight Cancer Panel, Illumina). Standard pipeline for genetic variant analysis and recommended international guidelines for clinical variant classification were applied4. All pathogenic (class 5) and probably pathogenic (class 4) variants were confirmed by Sanger sequencing5.

Results

The average coverage at 20X of the 255Kb target sequence was 96.2%. Besides the known mutations in LS genes, we found 7 pathogenic/probably pathogenic variants in 7 patients associated to 3 hereditary cancer

syndromes: hereditary breast cancer (*BRCA1*, *ATM*, *NBN*, *FANCA*, *FANCI*)6, neurofibromatosis type 1 (*NF1*) and hereditary leiomyomatosis (*FH*). These results show that the prevalence of MINAS is about 8% (7/84).

Conclusions

The prevalence of MINAS among LS patients is relevant. The underlying genetic landscape in LS becomes more complex than expected. Further analyses are needed to clarify the clinical impact of these findings.

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The mutational and clinical spectrum of TUBA1A tubulinopathy

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In three unrelated individuals with developmental delay we identified 3 heterozygous de novo missense variants in *TUBA1A* by exome sequencing, two previously described, c.518C>T, p.(Pro173Leu) and c.641G>A, p.(Arg214His), and one novel variant, c.1307G>A, p.(Gly436Asp).

The *TUBA1A* gene is a member of the large tubulin gene family. The tubulin proteins are essential for the formation of microtubules, cytoskeletal structures involved in various cellular processes including chromosomal segregation, intracellular transport and neuronal migration. During embryonal brain development, the tubulin alpha-1A protein encoded by the *TUBA1A* gene is a major alpha tubulin component in the microtubule architecture [1, 2]. Disease associated variants in *TUBA1A* and other members of the tubulin

gene family have been reported to cause a heterogeneous group of disorders with cortical and subcortical malformations and a variety of clinical features, collectively referred to as "tubulinopathies" [3].

We now extensively reviewed the current literature and variant databases to provide a standardized synopsis of neuro-radiological findings and clinical features of *TUBA1A* tubulinopathy based on the Human Phenotype Ontology (HPO) [4]. The disease associated variants of *TUBA1A* tubulinopathy were standardized to the Human Genome Variation Society (HGVS) nomenclature [5].

Thus far, a total of 61 distinct heterozygous missense variants in 83 individuals with *TUBA1A* tubulinopathy and 20 fetal cases have been reported. Further 56 variants were retrieved from databases. The main clinical spectrum of *TUBA1A* tubulinopathy included anomalies of the corpus callosum (94%), lissencephaly (60%), microcephaly (75%), developmental delay (58%) and epilepsy (41%). Fetal cases show a more severe manifestation with agenesis of corpus callosum in 80% (vs 18% in born individuals) and agyria in 75% (vs. 14%). Most of these common features were shared by the three cases with *TUBA1A* mutations described here. Moreover, we analyzed spatial clustering of all variants in the linear gene model and also used publicly available tertiary protein structure data of *TUBA1A* to provide an insight into the variant distribution. Based on their potential structural function variants were further classified in different groups [6, 7]. Most of the reported variants (54%) likely interact with microtubule-associated proteins, while 20% affect protein folding. Finally, we performed genotype-phenotype analysis. Variants at recurrently mutated residues are associated with a specific pattern of brain malformations, indicating a genotype-phenotype correlation. However additional clustering of phenotype and variant position with publication indicates probable observer bias. Further adoption and enforcement of standardized ontologies and nomenclatures is needed to make the computational analysis of genotype-phenotype relations feasible, thus improving the understanding of rare diseases like tubulinopathies.

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Amplification-free targeted SMRT sequencing using CRISPR/Cas9 for studying repeat expansions in Huntington's disease

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Background

Targeted re-sequencing is a widely used approach in next generation sequencing. Although being a powerful and cost-effective tool it has its limitations. Amplification of DNA is required in most available methods during library preparation [1-4], which can be challenging for example when targeting low complexity regions or repetitive sequences.

Methods

Here, we use a novel amplification-free method for targeted enrichment utilizing the CRISPR/Cas9 system[5] and SMRT sequencing for studying the CAG repeat in the huntingtin (*HTT*) gene, where expanded repeat alleles are associated with huntington's disease [6]. Guide RNA (gRNA) was designed in proximity to the *HTT* CAG repeat and was used for directed cleavage of PacBio sequencing libraries (SMRTbells). Capture adapters could subsequently be ligated to digested SMRTbells and magnetic beads were used to enrich for SMRTbells containing the capture adapter. The enriched SMRTbell libraries were sequenced using the PacBio RSII instrument.

Results

We applied this method to twelve patient samples and the CAG repeat count for 23 out of the 24 alleles agreed with previous results based on fragment analysis. Somatic variation of the CAG repeats was observed within the samples, with the largest variability found in the highly expanded alleles ($(CAG)_n>50$). Furthermore, the polymorphic CCG repeats in the flanking regions of the CAG repeats could be analyzed in detail. With this method analysis of large repeat expansions and somatic variation could be performed with confidence, while it is fraught with challenges using fragment analysis and short-read technologies. In addition to on-target reads, off-target effects by the CRISPR/Cas9 could be identified in sequences with high homology to the guide RNA. The protocol allows multiplexing of targets, and barcoding of samples are within the reach of the method. Sequencing of native DNA also enables detection of epigenetic modifications in the data but is yet to be explored.

Conclusions

In conclusion, with the combination of this amplification-free technique and the long reads of PacBio, we are able to accurately sequence through repeat expansions that are difficult to investigate using PCR-based methods.

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TARGETED SEQUENCING WITH EXPANDED GENE PROFILE ENABLES HIGH DIAGNOSTIC YIELD IN NON-5Q-SPINAL MUSCULAR ATROPHIES

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Spinal muscular atrophies (SMA) is a heterogeneous group of disorders characterized by muscular atrophy, weakness, and hypotonia due to suspected lower motor neuron (LMN) degeneration. In a large cohort of 3465

individuals suspected with SMA submitted for *SMN1* genetic testing to our routine diagnostic laboratory, 48% carried a homozygous *SMN1* deletion, 2.8% a subtle mutation and an *SMN1* deletion while 49.2% remained undiagnosed. Recently, several other genes implicated in SMA/LMND have been reported. Despite several efforts to establish a diagnostic algorithm for non-5q-SMA, data from large-scale studies are not available. We tested the clinical utility of targeted sequencing in non-5q-SMA by developing two different gene panels. We first analysed 30 individuals with a small panel including 62 genes associated with LMND using IonTorrent-AmpliSeq target enrichment. Then, additional 65 individuals were tested with a broader gene panel encompassing up to 479 genes implicated in neuromuscular diseases (NMD) with Agilent-SureSelect target enrichment. The NMD panel provided a higher diagnostic yield (45%) than the restricted LMND panel (13%). Non-diagnosed cases were further subjected to exome or genome sequencing which solved 9.4%. In conclusion, individuals with non-5q-SMA are efficiently diagnosed by using large gene panels covering a broad NMD spectrum.

Targeted next generation sequencing for RASopathies diagnosis – application of functional in silico and in vitro testing to novel likely pathogenic variants.

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Introduction: The RASopathies are a group of rare, developmental genetic disorders such as: Noonan syndrome (NS), Noonan syndrome with multiple lentigines, Costello syndrome, cardio-facio-cutaneous syndrome and neurofibromatosis type I. These disorders are caused by germline mutations in genes that encode components or regulators of the RAS/MAPK pathway. The RAS/MAPK pathway is one of the basic signal transduction pathways that transmit the external signal to the cell nucleus and specifically regulates gene expression. Due to the genetic and clinical heterogeneity of RASopathies, it seems reasonable to apply targeted next generation sequencing (NGS) for the diagnosis of these disorders.

Material and methods: One hundred fifty three (153, including 100 patients with excluded *PTPN11*, *RAF1* and *SOS1* mutations) patients with primary NS clinical diagnosis were tested with custom designed NGS panel (SeqCap EZ Choice Library, Roche Diagnostics). The NGS panel included canonical RASopathy genes, as well as novel NS genes (*RRAS*, *RASA2*, *SPRY1*, *MAP3K8*, *SOS2*, *LZTR1*, *PPP1CB*). The libraries were run on MiSeq Sequencer (2x75cycles) and the data was analyzed using dedicated bioinformatic pipeline. Likely pathogenic variants, not previously described, were analyzed using *in silico* prediction algorithms. For selected variants are still making a functional *in vitro* analysis (ERK activation study).

Results: In 30 patients, pathogenic / likely pathogenic variants were identified in canonical RASopathy genes, including novel variants in *RAF1* (c.236A>G, p.His79Arg; c.505G>C, p.Gly169Arg; c.1082G>C, p.Gly361Ala), *RIT1* (c.67A>G, p.Lys23Glu; c.91G>C, p.Gly31Arg; c.90_91delinsAT, p.Gly31Cys) and *NRAS* (c.457G>A, p.Glu153Lys). All these variants were predicted to be pathogenic with *in silico* analysis and have been *de novo* in families where the parents were available for the analysis. In addition, pathogenic / likely pathogenic variants were identified in 10 and 4 patients within *NF1* and *CBL* genes, respectively. Also, variants in *RASA2*, *MAP3K8*, *LZTR1* or *A2ML1* genes were found in single patients. No variants in *SOS2* or *PPP1CB* were found.

Conclusions: Targeted NGS is a useful method in the diagnostics of RASopathies that allows to detect novel pathogenic variants in genes of the RAS/MAPK pathway. However, further functional analysis allows to confirm pathogenicity of the identified variants.

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Identification of putative functional human circular RNAs

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Background: circular RNAs are highly abundant but poorly understood1, and although a few are known to be functional2–4, prioritising specific transcripts for functional assessment is problematic1. Recently, my laboratory has used in-silico analysis to infer that >98% or transcripts from RMST and FIRRE, two known lncRNAs with functions in early development, are circular5. They have also established that anucleate platelets are enriched in circRNAs 17- to 188-fold relative to nucleated tissues, making them ideal for comparative analyses6.

Aims and methods: confirm the inferred in-silico information about RMST and FIRRE through MiSeq amplicon sequencing, and investigate through comparative sequencing analysis conservation of structure and expression levels of platelet circRNAs in human, mouse and rat, to identify possible functional circular transcripts.
Results: I confirm RMST and FIRRE exons structure, and I identify additional 5 unannotated exons, a novel internal promoter and a total of 25 distinct circRNAs from FIRRE locus. I also establish that expression levels of circRNAs, can be as conserved as the expression of linear exons, consistent with the pattern of circRNAs splicing being of functional relevance. I also define 21 circRNAs conserved between the 3 species which are highly expressed in human nucleated tissues. These include HIPK3 and ZNF292, for which evidence of functionality has already been independently generated, suggesting this list of targets may be enriched for functional circRNAs.
Conclusion: circRNAs identified and characterised here are prime candidates for functional analyses. However, the identification of multiple FIRRE promoters and transcripts highlights the complexity of circRNA loci.

Whole ROR2 gene deletion uncovering a pathogenic mutation in a patient with autosomal recessive Robinow syndrome.

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Robinow Syndrome is a genetically heterogeneous disorder characterized by a triad of facial dysmorphisms including hypertelorism, short stature and genital hypoplasia with extreme clinical variability (Mazzeu et al., 2007). More severe bone involvement and marked short stature are observed in the autosomal recessive form of the syndrome (RRS [MIM 602337]) caused by biallelic mutations in *ROR2* (receptor tyrosine kinase-like orphan receptor 2) (Afzal et al., 2000; van Bokhoven et al., 2000). Mutations in DVL1, DVL3, WNT5A and FZD2 have been identified in a subset of patients with autosomal dominant Robinow syndrome (Pearson et al., 2010; Bunn et al., 2015;White et al., 2015, White et al., 2016; White et al., 2018. These genes encode for components of the Wnt signaling complex which play a key role in the regulation of cell differentiation and patterning. Here we report a patient with recessive Robinow syndrome caused by a whole *ROR2* deletion and a hemizygous mutation in the other allele.
Patient is a two-year old boy born to non-consanguineous parents with typical recessive Robinow syndrome phenotype, referred to our genetic service for molecular confirmation of the diagnosis. Mutation screening was performed by next generation sequencing (NGS) using ION PGM™ Inherited Disease Panel as described by the manufacturer. Investigation of deletions and duplications of *ROR2* was performed by MLPA using kit P179. Chromosome Microarray analysis was performed for delimitation of the deleted segment using the

Affymetrix 750K platform.

NGS revealed a single pathogenic mutation in *ROR2* (c.1970G>A, p.Arg657His), allegedly in homozygosity. Patient was reportedly homozygous for other polymorphic variants in *ROR2*. Considering that the parents were not consanguineous we performed MLPA that revealed a deletion of all probes for *ROR2*. Chromosome microarray analysis delimited the deletion to a 470 Kb on 9q22 (arr[hg19] 9q22.31(94,381,136-94,851,388)x1). The deletion includes *ROR2* and *SPTLC1* genes.

The patient here described presents a chromosomal microdeletion including *ROR2* gene uncovering a pathogenic mutation on the other allele of *ROR2*. These findings corroborate the diagnosis of RRS and sheds light to the importance of a criterious evaluation of NGS data for differentiation of hemizygous and homozygous mutations.

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Diagnostic Odyssey of Selected Patients with rare Neurogenetic Disorders: An Experience from an Adult Neurology Unit in a Tertiary Care Centre in Oman

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Background: There is a high frequency of consanguineous marriages in Oman where certain neurogenetic disorders pose particular diagnostic challenge due to rare, unfamiliar and pleiotropic clinical features. The socio-economic burden of diagnostic odyssey, the figurative journey of the patients and families starting from the symptom onset or initial hospital visit to the time of receiving the ultimate confirmatory diagnosis, is considerable. The affected patients may receive inappropriate diagnoses, treatment or face emotional distress and feeling of isolation leading to hospital shopping abroad during the period of diagnostic uncertainty.

Objective: To document the diagnostic odyssey of a selected set of Omani patients with rare neurogenetic disorders encountered in the adult Neurology unit.

Design/Methods: We reviewed the electronic case records of a few selected patients in whom molecular genetic confirmation of a heterogeneous list of neurogenetic disorders was attempted. The initial working and final diagnoses, odyssey duration, number and cost of tests obtained before genetic confirmation were recorded.

Results: In two affected patients of consanguineous parentage, with an initial working diagnosis of complicated axonal Charcot Marie Tooth disease (CMT) and negative CMT panel result, the final diagnosis of recessive distal motor neuropathy with pyramidal signs due to a novel SIGMAR1 mutation (c.238C>T; p.Gln80*) was reached in their second decade of life (with a diagnostic delay of 6 years) utilizing homozygosity mapping and whole exome sequencing (WES). The other rare diagnoses reached in other Omani kindred of different families included recessive distal motor neuropathy with impaired pallesthesia due to a novel DNAJB2 variant, Rett syndrome due to a novel MECP2 variant and gestational hemiplegic migraine due to a novel SLC1A3 variant using multigene test panel respectively; Cockayne syndrome type II with ataxia due to a novel splice site variant in ERCC6 gene, CAPOS due to a hetezygous mutation in ATP1A3, and SCAN1 due to TDP1 mutation utilizing WES. The direct cost incurred from the initial non-confirmatory genetic tests was up to 2290 Euros per patient and the odyssey duration ranging beyond 10 years in some patients. In general, patients visited several hospitals abroad for second opinion without securing a confirmed diagnosis, the cost of which could not be retrieved from our case records. We also encountered a few patients (other than Wilson disease) with potentially treatable disorders. In a patient with encephalopathy mimicking non-convulsive status epilepticus, the initial working diagnosis of hyperammonemic encephalopathy due to a urea cycle disorder led to the institution of standard hyperammonemia treatment protocol including intravenous dextrose and ammonia scavenger. Had the ultimate diagnosis of adult onset citrullinemia due to

citrin deficiency (a novel SLC25A13 exon 5 deletion) made earlier, she could have been saved from the dextrose induced fatal worsening of hyperammonemia by restricting carbohydrate/dextrose. On the other hand, a young adult with known arginase deficiency (due to a homozygous c.914G>T (p.Gly305Val) in exon 8 of *ARG1*gene) presented with hyperammonemic encephalopathy (triggered by high protein diet) that also mimicked non-convulsive status epilepticus. He recovered with dextrose and ammonia scavenger without recourse to anticonvulsant maintenance. Another young adult with recurrent stroke like episodes was genetically confirmed to have MELAS (mitochondrial encephalopathy with lactic acidosis and stroke like episodes) and he responded to l-Arginine without any further stroke recurrence. In a patient with neuronal brain iron accumulation thought to be due to beta-propellar protein associated neurodegeneration and another case of leukoencephalopathy with cysts and calcification, the genetic diagnosis remained inconclusive even after WES.

Conclusions: Our case series, with a wide variety of neurogenetic disorders including some potentially treatable conditions, illustrate the importance of recognizing the socioeconomic burden of diagnostic odyssey. There is an imminent need for initiating rare disease diagnosis program in Oman to end the diagnostic delay.

Keywords: Neurogenetic disorders, diagnostic odyssey, Omani kindred

Design and MinION testing of a nanopore sequencing specific gene panel for chronic lymphocytic leukemia

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INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a clinically heterogeneous disease originating from B lymphocytes that may differ in activation, maturation state, or cellular subgroup. It is the commonest form of leukemia in Europe and North America and has a very variable course, with survival ranging from months to decades. Recently, several important observations related to the biologic significance of IgVH mutational status and associated ZAP-70 overexpression, disrupted p53 function, and chromosomal aberrations have led to the ability to identify patients at high risk for early disease progression and inferior survival.

Oxford Nanopore Technologies recently released the MinION, an USB-interfaced sequencer. MinION is a single-molecule nanopore sequencer from Oxford Nanopore Technologies connected to a laptop through a USB 3.0 interface. Sequencing is performed by the moving of individual DNA strands through biologic nanopores on a chip, where an electric field is applied and electrical signal variations are recorded.

In the last years, next generation sequencing (NGS) methods have identified a wide range of gene mutations (e.g., TP53, NOTCH1, SF3B1, and BIRC3) which have improved our knowledge about CLL development, allowing to refine both the prognostic subgroups and better therapeutic strategies. Consequently, it is reasonable to argue that integration of the newly discovered genetic lesions into a comprehensive prognostic model based on both chromosomal abnormalities and gene mutations would help to improve prognostication of patients.

In this study, we used multiplex long PCR-based strategy followed by MinION sequencing to identify single nucleotide variations (SNV) and insertions/deletions (indels) in 5 frequently mutated genes in CLL: TP53, NOTCH1, BIRC3, SF3B1and MYD88.

METHODS AND MATERIALS

Ten new diagnosed CLL patients and 2 healthy donors (HD) were included in the study. The enrichment strategy we adopted to primarily select our targets was multiplex long-PCR. For each DNA sample two multiplex long-PCRs were performed with the two primer pools using the PrimeSTAR GXL DNA Polymerase (Takara Bio Inc.). Library was prepared according to the 2D Native barcoding genomic DNA (SQKLSK 208) protocol and barcoded with the ligation of nanopore-specific Native Barcodes. The sequencing mix was loaded on MinION flowcell FLO-MAP106 and sequenced for 48h. Reads were aligned on GRCh37 human reference genome with the BWA-MEM method using specific Nanopore platform parameters. Single nucleotide variants (SNV) and insertions/deletions (indels) detection was separately performed with the Varscan tool, and the VCF files obtained were annotated with ANNOVAR tool. The filtered variants were then validated using Sanger Sequencing (SS) or Allele Specific Oligonucleotide PCR(ASO-PCR).

RESULTS: In our experiment, the library of 12 samples was loaded on MinION for sequencing. On a total of 106543 fast5 total files were produced 48599 passed 2D filters, and 46451 had a recognizable barcode. Coverage analysis showed that the range of read depth was inversely related to the amplicon size, with the smaller amplicons having a higher coverage (up to about 2100x).

To reduce the chance for producing false positive during the variant calling due to the relatively high error rate of the MinION we decided to compare the variants of each CLL patient with those called in the 2 HD samples simultaneously sequenced. Variants shared by at least one of the two controls were retained only if the allele rate was significantly higher. Shared and unique variants were further filtered by allelic ratio (>10%for SNVs, >15% for indels), effect and frequency in the population.

Overall, 8 pathogenic mutations were detected in 6 patients, with two 2 patients harboring concurrently 2 mutations; in details, 6 mutations were SNV, whereas the main hotspot deletion of NOTCH1 in CLL (c.7541_7542del) was detected in 2 patients.

All mutations detected by MinION sequencing were also validated with SS except for 2 variants having a mutation allelic ratio below the detection limit of SS which were validated by ASO-PCR.

DISCUSSION AND CONCLUSIONS

MinION measures changes in electrical current as individual strands of DNA pass through one of its 500 protein tiny pores. To date, it is the only technology that directly measures a single DNA strand rather than incorporation events relative to a template strand. Moreover, speed, single-base sensitivity, and long read lengths make nanopores a promising technology for high-throughput sequencing. Using MinION we have designed and tested a custom gene panel including TP53, BIRC3, NOTCH1, SF3B1 and MYD88 that are the most frequently mutated genes in CLL. Mutational analysis of these genes is important for prognosis and patient management. Our panel allows the target enrichment by long template PCR producing amplicons with compatible size for MinION sequencing. MinION platform is surely more affordable compared to other NGS platforms and offers a sensitivity that is slightly higher than Sanger Sequencing. Indeed, we were able to detect variants around to 10% of allelic ratio. In summary, we demonstrate that MinION is a suitable tool for the detection of mutations in these genes in patients affected by CLL. Although MinION is still not adequate for substituting other NGS technologies, it can be a useful strategy for pre-screening analyses before Sanger sequencing validation. However, the constant improvements of nanopore technology promise an exclusive and convenient use of MinIONin the immediate future

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Drosophila as a model system to investigate cell division

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The fruit fly *Drosophila melanogaster* has been used for over a century for studying basic biological processes. Its ease to culture in laboratory conditions and rapid generation time, together with the sophisticated array of genetic and molecular tools that have evolved for analysis of gene function have made this organism one of the most fruitful experimental models for studies in cell and developmental biology, neurobiology and behavior, evolutionary and population genetics.

In our lab, we are interested in identifying and characterizing genes required for cell division, chromosome behavior and spindle assembly. Several *Drosophila* tissues are easily amenable to investigate different types of cell division, including asymmetric division of neuroblasts, meiosis in testes and oocytes, and syncytial mitosis in early embryos. In addition, *Drosophila* cell lines are available for *in vitro* cell culture techniques. To identify novel players in cell division we use both classical forward genetics, identifying genes of interest

by mutagenesis screens, and targeted gene silencing by RNA interference (Perrimon et al., 2010). Generation of transgenic flies expressing functional fluorescently (e.g. GFP) - tagged mitotic proteins are used for rescue experiments and *in vivo* analysis. In the attempt to understand the complex interactions that occur during mitosis, we have also developed an effective protocol based on affinity purification and mass spectrometry (AP-MS), and analysis based on the isolation of GFP-tagged proteins and their interactors in the developing embryo (Palumbo et al., 2015), which undergoes a series of rapid, synchronous nuclear divisions in the absence of zygotic transcription (Foe and Alberts, 1983). In addition, we use *Drosophila* embryos expressing GFP-labeled proteins to follow live dynamics using confocal microscopy. The embryo system is also exploited to microinject either antibodies or inhibitors to study the effect of the loss or perturbation of the function of specific proteins (Brust-Mascher and Scholey, 2009).

The use of this combined approach employing genetics, cell biology, cell image analysis and proteomics has allowed us to identify a number of essential genes required for different aspects of cell division.

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Implementation of a Custom-Designed Targeted Next-Generation Sequencing Panel for the Diagnostics of Inherited Bone Marrow Failure Syndromes

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Inherited bone marrow failure syndromes (IBMFS) composed of great number of clinically similar but yet genetically heterogeneous diseases with reduction of one or multiple hematopoietic cell lines in bone marrow as a main feature. Next-Generation sequencing (NGS) facilitated the discovery of novel germline mutations that are the cause of IBMFS.

The aim of this study was the elaboration and implementation of a custom-designed 173-genes NGS-panel into routine diagnostics.

We performed NGS-sequencing for 67 patients with different referral diagnosis: Diamond-Blackfan anemia (21); aplastic anemia (23); Fanconi anemia (6), myelodysplastic syndrome (7); bone marrow failure (3), idiopathic thrombocytopenia (3), dyskeratosis congenita (1), severe congenital neutropenia (1), Shwachman-Diamond Syndrome (1), Fisher-Evans syndrome (1). Sample preparation was carried out with KAPA HTP Library Preparation Kit Illumina (Roche, Switzerland) and tagret hybridization enrichment technique via custom probe panel SeqCap EZ Choice Library (Roche, Switzerland). Obtained libraries were sequenced on MiSeq platform (Illumina, USA).

We revealed 34 rare genetic variants that could relate to the cause of the disease (13 – pathogenic, 11 – likely pathogenic and 10 variants of uncertain significance). Among 67 patients the diagnosis was confirmed in 19 and likely confirmed in 7 cases. Despite the reason that the given target panel was meant to reveal germ line variants, in order to perform differential diagnostics the genes where somatic variants can lead to myelodysplastic syndrome were included. For detection of the somatic mutations it is necessary to gain mean coverage depth more than 500x, however current study design with mean depth further than 200x allows to suspect the presence of somatic variants. Thus, total efficiency of revealing causative variants is 38.8%. Meanwhile, among 28.4% the reason was confirmed and in 10.4% likely confirmed.

The use of NGS for diagnostic of IBMFS significantly expands the search range while results can influence the patient's clinical management. Furthermore, identification of IBMFS genetic reason plays important role in genetic counseling of patient's family members. Obtained results shows high diagnostic yield and allows to apply this custom gene panel in clinical practice.

Mutation profiling of colorectal adenomas from patients with MSH3-related adenomatous polyposis

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Colorectal Cancer (CRC) is a genetic disease, arising due to the stepwise accumulation of somatically acquired mutations in critical cancer genes. The identification of the relevant genes and pathways is important to understand the pathophysiology of tumorigenesis, to identify clinically relevant biomarkers, and to find new drug targets for personalised cancer treatment. In 3-5%, the CRC occurs on the basis of an inherited monogenic tumour predisposition syndrom such as Lynch syndrome or a gastrointestinal polyposis caused by germline mutations in a cancer gene.

Adenomatous polyposis syndromes of the colorectum are precancerous conditions characterised by the presence of dozens to thousands of adenomatous polyps, which, unless detected early and removed, invariably result in CRC. In up to 50% of cases, no causative germline mutation can be identified in established genes such as APC or MUTYH. Recently, an exome study of patients with unexplained adenomatous polyposis revealed for the first time biallelic MSH3 germline mutations in two unrelated individuals and affected siblings and thus could describe a novel, rare, autosomal recessive monogenic subtype of adenomatous polyposis. Besides of gastrointestinal tumours, the patients exhibit several extraintestinal malignancies.[1]

MSH3 is a member of the mismatch repair (MMR) system, a crucial pathway that corrects base-base and indel mispairs occuring as a result of replication errors, thus increasing the fidelity of DNA replication.[2-4] Canonical members include the four MMR proteins MLH1, MSH2, MSH6, and PMS2. MSH2 forms a heterodimer with MSH6 (MutSß) and also with MSH3 (MutSα), both recognizing mispaired bases. In humans, both MutSβ and MutSα play a role in repairing small insertion/deletion loops with MutSα showing a stronger affinity for recognizing larger base-indel loops with up to ten nucleotides.[5; 6] Microsatellite instability (MSI) at loci containing mono- and dinucleotide repeats is a hallmark of MMR-deficient tumours. However, MSH3-deficient cells show MSI at loci containing dinucleotide and tetranucleotid repeats instead, resulting in a specific type of MSI which is found in approximately 50% of sporadic CRC.[7; 8] Interestingly, examination of colorectal tumor tissue of the patients with MSH3-associated polyposis demonstrated this specific type of MSI and also loss of nuclear MSH3 expression in both normal and tumour tissue.[1]

In the present study, we aim to describe mutational signatures of colorectal polyps (adenomas) from these patients with biallelic MSH3 germline mutation to characterise the mutational pattern and to identify potential driver genes and pathways of MSH3-related tumorigenesis. Therefore, we performed exome sequencing and array analysis of 2-3 adenomas and matched normal tissue (normal colorectal mucosa or leukocyte DNA) in each of three patients. Tumor-specific somatic variants are filtered in comparison with normal tissue. In a first step we analyse and compare the spectrum of point mutations in the polyps, subsequently followed by the analysis of structural variants. We hope to identify early mutations in specific signaling pathways to get insights into early steps of carcinogenesis. In particular, we are interested to see whether or not specific mutations at sights of di- and tetranucleotid repeats in coding genes can be observed. Hopefully, the results will contribute to a better understanding of MSH3 deficiency on tumorigenesis.

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Glioma diagnostics using targeted next-generation sequencing

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Introduction

Gliomas are the most common type of primary central nervous tumors in adults and classification of gliomas has for many years only been based on histological criteria according to the World Health Organization (WHO) classification of tumors of the central nervous system. In the revised WHO classification of CNS tumors published in May 2016 [1], gliomas are now defined by both histology and 3 molecular biomarkers; IDH1/2 (isocitrate dehydrogenase), ATRX (alpha-thalassemia/mental retardation syndrome X-linked) and co- deletion of chromosome arms 1p and 19q, constituting so-called integrated diagnoses. Targeted next-generation sequencing (NGS) gene panels offer promising perspectives to approach the revised WHO classification [2-5]. Recently, 20 of the most common genes in gliomas have been selected for a customized targeted NGS glioma panel by Reifenberger and colleges [6]. In a retrospective study, we tested the diagnostic value of this targeted NGS 20-gene glioma panel for up-front brain tumors diagnostics.

Materials and Methods

A total of 44 formalin-fixed, paraffin-embedded tissue samples from grade II and III gliomas, collected in 2005-2014 were analyzed. All the gliomas were previously classified according to the WHO CNS classification from 2007 by neuropathologists. Initially the IDH1 (R132H), ATRX and 1p/19q status was clarified by immunohistochemistry/fluorescence in situ hybridization (FISH). Retrospective targeted NGS analyses were afterwards performed on all the 44 IDH-wildtype tumors and the NGS results were subsequently used to classify the gliomas according to the 2016 WHO criteria. The diagnostic value of the glioma panel was evaluated in relation to the effectiveness of identifying the required genetic alterations; IDH1/2 and ATRX and other supporting genetic alterations (TP53, EGFR, PTEN, TERT etc.) included in the new integrated WHO classification of gliomas.

The customized targeted NGS glioma panel included coding sequences (cds) and mutational hotspot (hs) of the following 20 genes: ATRX(cds), BRAF (hs), CDKN2A (cds), CDKN2B (cds), CDKN2C (cds), CIC(cds),

EGFR(cds), FUBPI(cds), H3F3A(hs), IDH1(hs), IDH2(hs), NF1(cds), NF2(cds), NRAS(cds), PIK3CA(cds), PIK3RI(cds), PTEN(cds),RB1(cds), TERT(hs) TP53(cds). DNA was extracted from 10 µm paraffin slides and quantified by qPCR. Libraries were prepared using the Ion AmpliSeq Library Kit 2.0 and 5 ng template DNA. Sequencing was performed using the Ion Torrent PGM System with Ion PGM Sequencing 200 Kit v2 (Life Technologies, Carlsbad, CA, USA). Data analysis including base calling, quality scoring, trimming, demultiplexing, and alignment was performed using standard Torrent Suite v5.0 workflows. The analysis results were also manually assessed.

Results

In total 44 gliomas were analysed with the targeted NGS 20-gene glioma panel, revealing a total of 108 genetic aberrations. In 5 tumors no genetic aberrations were found. The top 3 most commonly mutated genes were; TP53 (14%), EGFR (12%) and PTEN (11%). In 11 out of the 44 tumors an IDH1 or IDH2 mutation were found that was not detected by immunohistochemistry. Regarding ATRX additionally mutations were found in 5 out of 44 tumors.

In the group of astrocytic tumors, 3 of 6 diffuse astrocytomas were found to be IDH-mutated, whereas only 2 of 12 anaplastic astrocytomas were IDH-mutated. The remaining anaplastic astrocytomas were reclassified as 5 IDH-wildtype, anaplastic astrocytoma and 5 IDH-wildtype glioblastomas. The new WHO classification requires the demonstration of both IDH mutation and 1p/19q co-deletion for the diagnosis of oligodendroglioma and anaplastic oligodendroglioma [1]. Only 1 of the 4 oligodendrogliomas had both an IDH mutation and a 1p/19q co-deletion and could be classified as an IDH-mutated, 1p/19q co-deleted oligodendroglioma. The remaining 3 tumors were found without a 1p/19q co-deletion and based on IDH status classified as IDH-wildtype diffuse astrocytomas and IDH-mutated, anaplastic astrocytoma. For the anaplastic oligodendrogliomas only 3 out of 11 demonstrated both IDH mutation and 1p/19q co-deletion and were classified as IDH-mutated, 1p/19q co-deleted anaplastic oligodendrogliomas. The remaining 8 tumors were reclassified as IDH-wildtype glioblastomas. All tumors in the mixed group comprising 3 oligoastrocytomas and 8 anaplastic oligoastrocytomas were reclassified to other diagnoses regarding the status of IDH1/2 and 1p/19q. All the oligoastrocytomas were IDH mutated, expressed 1p/19q co-deletion and were classified as IDH-mutated, 1p/19q co-deleted oligodendrogliomas, whereas the anaplastic oligoastrocytomas were found without both IDH mutation and 1p/19q-codeletion and classified as IDH-wildtype glioblastomas.

Conclusion

In this retrospective study we tested the diagnostic value of a targeted NGS 20-gene glioma panel for up-front glioma diagnostics. We found that the targeted NGS panel was a useful diagnostic tool, especially for detection of IDH mutations that escape detection by immunohistochemistry. Demonstration of IDH1 or IDH2 mutation together with 1p/19q co-deletion by FISH is required by the new WHO classification of oligodendrogliomas

and anaplastic oligodendrogliomas. The identification of these molecular biomarkers is important due to significant differences in prognosis and treatment between oligodendrogliomas and other glial tumors.

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A web tool for investigation variants in NGS diagnostic concerning disruptions in the collected human high-confidence microRNA binding sites

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Key words: Regulation of gene expression, microRNA-mRNA interactions, microRNA, miRNA, NGS, nucleotide variants

Background: microRNAs are small noncoding RNAs that play a key role in the regulation of gene expression. Today it is known more than 2’500 human microRNAs, while a majority of microRNA-mRNA interactions remain unidentified. A common method for determining the microRNA-mRNA interactions is using prediction programs that show a small intersection of the results and are poorly consistent with known experimental data [1]. Therefore, using experimentally obtained data of the microRNA binding regions should be the most promising approach in order to identify new molecular mechanisms for the etiopathogenesis of hereditary diseases.

Methods: We collected results of 79 AGO2-CLIP-seq data from 9 different cell lines and also took data from two modified CLIP-seq studies (CLASH, CLEAR-CLIP) [2,3] that straightforwardly detect microRNA–mRNA pairs as chimeric reads. Expression levels for mRNAs and microRNAs were retrieved from FANTOM5 and GEO.

Results: Our analysis revealed 46,8 thousand high confidence microRNA binding regions that were established in at least two different experiments. These regions were formed by 15 thousand genes and 465 known microRNAs. While 99% of the high confidence microRNA binding regions were smaller than 150nt, there are a few extended regions. These regions have a high density of supported experiments and assume to be a cluster that could interact with many different microRNAs. Thus, the longest region (631nt) in autosomoses was supported by results from the 54 different experiments in 9 different cell lines. The analysis of high-confidence microRNA binding sites revealed tissue-specific interactions for two predominate cells: HEK293 and Huh7.5. On the other hand, we obtained a group of "house-keeping" microRNA binding regions that were identified in predominant cells.

Expression analysis demonstrated the distinguish in expressed and interacted microRNAs across two different cells. We revealed two interesting group of microRNAs: specific that interacted with a few microRNAs while they have a high expression level and pretend to be a high-specific gene expression regulator and promiscuous microRNAs that have opposite characteristics. A half of the top of specific microRNAs is the same in the two cells. Both groups of microRNAs have significantly higher conservation score, while all 989 identified microRNAs have the less conservative score but higher than on average conservative score between all known mature microRNAs.

The identified high-confidence microRNA binding regions have been arranged in the tool (available online: http://score.generesearch.ru/services/mirna/). Hence, it will be a valuable resource that should provide additional insights into the identification new molecular mechanisms of hereditary diseases caused by breaking in the microRNA-mRNA interactions.

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MAGED2 associated polyhydramnios and transient Bartter-like syndrome

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Mutations and deletions in the X-chromosomal Melanoma Antigen Family D2 (MAGED2) gene were recently discovered as a novel cause for a transient form of the antenatal Bartter-syndrome in connection with a severe form of polyhydramnios leading to prematurity and an increased risk for stillbirth. A severe form of the Bartter-syndrome with salt-wasting and polyuria has been observed postnatally, which resolved within a few weeks. Staining of a fetal kidney with MAGED2 mutation showed a reduced apical abundance of the ion channel proteins Na-K-2Cl-Cotransporter (NKCC2) and Na+-Cl-cotransporter (NCC), which has been shown in further cell culture experiments as consequence of impaired protein maturation (Laghmani et al., 2016).
In our studies we were able to identify further mutations in the MAGED2 gene of male infants, whose gestation was complicated by idiopathic polyhydramnios. Besides mutations affecting correct mRNA splicing, we identified the first real missense mutation resulting in the expression of a full-length-protein with an arginine to cytosine substitution at protein position 446 (R446C). Subcellular fractionation as well as immunostaining experiments revealed altered protein MAGED2 localization within HEK293T and Cos-7 cells with an absence in the nucleus.
Furthermore, we identified interaction partners of MAGED2 via interactome and co-immunoprecipitation analysis. We identified GNAS as one interaction partner that binds wild-type but not R446C-MAGED2 protein. Furthermore, MAGED2 increased GNAS stability in a CHX assay. GNAS encodes the alpha-subunit of the stimulatory G protein (Gsα), which is a signaling protein able to increase NKCC2 and NCC expression and maturation via a cAMP-dependent pathway. As GNAS knock-out in mice had already been shown to result in impaired membrane expression of NKCC2, this could represent a pathomechanism of mutated MAGED2.
To further investigate the function of MAGED2 in cAMP-dependent pathways, we knocked down MAGED2 in the murine collecting duct cell line mpkCCD. This cell-line is sensitive to vasopressin (dDAVP) exposure which leads to an increase of intracellular levels of cAMP and expression of the channel protein AQP2. Short-term treatment of mpkCCD cells with dDAVP showed altered cAMP levels as well as altered levels of phosphorylated *cAMP response element-binding protein* (CREB) in the MAGED2 knock-down cells, which displays a mediating transcription factor in the vasopressin induced cAMP pathway.
Long-term treatment of mpkCCD cells with dDAVP led to significantly increased levels of AQP2 in the MAGED2 knock-down cells on transcriptional and translational level.

Congenital Central Hypoventilation Syndrome: A rare cause of recurrent neonatal apnoea

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Congenital central hypoventilation syndrome (CCHS) is a disorder of respiratory and autonomic regulation associated with mutations in the *PHOX2B* gene, and is rarely diagnosed in the South African setting. We describe two unrelated neonates with CCHS and identical *PHOX2B* pathogenic variants. We highlight similarities and differences in their phenotype and comment on the relevance of *PHOX2B* mutation testing in the South African context.

Both our cases presented within the first day of life with episodic apnoea. Despite requiring minimal ventilatory support, weaning from mechanical ventilation resulted in recurrent episodes of apnoea and hypercarbia. In addition, Case 2 demonstrated autonomic instability and hypoglycaemia. Common causes for neonatal apnoea were systematically excluded for both, which prompted genetic consult for suspected CCHS. In both individuals, PCR and fragment analysis with confirmatory sequence analysis demonstrated the presence of a pathogenic heterozygous expansion of the 20-residue polyalanine tract in exon 3 to 27 alanines. The mother of Case 1, who retrospectively reported autonomic symptoms during pregnancy, was homozygous for the normal 20-residue alanine allele. Both our cases continue to require long term ventilation via tracheostomy.

The suspicion of CCHS is prompted by persistent hypoventilation and autonomic nervous system dysregulation in neonates without a cardiopulmonary, metabolic, neuromuscular or brainstem cause to account for the phenotype. Access to *PHOX2B* mutation analysis remains challenging in a resource limited setting. Genetic confirmation of a pathogenic variant in *PHOX2B* provides a definitive diagnosis of CCHS for parents and treating clinicians, which may alleviate uncertainty, continuous, unnecessary, costly investigations and guide long term medical management and prognosis. Although most pathogenic mutations occur *de novo*, somatic mosaicism may occur in asymptomatic parents. This has potential ramifications for parental health, in addition to recurrence risk implications, and provides additional motivation for genetic testing. The variable phenotype and risk of early neonatal death suggests that CCHS may be less rare than is currently assumed.

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Asparagine synthetase deficiency – a case report

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Introduction

Asparagine synthetase deficiency (ASNSD) is a rare neurometabolic disorder, with autosomal recessive inheritance, caused by mutations in *ASNS* gene. Asparagine synthetase catalyses the transfer of ammonia, from glutamine to aspartic acid, producing asparagine.

The main clinical characteristics include congenital or postnatal microcephaly, severe global developmental delay and epileptic encephalopathy. Acoustic startles (hyperekplexia) and non-habituation of the head-retraction reflex when present may be important clues. Other neurological manifestations, such as axial hypotonia, hypertonia, clonic tremors and spastic tetraplegia, can occur.

MRI shows mainly cerebellar atrophy/hypoplasia, simplified gyral pattern, pontine hypoplasia, cerebral atrophy and delayed myelination, but other signs may be present.

Plasma asparagine levels may be normally low and CSF asparagine levels may be low; however, normal levels of this non-essential amino acid do not exclude the disease. Of the few cases described in the literature, the diagnosis was made by NGS technology, confirming the biallelic mutations in ASNS gene.

ASNSD is a slowly progressive neurodegenerative disorder with no treatment available. Although it minimally improved mental status/consciousness, treatment with high doses of asparagine has led to worsened seizures.

We present a case of an ASNSD diagnosed very recently at our center.

Case report

6 years-old male followed at our Genetics clinic since he was born due to congenital microcephaly. He is the first living child of healthy consanguineous parents (second-cousins). They had a miscarriage and a stillbirth of a female foetus with normal karyotype presenting with mild facial dysmorphisms and x-ray and bone histology anomalies, possible compatible with bone dysplasia with angulated bones. *FGFR3* gene study was normal.

With time, this child has shown severe global developmental delay, refractory epilepsy, spastic tetraplegia, mild facial dysmorphisms and scoliosis.

Brain MRI revealed congenital microcephaly with a simplified gyral pattern associated with delayed myelination, corpus callosum hypoplasia/hypogenesis, pontine and mesencephalon hypoplasia, and cystic lesions on the subcortical white matter of the temporal poles.

Biochemical studies revealed plasma asparagine levels in the normal range [54 µmol/L (N: 36-191)], as well as normal results for 7-DHC, VLCFA, organic acids and transferrin isoelectric focusing test. Karyotype and molecular cytogenetic tests were normal. Several genes had been studied, namely gene *ARX*, *ASPM* and a gene panel for brain morphogenesis defects, all normal. Finally, a clinical exome was performed, revealing a novel missense variant c.30T>G (p.Ser10Arg), in homozygosity in *ASNS* gene. Parental study revealed the carrier status for both. After this result, a second plasma aminoacids determination revealed mildly reduced levels of asparagine [24 µmol/L (N: 26 – 64)]. An attempted lumbar punction failed because of severe scoliosis.

Conclusions

With this work, the authors wish to stress the importance of re-evaluating undiagnosed patients, as well as the diagnostic tools available at a specific time. In this case, the diagnosis was not possible with metabolic studies, neither with the NGS panel for microcephaly (which did not include this gene). Clinical exome sequencing provided the diagnosis and the possibility to offer prenatal testing for future pregnancies to the parents. Furthermore, sharing information on this rare disease with the scientific community may help earlier diagnosis in other patients and better characterization of the disease.

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Clinical and genetic analyses of a Dutch cohort of 40 patients with a nephronophthisis-related ciliopathy

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Objective: Nephronophthisis is an autosomal recessive ciliopathy and an important cause of end-stage renal disease in children and young adults. Diagnostic delay is frequent in nephronophthisis. The aim of this study was to gain insight in the clinical characteristics, early symptoms and genetic defects associated with nephronophthisis in a Dutch cohort of 40 patients with a nephronophthisis-related ciliopathy to improve early diagnostics and genetic counseling.

Methods: A cohort of 52 patients from 45 families with a (suspected) renal ciliopathy including 40 patients with a nephronophthisis-related ciliopathy was recruited at eight university medical centers in The Netherlands and via the webpage www.kouncil.nl. Comprehensive clinical and genotypic data were recorded in the Nephronophthisis Registry. Thirteen patients with a nephronophthisis-related ciliopathy without a molecular diagnosis were analyzed for deletion/duplication of the *NPHP1* gene and gene panel sequencing and/or whole-exome sequencing were performed.

Results: Of 40 patients with a nephronophthisis-related ciliopathy, 50% had isolated nephronophthisis and 43% had a syndrome diagnosis. Prevalent ultrasound findings included increased echogenicity (65%), renal cysts (43%) and abnormal corticomedullary differentiation (32%). The most frequently reported presenting signs and symptoms were fatigue (42%), polydipsia and polyuria (33%) and hypertension (21%), which should prompt evaluation of renal function. Renal biopsies were performed in eight patients and showed nonspecific signs of chronic kidney disease. Genetic testing yielded a molecular diagnosis in 38% of the patients tested within our cohort. In addition, we identified a heterozygous variant in the candidate ciliary gene *NINL* that requires further validation.

Conclusion: Our findings in a cohort of 40 Dutch patients with a nephronophthisis-related ciliopathy will aid early diagnostics and guide genetic counseling for improved patient care.

Does the human SMA protective modifier Plastin 3 escape X-inactivation?

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Plastin 3 (*PLS3*) is a protective modifier of spinal muscular atrophy (SMA). SMA is a devastating and frequent motor neuron disorder caused by homozygous loss of the Survival of Motor Neuron 1 (*SMN1*) gene and insufficient functional SMN protein produced by the *SMN2* gene. Several families with asymptomatic individuals that share the *SMN1* deletion and the same copy number of *SMN2* as their affected siblings have been identified. In seven families, *PLS3* has been found to be upregulated in asymptomatic homozygously *SMN1*-deleted individuals and thus to protect against SMA. PLS3 is a Ca^{2+}-dependent F-actin binding and –bundling protein, which is able to restore F-actin levels in SMA condition. By this, it is able to rescue axonal growth defects, the structure and function of the neuromuscular junctions and motor neuron function in several SMA model systems. *PLS3* is upregulated in approximately five percent of the healthy human population, while the reason for the upregulation is still elusive. Interestingly, all asymptomatic individuals protected by increased *PLS3* levels are women. As *PLS3* is an X-linked gene, we hypothesised that *PLS3* might escape the X-chromosomal inactivation. By analysing the transcriptomes of single cell clones of iPSC-derived motor neurons of asymptomatic individuals, we were able to detect biallelic expression of *PLS3* in the asymptomatic individuals. *PLS3* (hg19, ChrX: 114,827,819-114,885,179 bp) is localized on the X-chromosome next to the unique macrosatellite *DXZ4* (hg 19, ChrX: 114,867,433 – 114,919,088 bp), which is essential for the X-chromosomal inactivation. The macrosatellite has a highly variable repeat number in humans ranging from 50 to 100 copies of a 3 kb repeat monomer. Surprisingly, *DXZ4* is associated with heterochromatic epigenetic features on the active female X-chromosomes, while it has euchromatic features on the inactive X-chromosomes and the locus of *DXZ4* is therefore open for transcription.

Based on this, we hypothesised that the repeat number of *DXZ4* can modulate the expression of *PLS3* and other genes in its molecular neighbourhood on the inactivated X-chromosome in women, facilitating the escape of those genes from X-chromosomal inactivation. We used molecular combing to analyse the repeat number of *DXZ4* comparing samples from individuals that differ in their expression of *PLS3*. This method uses multi-colour DNA probes to mark repeat regions on linear stretched DNA molecules. We were able to quantify the repeat number of the macrosatellite in SMA affected and healthy individuals and compared the results to *PLS3* expression levels measured by qPCR.

We were able to distinguish both alleles in female individuals. Women with a high expression of *PLS3* showed a copy number of 89 to 100 repeats for at least one allele. The larger allele in women with low *PLS3* expression showed a copy number of 43 to 66 repeats. We performed a multivariate analysis of variance (MANOVA) comparing the *DXZ4* copy number and the *PLS3* expression as dependent variables between females that differ in their *PLS3* expression and found a significant difference ($P \approx 3.8*10^{-3}$) between the two groups.

Our data strongly suggest 1) that asymptomatic highly *PLS3* expressing *SMN1*-deleted women carry one allele with an elongated macrosatellite and 2) that the length of the macrosatellite *DXZ4* influences *PLS3* expression by an escape from X-inactivation.

Utility of whole exome sequencing in the genetic analysis of Hungarian ALS-patients

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Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. Genetic factors play a key role in ALS and uncovering its genetic background may bring us closer to fully understand its pathomechanism. Therefore, the aim was to identify rare damaging variants in major and minor genes involved in pathways annotated to ALS and in genes of other neurogenetic disorders.
Patients and methods: 21 Hungarian patients with sporadic ALS were enrolled in the study. They all fulfilled the revised El Escorial and the Awaji-shima criteria for ALS. Prior to whole exome sequencing, the patients were prescreened for 7 major ALS genes: C9ORF72 repeat expansion, SOD1, ANG, FUS, SETX, TARDBP and UBQLN2. Exome sequencing was carried out by using Illumina NextSeq sequencer and data analysis was performed according to the best practices to identify single nucleotide variants and small insertions/deletions. Variant filtering was performed to identify damaging variants in ALS "priority" genes (n=32), ALS "candidate" genes (n=98) and other neurogenetic disease genes (n=125). The detected variants were confirmed by Sanger sequencing.
Results: In ALS "priority" genes, exome sequencing revealed a novel non-synonymous variant in the NEFH gene that encodes neurofilament heavy polypeptide; a novel nonsynonymous variant in the MATR3 gene that is specific to the matrin-3 isoform 1; a previously described pathogenic nonsense mutation in the alsin (ALS2) gene that leads to premature stop codon and may affect endosomal and vesicle transport; a rare variant in the SPG11 gene with uncertain significance; and finally a novel and a known variant in the NEK1 gene, encoding NIMA-related kinase-1, that has recently been associated with ALS in the Caucasian population. Further novel and rare recurrent variants have been detected in 10 ALS "candidate" genes and in 7 other neurogenetic disease genes. None of the detected variants were present in healthy Hungarian and Austrian controls.
Conclusion: Potentially disease-causing variants in ALS "priority" genes have been detected in 28% (6/21) of this sporadic cohort. While the disease causing role of several mutations identified in this study has been previously well-established, other variants may show reduced penetrance or may be rare benign variants. Our study provides further insight into the genetic etiology of this heterogenous disease.
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Utility of multiplex ligation-dependent probe amplification on patients with acute myeloid leukemia. A case report study

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We present a patient diagnosed on September 2017 with Myelodysplastic Syndrome (MDS). On December 2017 the number of myeloid blast cell increase at 60%. The patient was diagnosed with secondary Acute Myeloid Leukemia (sAML). In addition to the routine laboratory analysis performed, we performed the cytogenetic analyses, and the patients had a normal karyotype, without abnormalities. In addition, we investigated FLT3 ITD, FLT3 D835, DNMT3A (R882H and R882C) and NPM1(c.863_864insTCTG/ CATG) mutations. The patient was negative for the mentioned mutations. The patient's condition worsened. The multiplex ligation-dependent probe amplification (MLPA) analyses was performed using the SALSA MLPA P377 Hematologic Malignancies probemix. The Coffalyser program was used for interpretation of CNVs (copy number variations). Our patient presented three CNVs: del 5q32-34, del 7p12.2, del 7q31.2-36.2. We repeated the analyzes one more time and we found the same CNVs. For del 7p12.2 confirmation we performed the MLPA analyses by using SALSA MLPA P202 IKZF1 (IKAROS) probemix and for del 5q32-34 and del 7q31.2-36.2 by using SALSA MLPA P414 MDS probemix. By using the new probemixes we confirmed the deletions observed. Unfortunately, the patient has not survived. Deletion of 5q or 7q are associated with rapid disease progression, poor outcome and survival, and with poor prognosis.

Keywords: AML, MLPA, molecular, cytogenetics

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NOVEL VARIANTS IN ADAMTS13 DETECTED BY NEXT GENERATION SEQUENCING (NGS) IN A PATIENT WITH ATYPICAL THROMBOTIC THROMBOCYTOPENIC PURPURA

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Introductonn NGS is a useful tool to diagnose genetc defects in patents with atypical course of congenital disorders.

Matherials and methodsn We report a boy who presented at the age of 7 months with hemolytc anemia, thrombocytopenia and no neurological and renal dysfuncton or evidence of thrombosis. He initally responded to steroids and rituiimab treatment. Subseuuently the relapse of the disease was accompanied by hypogammaglobulinemia. Various primary immunodefciencies were eicluded via Sanger seuuencing. Eventually the boy died of intracranial bleeding and sepsis. The clinical eiome seuuencing (TruSightOne, Illumina) was performed (MiSeu, Illumina) in the patent. Detected variants were fltered, their signifcance was assessed according to the ACMG recommendatons. Candidate variants were confrmed in the patent and his healthy parents by Sanger seuuencing, their pathogenicity was tested by in silico analysis.

Resultsn In the patent we detected two novel complei heterozygous variants in ADAMTS13(NM_139025) genen c.947_948delinsTT, p.G316V and c.1143_1144delinsC, p.A381fs. Each was inherited from respectve parent. Both variants have not been previously reported in the literature and mutatons databases. Substtuton p.G316V was predicted to be pathogenic by in silico analysis.

Conclusionn Familial thrombotc thrombocytopenic purpura (TTP) is a rare autosomal recessive disorder caused by mutaton in the ADAMTS13 gene, encoding the von Willebrand factor-cleaving protease and characterized by microangiopathic hemolytc anemia, thrombocytopenia, neurologic and renal complicatons due to microthrombi formaton. TTP was not suspected in the patent who died before the correct diagnosis was made. Quick access to NGS is crucial in severe congenital disorders

Identification of compound heterozygous variants in PIGQ in a girl with early infantile epileptic encephalopathy, dysmorphic features and multiple congenital anomalies

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Glycosylphosphatidylinositol (GPI) is a glycolipid posttranslational modification utilized by cells to anchor proteins to the cell surface. There are over 150 known GPI anchored proteins which play vital roles in development and neurogenesis (PMID 22265715). Mutations in the genes responsible for GPI anchor formation have been identified leading to emergence of the inherited GPI deficiencies (IGDs), considered a subgroup of congenital disorders of glycosylation. IGDs present with a wide, but severe, clinical presentations often with profound neurologic impairment, early infantile epileptic encephalopathy (EIEE) and congenital anomalies (PMID 26996948). GPI anchor formation is a complex process requiring at least 10 enzymatic reactions and over 20 proteins including multiple members of the phosphatidylinositol glycan anchor biosynthesis (PIG) gene family (PMID 24727937).

Here, we report an infant who presented with fetal hepatomegaly and polyhydramnios and postnatal dysmorphic features, EIEE and multiple congenital anomalies. Clinical whole exome sequencing was negative however research re-analysis of this data identified compound heterozygous mutations in the gene phosphatidylinositol glycan anchor biosynthesis, class Q (*PIGQ*) (NM_148920.2: c.1130_1168del;p.Ala377_Ser389del and c.1345G>C;p.Gly449Arg). *PIGQ*, formerly called *GPI1*, encodes a subunit of the first enzymatic step of GPI anchor formation. Flow cytometry of patient granulocytes identified reduced GPI anchor protein expression markers on the cell surface compared with controls. A single patient has previously been reported with EIEE and a homozygous splice site mutation thought to affect *PIGQ* function (PMID 24463883). The patient reported here is the second affected individual reported to date, providing further evidence of the disease-gene association and expanding our understanding of the clinical spectrum of *PIGQ* mutations. The heterogeneous presentation makes clinical diagnosis difficult and warrants early implementation of genome-wide sequencing to obtain a diagnosis and direct management.

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