EUROPEAN SOCIETY OF HUMAN GENETICS

European School of Genetic Medicine

4th Course in

Next Generation Sequencing

Bertinoro - Italy

May 13-16, 2015

Bertinoro University Residential Centre
Via Frangipane, 6 – Bertinoro

Course Director:
J. Veltman (Nijmegen, The Netherlands)

Course Co-Directors:
Gilissen C. (Nijmegen, The Netherlands), Pippucci T. (Bologna, Italy)

EUROPEAN SCHOOL OF GENETIC MEDICINE

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

CENTRO RESIDENZIALE UNIVERSITARIO Bertinoro

4th Course in

Next Generation Sequencing

Bertinoro – Italy May 13-16, 2015

CONTENTS

4th COURSE IN NEXT GENERATION SEQUENCING

Bertinoro University Residential Centre
Bertinoro (Italy), May 13-16, 2015

Arrival: Tuesday May 12th

Wednesday, May 13

Morning Session: Introduction to Next Generation Sequencing

9.00 – 9.15	Evolution of Human/Medical Genetics: Sixty Years of History **Giovanni Romeo**
9.15 – 10.15	Next Generation Sequencing basics, part 1 **Joris Veltman**
10.15 – 10.45	**Coffee Break**
10.15 – 11.00	Next Generation Sequencing basics, part 2 **Joris Veltman**
11.00 – 12.45	Bioinformatic basics **Christian Gilissen**
12.45– 13.30	**Lunch Break**

Afternoon Session:

13.30 –14.00	Poster Viewing Session
14.00 – 16.00	Concurrent Workshops: Computer practical: Variant identification (**C. Gilissen** & **T. Pippucci**) Workshops by speakers
16.00-16.30	**Coffee Break**
16.30 – 18.00	Concurrent Workshops Computer practical: Variant identification (**C. Gilissen** & **T. Pippucci**) Workshops by speakers

Thursday, May 14

Morning Session: Applications to disease gene identification & diagnostics

9.00 – 10.00	Application of NGS for the diagnosis of neonatal diabetes **Elisa de Franco**
10.00 – 11.00	The Sardinia project **Carlo Sidore**
11.00 – 11.30	**Coffee Break**
11.30- 12.30	De novo mutations in human genetic disease **Joris Veltman**
12.30 – 13.30	WGS for non-coding mutations in congenital disorders **Malte Spielmann**
13.30 – 14.30	**Lunch Break**

Afternoon Session:

14.30 – 16.00	Concurrent Workshops Computer practical: Disease gene identification (**C. Gilissen** & **T.Pippucci**) Workshops by speakers
16.00-16.30	**Coffee Break**
16.30 – 18.00	Concurrent Workshops Computer practical: Disease gene identification (**C. Gilissen** & **T.Pippucci**) Workshops by speakers

Friday, May 15

Morning Session: Applications to common disease & cancer

9.00 – 10.00	Bioinformatic strategies & ontology's **Peter Robinson**
10.00 – 11.00	Exome diagnostics in intellectual disability **Anita Rauch**
11.00 – 11.30	**Coffee Break**
11.30 – 12.30	Cancer genomics **Moritz Gerstung**
12.30 – 13:30	Cancer Genomics in the Clinic **Matthew Parker**
13:30 – 14.30	**Lunch Break**

Afternoon Session:

14.30 –15.00	Poster Viewing Session
15.00 – 16.30	Concurrent Workshops Computer practical: Diagnostic NGS (**C. Gilissen** & **T. Pippucci**) Workshops by speakers
16.30-17.00	Coffee Break
17.00 – 18.30	Concurrent Workshops Computer practical: Diagnostic NGS (**C. Gilissen** & **T. Pippucci**) Workshops by speakers

Saturday, May 16

Morning Session: Genome technologies

9.00– 10.00	Single-cell genomics unveils genetic heterogeneity in health and disease, and enables novel clinical applications **Thierry Voet**
10.00 – 11.00	Handheld diagnostics on nanowires **Jonathan O'Halloran**
11.00 – 11.30	**Coffee Break**
11.30 – 12.00	Best Posters Presentations by students
12.00 – 12.30	Wrapping up of the course **(Joris Veltman**)
12.30	**Lunch**

Departures

ABSTRACTS OF LECTURES

Wednesday, May 13

Evolution of Human/Medical Genetics: Sixty Years of History

Giovanni Romeo
University of Bologna & European School of Genetic Medicine, Italy

The main steps in the evolution of contemporary Human/Medical Genetics will be summarized and each step will be linked to the professional history of a scientist or clinician who left a mark in our field. Along with the research blooming in this period, some educational initiatives were developed in America and Europe, like the Short Course in Medical Genetics started by V.A. McKusick in Bar Harbor, Maine-USA, in 1960, the European School of Genetic Medicine started in Sestri Levante, Italy, in 1988 and the Latin American School of Human and Medical Genetics started by Roberto Giugliani in Caxias do Sul, Brazil, in 2005. During the same years these courses and schools trained thousands of young geneticists coming from all over the world.

Next Generation Sequencing basics

Joris A.Veltman
Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

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

Recommended reading:

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

Bioinformatic basics

Christian Gilissen
Department of Human Genetics -
Radboud University Nijmegen Medical Centre, the Netherlands

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

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

[1] Unlocking Mendelian disease using exome sequencing. Gilissen C, Hoischen A, Brunner HG, Veltman JA. Genome Biol. 2011 Sep 14;12(9):228. doi: 10.1186/gb-2011-12-9-228. Review. PMID: 21920049.

[2] Diagnostic exome sequencing in persons with severe intellectual disability. de Ligt J, Willemsen MH, van Bon BW, Kleefstra T, Yntema HG, Kroes T, Vulto-van Silfhout AT, Koolen DA, de Vries P, Gilissen C, del Rosario M, Hoischen A, Scheffer H, de Vries BB, Brunner HG, Veltman JA, Vissers LE. N Engl J Med. 2012 Nov 15;367(20):1921-9. PMID: 23033978.

[3] A post-hoc comparison of the utility of Sanger sequencing and exome sequencing for the diagnosis of heterogeneous diseases. Neveling K, Feenstra I, Gilissen C, Hoefsloot LH, Kamsteeg EJ, Mensenkamp AR, Rodenburg RJ, Yntema HG, Spruijt L, Vermeer S, Rinne T, van Gassen KL, Bodmer D, Lugtenberg D, de

Reuver R, Buijsman W, Derks RC, Wieskamp N, van den Heuvel B, Ligtenberg MJ, Kremer H, Koolen DA, van de Warrenburg BP, Cremers FP, Marcelis CL, Smeitink JA, Wortmann SB, van Zelst-Stams WA, Veltman JA, Brunner HG, Scheffer H, Nelen MR. Hum Mutat. 2013 Dec;34(12):1721-6. PMID: 24123792.

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

Application of NGS for the diagnosis of neonatal diabetes

Elisa de Franco
University of Exeter Medical School, UK

For genetically heterogeneous diseases, genetic testing was traditionally employed to confirm a clinical diagnosis made by a clinical expert and based on the disease course or a cluster of clinical features. This strategy focused on analysis of one or a few genes selected according to clinical features. This approach is changing as improved sequencing methods enable simultaneous analysis of multiple genes.

Neonatal diabetes diagnosed before 6 months of age is a clinically and genetically heterogeneous disease, with 23 genetic causes identified so far (Ref). Being diagnosed soon after birth, neonatal diabetes is the presenting feature of many discrete clinical phenotypes defined by different genetic aetiologies. The most common cause of neonatal diabetes are mutations in the potassium channel subunit genes ABCC8 and KCNJ11 (Ref). Patients with neonatal diabetes caused by a potassium channel gene mutation are sensitive to sulfonylurea treatment and therefore their clinical management can be improved by replacing insulin with oral agents (Ref). This highlights the importance of an early genetic diagnosis in neonatal diabetes and international guidelines suggest immediate referral for genetic testing as soon as a clinical diagnosis of neonatal diabetes is made (Ref).

Three targeted next-generation sequencing assays have been developed for genetic testing of monogenic diabetes (Ref), including our panel which includes all the 22 known neonatal diabetes genes (Ref). We used this targeted next-generation sequencing assay to test all the genetically undiagnosed patients in a large, international cohort of 1020 neonatal diabetes patients with the objective of evaluating the impact of early, comprehensive genetic testing in this disease.

A genetic diagnosis was obtained in 840 patients (82%). Our data show that patients are now referred soon after presentation with neonatal diabetes and this has implications for the clinical phenotype. For example, among patients with genetically diagnosed Wolcott-Rallison syndrome (a syndromic form of neonatal diabetes) just 12% of those tested within 3 months from diagnosis had syndromic diabetes vs 82% of those referred later (over 4 years after diabetes presentation, $p<0{\cdot}0001$) in whom skeletal and/or liver involvement was common. Similarly, for patients with transient neonatal diabetes only 10% of those tested early (less than 3 months from diagnosis with diabetes) had remitted compared to 100% ($p<0{\cdot}0001$) of the later referrals.

Comprehensive testing using next generation sequencing allows early genetic diagnosis in patients with neonatal diabetes, often before development of specific clinical features. We anticipate that the future of care in neonatal diabetes will increasingly rely on the results of genetic testing with the genetic diagnosis not only informing a clinician of the likely course and best treatment for the diabetes but also predicting development

of additional clinical features. This represents a new paradigm with genetic testing defining, rather than just confirming, the clinical diagnosis.

The Sardinia Sequencing Project

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

Genome-wide association studies have increasingly furthered our understanding of the molecular basis of many complex traits by finding, through genotyping and imputation, loci associated with many different traits. However, studies based on variants present in common genotyping arrays and imputation panels may not capture the fraction of human genome variation that is rare or geographically restricted and unique to specific populations. To advance our understanding of the genetics of a variety of traits in the Sardinian population, we are studying a sample of 6,602 individuals recruited from the population of a cluster of 4 small towns in Sardinia. Using whole genome sequencing, we sequenced DNAs from 3,514 Sardinian individuals enrolled either in this project or in a parallel project on autoimmune diseases, at an average depth of coverage of ~4X. We successfully identified and genotyped >23M single nucleotide polymorphisms (30.6% of them novel, not in dbSNP v135) with an error rate of 0.15%. To increase the power to detect association, we are using the haplotypes generated by sequencing of these individuals to impute missing genotypes in the remaining 6,602 already genotyped with an integrated map of 4 GWAS Illumina arrays (ImmunoChip, CardioMetaboChip, ExomeChip and OmniExpress). Strikingly, imputation using our Sardinian reference panel shows greatly increased accuracy when compared to an equal size reference panel of European haplotypes generated by the 1000 Genomes Project (average imputation accuracy, rsqr=0.9 compared to 0.75 for alleles with frequency 1-3%). With a larger reference panel, imputation accuracy of variants with frequency 1-3% reaches 0.94 %, giving us the possibility of analyzing the rare frequency domain in the Sardinian population.

As an example of the advantages of analyzing population specific rare variation, we will discuss the Q39X mutation in the *HBB* gene, which is common in Sardinia (MAF ~5%) but very rare elsewhere. The variant is associated with a variety of blood phenotypes. For LDL cholesterol, this variant has the second largest effect among the variants previously found with standard GWAS analysis. Our approach thus increases the power of detecting population specific association.

De novo mutations in human genetic disease

Joris A.Veltman
Department of Human Genetics, Radboud University Nijmegen Medical Centre,
Nijmegen, The Netherlands

Severe disorders with an onset early in life affect fitness, many of them can be considered reproductively lethal. The natural selection against the spread of these disorders in the population should result in a reduction of the occurrence of these disorders, unless these disorders occur de novo by some mechanism. One way in which genetic disorders can arise de novo is by de novo mutations occurring in the germline in each new generation. Unbiased approaches such as exome and genome sequencing, if applied to patients and their unaffected parents, allow us now for the first time to study the presence, frequency and impact of all types of de novo mutations (SNV, indels, CNVs) on genetic disease. In this presentation I will discuss the de novo mutation hypothesis, and recent insight into the role of de novo mutations in rare and common genetic disease. In addition, I will discuss ways to identify the paternal origin of these mutations, and show how these studies have pointed to increased paternal age as a risk factor for disorders caused by de novo mutations.

Recommended reading:

1. Roach JC, Glusman G, Smit AF, Huff CD, Hubley R, Shannon PT, Rowen L, Pant KP, Goodman N, Bamshad M, Shendure J, Drmanac R, Jorde LB, Hood L, Galas DJ. Analysis of genetic inheritance in a family quartet by whole-genome sequencing. Science 328: 636-9 (2010).
2. Veltman JA and Brunner HG. De novo mutations in human genetic disease. Nat Rev Genet 13: 565-75 (2012).
3. Kong A, Frigge ML, Masson G, Besenbacher S, Sulem P, Magnusson G, Gudjonsson SA, Sigurdsson A, Jonasdottir A, Jonasdottir A, Wong WS, Sigurdsson G, Walters GB, Steinberg S, Helgason H, Thorleifsson G, Gudbjartsson DF, Helgason A, Magnusson OT, Thorsteinsdottir U, Stefansson K. Rate of de novo mutations and the importance of father's age to disease risk. Nature 488: 471-5 (2012).

WGS for non-coding mutations in congenital disorders

Malte Spielmann
Institute for Medical Genetics and Human Genetics, Charité Universitätsmedizin, Berlin, Germany and Max Planck Institute for Molecular Genetics, Berlin, Germany

High-throughput genomic technologies are currently revolutionizing human genetics. So far the focus has been on the 1.5% of the genome, which is coding, in spite of the fact that the great majority of genomic variants fall outside the coding regions. Now we are amidst a paradigm-shift: Exome sequencing is feasible in a clinical setting, yet there are thousands of well-defined or suspected disorders for which the genetic basis remains unknown. The next frontier is the non-coding sequence of the human genome. Therefore whole genome sequencing will be necessary in many cases.

While Exome sequencing studies have failed to identify coding mutations in 40% of the cases, there is evidence from recent efforts to annotate the non-coding sequence that over 80% of the genome is biochemically active. NGS-based studies of DNA: DNA interactions have provided insight into the general conformation of the non-coding genome, as well as interactions between promoters and distant-acting transcriptional enhancers. Recent data from genome-wide chromosome conformation capture analysis indicate that the human genome is divided into conserved megabase-sized self-interacting regions called topological associated domains (TADs). These TADs consist of sequence regions that enhance and/or silence the expression of nearby genes and form the regulatory backbone of the genome. The TADs are separated by regulatory boundary elements or barriers.

We have investigated how structural variations such as deletions, duplications, and inversions, can interfere with DNA folding, TAD formation and gene regulation and how this may be related to congenital disease.

Using a computational approach we will show how copy-number variations can potentially alter the TAD architecture of the human genome by deleting the barriers and thereby allowing enhancers from neighboring domains to ectopically activate genes causing misexpression and disease, a mutational mechanism that has recently been termed enhancer adoption. Our results show that up to 11.8% of the deletions in the DECIPHER database of chromosomal imbalances could be best explained by enhancer adoption or a combination of enhancer adoption and gene-dosage effects.

Using whole genome sequencing and array CGH we have identified the molecular cause of several new phenotypes associated with structural variations at different loci including *PITX1, EPHA4, PAX3, HOXD13,* and *FGF8*. We will focus on balanced rearrangements or the functional impact of deletions and duplications that are limited to non-coding DNA. We re-engineered structural variations of various sizes (up to 1.6 Mb) in mice using a modified CRISP/Cas protocol and examine them for their phenotype. DNA:DNA interactions were analyzed using 4C-seq in mouse embryonic tissues. Similar studies were performed in corresponding human cell lines. Our results show that structural variations can change the TAD order of the genome thereby inducing aberrant contacts between enhancers and promoters which in turn results in misexpression

and morphogenetic changes. Our studies provide a framework to understand the impact of structural variations on genomic integrity and help to predict their pathogenicity.

More recently we started using whole genome sequencing to screen cohorts of patients with congenital malformation syndromes such as split hand foot malformation (SHFM) and other limb defects for the systematic discovery of non-coding mutations and structural variations. We show that mutations and structural variations outside of the coding genome can interfere with normal gene regulation by disrupting the regulatory landscape. Therefore, the regulatory landscape of the genome has also to be taken into consideration when investigating the pathology of human disease.

Friday, May 15

Bioinformatic strategies & ontology's

Peter Robinson
Institut für Medizinische Genetik und Humangenetik Charité - Universitätsmedizin Berlin
Germany

Once we have aligned and Q/C'd whole-exome data, and called the variants, the major challenge remains of decided which of the variants might be responsible for the disease we are investigating. Standard pipelines evaluate all variants as to their rarity and known or predicted pathogenicity, and if possible compare results in multiple affected individuals or intersect candidate variants with linkage intervals. Nonetheless, often hundreds of candidate variants remain, and it is often simply not possible to make the diagnosis in a clinical setting or to identify a novel disease gene in research environments. A very active area in bioinformatics research is gene prioritization, that is, ranking candidate genes with respect to their potential relevance to the disease. Compared to previous efforts at prioritization, exome data allows us to intersect an evaluation of the variants with an evaluation of the relevance of candidate genes for the disease.

In this talk, I will present recently published as well as submitted work that employs an analysis of phenotypic similarity or of protein protein interaction networks to prioritize genes. I will begin with a discussion of the Exomiser project (https://www.sanger.ac.uk/resources/databases/exomiser/). The observation that each of our genomes contains about 100 genuine loss-of-function variants makes identification of the causative mutation problematic when using these strategies alone. Here, we propose using the wealth of genotype to phenotype data that already exists from model organism studies to assess the potential impact of these exome variants. We introduce PHenotypic Interpretation of Variants in Exomes (PHIVE), an algorithm that integrates the calculation of phenotype similarity between human diseases and genetically modified mouse models with evaluation of the variants according to allele frequency, pathogenicity, and mode of inheritance approaches in our Exomiser tool. Large-scale validation of PHIVE analysis using 100,000 exomes containing known mutations demonstrated a substantial improvement (up to 54.1-fold) over purely variant-based (frequency and pathogenicity) methods with the correct gene recalled as the top hit in up to 83% of samples, corresponding to an area under the ROC curve of >95%. We conclude that incorporation of phenotype data can play a vital role in translational bioinformatics and propose that exome sequencing projects should systematically capture clinical phenotypes to take advantage of the strategy presented here. More recently we have developed a prioritization framework that takes advantage of a random walk analysis of protein-protein interaction networks. We again showed that this data induces a substantial improvement over purely variant based prioritization. Interestingly, different methods show advantages for different exomes, suggesting that future work show develop a framework for combining

different prioritization approaches. In the talk, I will explain the prioritization framework and the algorithms. A workshop will be offered in which we will explore how to use the programs.

As an additional offer to students of this course, it is possible to get a copy of a 150-page handout for a course I am teaching at the Free University of Berlin Bioinformatics Master program on exome sequencing, which includes a lot of practical computer exercises (command line, programming, scripting) for the entire exome pipeline (http://compbio.charite.de/contao/index.php/MedicalGenomics.html). The script is suitable mainly for bioinformaticians, send me a mail if you would like a copy.

Exome diagnostics in intellectual disability

Anita Rauch

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

Identification of disease causing mutations in genetically heterogeneous conditions such as intellectual disability by Sanger sequencing is time-consuming, costly and often unsuccessful. The advent of NGS techniques is paving the way for novel large scale approaches with an unforeseen diagnostic power. However, the plethora of variants of unknown significance detected by genome-wide approaches requires distinctive strategies to identify actually disease-related mutations. We recently showed that exome sequencing of patient-parent trios in sporadic cases of unspecific severe intellectual disability may unravel disease causing mutation in more than 50% of previously unsolved cases, with more than 30% of mutations in already known disease genes. Thereby it became also evident, that the current descriptions of phenotypes associated with mutations in a certain gene, are heavily biased towards certain recognizable patterns. However, while whole exome sequencing may currently provide theoretically the highest cost-efficient diagnostic power, it may miss mutations due to incomplete coverage of certain genes. Therefore in some phenotypes a “clinical exome” limited to a set of genes with currently known monogenic mutations may also be useful.

Cancer genomics

Moritz Gerstung

Cancer Genome Project - Wellcome Trust Sanger Institute - Wellcome Trust Genome Campus Hinxton, Cambridgeshire, UK

The availability of economic next-generation sequencing technologies has facilitated the study of the genomic changes of cancer cells at unprecedented detail. I will first summarise some of the recent advances in cancer genomics over the last decade. These include the discovery of novel mutagenic processes, subclonal diversity and also an ever growing list of cancer genes. At the example of a large cohort of 1540

patients with acute myeloid leukaemia I will then discuss some of the clinical ramifications of cancer genomes. These analyses demonstrate that patient survival is influenced by a large number of genomic lesions with each patient having a unique constellation of risk factors. A comprehensive analysis can greatly refine our understanding of patient risk, which will provide the basis for rational clinical decision support.

Cancer Genomics in the Clinic

Matthew Parker
Genomics England - Queen Mary's University London, UK

Whole genome cancer genomics is now reaching the clinic in the NHS in England with the help of the 100,000 genomes project. Gene panels and more recently exomes are relatively common place in clinical diagnostic laboratories around the country, but these have major shortcomings in the diagnosis and discovery of complex genomic lesions that are often present in cancer, it is therefore preferential to sequence the whole genomes of these patients. For example, whole genome sequencing and subsequent RNASeq of Ependymoma as part of the Paediatric Cancer Genome Project (PCGP) allowed the discovery of a highly penetrant somatic lesion in the Supratentorial subtype of this tumour. Through shattering and rejoining of chromosome 11 (chromothripsis) the C11orf95 and RELA genes are juxtaposed, either are directly fused through exon-exon translocations, exon-intron translocations or through an event which brings the genes close enough that the intervening DNA is spliced out of the final fusion product. Because the nature of chromothripsis the events that lead to the fusion of C11orf95 and RELA are different in every patient and therefore require either WGS, RNASeq or FISH for detection. The resulting fusions spontaneously translocate to the nucleus to activate NF-kB target genes and transform neural stem cells to form tumours in mice. Lessons learned from the PCGP allowed St Jude to embark upon an ambitious pilot project to use WGS, Exome and RNASeq to diagnose every cancer patient admitted to the hospital and replace current diagnostic testing. The 100,000 genomes project is in its pilot phase for cancer and assessments of fresh frozen (FF) vs formalin-fixed paraffin embedded (FFPE) material for use in whole genome sequencing is underway. FF material is the preferred substrate for WGS sequencing, the data produced is of good quality and relatively free from artefacts, however, FFPE produces many hurdles to obtaining a good whole genome sequence.

Saturday, May 16

Single-cell genomics unveils genetic heterogeneity in health and disease, and enables novel clinical applications

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

Single-cell genomics enables investigating the extent and nature of genomic and transcriptomic heterogeneity which occurs in both normal development and disease, and in addition provides new tools for clinical application. 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. 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]. Data on the application of these methods for the study of genetic heterogeneity acquired in cleavage stage embryogenesis and carcinogenesis will be presented. Additionally, 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. This and a visionary on the future will be presented.

1. Macaulay IC, Haerty W, Kumar P, Li YI, Hu TX, Teng MJ, Goolam M, Saurat N, Coupland P, Shirley LM, Smith M, Van der Aa N, Banerjee R, Ellis PD, Quail MA, Swerdlow HP, Zernicka-Goetz M, Livesey FJ, Ponting CP, Voet T. G&T-seq: parallel sequencing of single-cell genomes and transcriptomes. Nature Methods. 2015 Apr 27. doi: 10.1038/nmeth.3370.

Handheld diagnostics on nanowires

Jonathan O'Halloran
QUANTUMDx GROUP LIMITED, Newcastle, U.K

The world of DNA sequencing continues to improve and evolve, with data being generated at a remarkable pace. Alongside significant improvements in bioinformatics, the explosion in different technologies and techniques to perform DNA sequencing continues to boggle the mind and perhaps even confuse labs looking to invest in the technology. Jonathan will provide an overview of DNA Sequencing technologies presently in use, describing their methods, limitations and more. He will

also introduce the future of DNA sequencing by describing the next generation of devices soon to hit the market, including his own, a novel handheld DNA sequencer for targeted DNA Sequencing, specifically designed for infectious disease applications.

ABSTRACTS OF STUDENTS POSTERS

EARLY ONSET FAMILIAL ALZHEIMER-TYPE DEMENTIA ASSOCIATED WITH TAUOPATHY AND TDP-43 PROTEINOPATHY

John Alexander (2), Gabor G. Kovacs (1), Ognian Kalev (3), Shima Mehrabian (4), Petros Drineas (5), Thomas Ströbel (1), Peristera Paschou (2)

1: Institute of Neurology, Medical University Vienna, Austria
2: Department of Molecular Biology and Genetics, Democritus University of Thrace, Greece
3: Institute of Pathology and Neuropathology, Landes-Nervenklinik Wagner-Jauregg, Linz, Austria
4: Department of Neurology, Medical University Sofia, Sofia, Bulgaria
5: Department of Computer Science, Rensselaer Polytechnic Institute, USA

Objective: To describe the clinical, neuropathological, and genetic findings of a kindred with progressive Alzheimer-type dementia in which the proband had autopsy-confirmed tauopathy with TDP-43 proteinopathy.

Methods: Three family members were examined using exome sequencing and genetic anaylsis. We performed whole exome sequencing on 2 affected siblings and one unaffected aunt. By identifying identical by descent regions in both siblings, we uncovered a large number of candidate genes potentially involved in the etiology of the phenotype under study.

Results: Genetic analysis of the *GRN, TARDBP, APP; PSEN 1, 2* and *MAPT* genes excluded mutations. Genes that are found to harbor more variants shared identical by descent among the siblings, included *LRRK2, CSMD1, AHNAK2, PCNT*, and *TMEM176B.* According to our analysis of variants shared among the siblings there is an enrichment in genes involved in nervous system development, synaptic transmission, neurogenesis, muscle structuredevelopment, brain and forebrain development, lipid transport and localization, behavior, learning or memory. In addition, pathway analysis revealed pathways related to Alzheimerdisease.

Conclusion: This condition does not fit into any previously characterized tauopathy or TDP- 43 proteinopathy, and the clinical picture deviated from more frequent presentations like frontotemporal dementia with parkinsonism. Although further genetic studies may eventually disclose the etiology our results highlight the possibility that there is no single causative gene in this case study but a set of genes working together in different pathways contributing to the etiology of a complex clinical phenotype.

Supported by European Commission's 7th Framework Programme under GA No 278486,"DEVELAGE".

Targeted Next-generation Sequencing for Molecular Diagnosis of Mendelian Diseases in Qatar

Shruti Bhagat 1, Barathy Logendra 1, Giorgia Girotto 2, Moza K Alkowari 1, Khaled Abdulhadi 3, Ramin Badii 1, Paolo Gasparini 2, Ronald G Crystal 4, Ajayeb Al-Nabet 1

Sequencing technologies have grown exponentially in the past decade resulting in massively parallel sequencing platforms which are more efficient in terms of biochemistry, cost and time. This study aims to introduce a rapid molecular diagnostic test for hereditary hearing loss, a highly heterogeneous disorder, with a targeted custom-made 80 gene panel using the Ion Torrent™ personal genome machine. The ongoing study has identified pathogenic mutations in genes such as TECTA, CDH23, MYH14(DFNA4) and MYO15A in 26 Qatari individuals from 14 families at a mean coverage of 97% at 20X and 95% at 100X. The test platform will help reduce turnaround time and provide relevant information about disease causing mutations and unknown benign variants.

1Department of Laboratory Medicine and Pathology, Hamad Medical Corporation, Doha, Qatar

2Department of Medical Sciences, University of Trieste, Trieste, Italy

3Audiology and Balance Unit, National Program for Early Detection of Hearing Loss, Hamad Medical Corporation, Doha, Qatar

4Department of Genetic Medicine, Weill Cornell Medical College, New York, USA

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

Zeineb Bakey 1,2, Marie-thérèse Bihoreau 3, Rémi Piedagnel1 2, Laure Delestré 4, 1, 5, Catherine Arnould 1,2, Alexandre d'Hotman de Villiers 1,2, Joanna H Brown 6, Olivier Devuyst 7, Sigrid Hoffmann 8, Pierre Ronco 1, 2, 9, Dominique Gauguier 4,1,5,10 and Brigitte Lelongt 1,2

1 Sorbonne Universités, UPMC Univ Paris 06, UMR_S1155, F-75006 and 75020 Paris, France;
2 INSERM, UMR_S1155, F-75020 Paris, France;
3 Centre National de Génotypage, F Evry, France;
4 UPD Univ Paris 05, F-75006, Paris, France;
5 INSERM, UMR_S1138, CRC, F-75006 Paris, France;
6 The Wellcome Trust Centre for Human Genetics University of Oxford, Oxford,UK;
7 UCL Medical School, B-1200 Brussels, Belgium; and University of Zurich, CH-8057 rich, Switzerland;
8 Medical Research Center, University of Heidelberg, D-68167 Mannheim, Germany;
9 AP-HP, Hôpital Tenon, F-75020, Paris, France;
10 Institute of Cardiometabolism and Nutrition, University Pierre & Marie Curie, Hospital Pitié Salpetrière, Paris, France

BACKGROUND:

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

METHODS:

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

RESULTS:

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

CONCLUSIONS:

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

OXTR promoter methylation is related to social behavior in dogs

Zsofia Banlaki 1, Giulia Cimarelli 2,3, Zsofia Viranyi 2,3, Eniko Kubinyi 4,5, Jozsef Topal 4,5, Maria Sasvari-Szekely 1, Zsolt Ronai 1

1Semmelweis University, Department of Medical Chemistry, Molecular Biology and Pathobiochemistry; Budapest, Hungary
2Wolf Science Center; Ernstbrunn, Austria
3Messerli Research Institute, University of Veterinary Medicine Vienna, Medical University of Vienna, University of Vienna; Vienna, Austria
4MTA-ELTE Comparative Ethology Research Group; Budapest, Hungary
5Research Centre for Natural Sciences, Institute of Cognitive Neuroscience and Psychology, Hungarian Academy of Sciences; Budapest, Hungary

Oxytocin receptor (OXTR) is a key modulator of social behavior, affiliation and cognitive functions, as highlighted by animal studies. Polymorphisms of the OXTR gene are known to be related to behavioral traits both in humans and other mammals, however, little is known about the effect of epigenetic variations. In the present study, we use the domestic dog as a model system to investigate the possible role of DNA methylation within the promoter region. Using traditional pyrosequencing method, we first explored possible differentially methylated CpG sites in the canine OXTR promoter on a small heterogeneous sample of dogs and wolves of different age and keeping conditions. By testing 5 different primer pairs for amplification and 13 different sequencing primers, we identified 6 CpG sites of potential interest. Of these, two (covered by the same sequencing primer) were measured in triplicates in 217 pet border collies previously tested for behavioral reactions in various situations. We have found that methylation level of especially one of these CpG sites is significantly associated with reaction to threatening approach of an unfamiliar person ($p<0.001$). Our results thus underpin the assumed relationship between behavior and DNA methylation levels of genes related to the central nervous system and will hopefully contribute to our deeper understanding of the biological background of behavioral aspects.

Acknowledgements:
This work has been supported by Hungarian-Austrian collaborative funds OTKA ANN 107726 / FWF I 1271-B24 / WWTF CS11-026 and the Hungarian Research Fund OTKA K112138.

Targeted gene capture and exome sequencing identifies pathogenic variants for deafness

Maria Birkan 1, Zippora Brownstein 1, Amal Abu-Rayyan 2, Ofer Isakov 3, Fabio T.A. Martins 1, Orly Yaron 4, Noam Shomron 3, 4, Moshe Frydman 1, 5, Moien Kanaan, Karen B. Avraham1, 4

1Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; 2Department of Biological Sciences, Bethlehem University, Bethlehem, Palestine; 3Department of Cell and Developmental Biology, 4Functional Genomics Laboratory, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; 5Institute of Human Genetics, Sheba Medical Center, Tel Hashomer, Israel.

Identifying Mendelian disease genes remains a challenge in human genetics, in particular for heterogeneous pathologies such as deafness. Next Generation Sequencing (NGS), also known as Massively Parallel Sequencing (MPS), has become the optimal method to identify new deafness genes. Our approach combines Targeted Gene Capture (TGC) and MPS of 284 human deafness genes and orthologues of mouse genes associated with deafness, which enabled us to double the number of genes involved in hereditary hearing loss in the Israeli Jewish and Palestinian Arab populations (Brownstein et al 2011). In our current experiment we sequenced the exomes of deafness genes in 123 families. We captured the exonic regions of the genes using the Agilent Sure-Select Target Enrichment System and samples were multiplexed and sequenced in an Illumina HiSeq2500 at 100 bp paired-end reads. Bioinformatics analysis was used to align the sequences to the reference genome, filter out non-damaging variants and assign scores for potential pathogenicity. We

found novel variants in the genes MITF and GATA3 in patients with syndromic deafness. In addition, we found variants in ADGRV1, CDH23, CACNA1D, CLDN14, COCH, COL11A2, ESRRB, EYA4, LOXHD1, MYO6, MYO7A, MYO15A, OTOF, PAX3, SLC26A4, STRC, TECTA, TMC1, and USH2A in patients with deafness only. Functional assays are ongoing to validate the pathogenicity of the novel variants. Transfection of the SLC26A4 p.Leu117Phe variant in COS7 cells revealed mislocalization of the mutant to the endoplasmic reticulum, instead of the plasma membrane. Finally, a new gene for human deafness was identified, SLC12A6, encoding a cation-chloride co-transporter.

Spastic paraplegia 11, an example of a targeted NGSassay guided by highly suggestive clinical features.

Cristina Castro-Fernández 1,2,4, Manuel Arias Gómez 2,3, Luis Santomé Collazo1,2 , Jorge Amigo Lechuga 2,4 , Ángel Carracedo 1,2,4, Patricia Blanco-Arias, 1,2,4 Maria-Jesús Sobrido 1,2,4.

(1) Fundación Pública Galega de Medicina Xenómica-SERGAS
(2) Instituto de Investigación Sanitaria de Santiago-IDIS
(3) Servicio de Neurología – Complejo Hospitalario de Santiago de Compostela
(4) Grupo de Medicina Xenómica, Universidad de Santiago de Compostela

We report the clinical, neuroimagingand genetic characterization of a 25-year-old female with a clinical suspicion of spastic paraplegia 11 (SPG11). Hereditary spastic paraplegias(HSP) are a diverse group of disorders caused by mutations in over 70 loci and with different patterns of inheritance. SPG11is an autosomal recessive HSP caused by genetic variation in the spatacsingene, which has 100982 coding nucleotides distributed in 40 exons.The observation of atrophy of the corpus callosumin brain magnetic resonance studies can guide selectivemutation screening in HSP patients.

Methods:

Clinical methods: Clinical examination, familyhistory, brain magnetic resonance imaging (MRI) and electromyography were performed.Molecular methods:we carried out anSPG11targeted next generation sequencing study. The assay was designed using "AgilentSureSelect™ Target Enrichment System, whichextracts target regions from genomic libraries by hybridization to in-solution biotinylated cRNAprobes, or "baits".The targeted region included all coding exons and exon-intron boundaries (10bp), which were amplified by means of 231 probes using ligation-based sequencing (SOLiD platform, Life Technologies). meancoverage of the target region was921X, with 100% of the nucleotides covered ≥30X.Variant calling was performed with GATK and Lifescope 2.5.1 programs, variant annotation wascarried out with ANNOVAR(2014Feb24)and Exome Depth was used for deletion detection. The bioinformatic analysis included allele frequencies, functional conservation scores, and pathogenicity scoresby different algorithms, (SIFT, Polyphen, Mutation Taster, CADD score, and others).

Results:

We reported afemalewith a spastic paraplegiaaccompanied by pes cavus and dystonic foot position(age of onset20),family history was negative for spastic paraplegia or related neurological manifestations.Non-genetic causes were excluded through routine testing. MRIrevealed moderate cortical atrophy, pronounced corpus callosum atrophy and periventricular white matter hyperintensities. The electromyography showed a sensory motor axonal polyneuropathy.The patient was compound heterozygous for the following variants: A missense variant(NM_025137:c.6999G>C:p.Q2333H in exon 38)and a deletion involving 213 nucleotides in exon 29.Both variants were cConfirmed by Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA) analysis,respectively.

Discussion

Through highly selective, targeted NGS we have identified two novel mutations, an exonic deletion and a missense variant, confirming the diagnosis of SPG11. In silico predictions supports the pathogenicityof the p.Q2333H variant. Partial gene deletions have previously been reported in SPG11cases.Well oriented clinical diagnosisallows designinghighly specific targeted NGS assays in order to achieve robustdiagnostic results, while minimizing the risk of incidental findings. Sanger Sequencingwas the gold standard genetic diagnosis technique until recently;however with the NGS approach the entire SPG11target region can be completely covered, allowing to identifydifferent mutation types in a fast and efficient way.

Bicuspid aoRtic vAlVe gEnetic research – the BRAVE study

Radek Debiec

Bicuspid aortic valve (BAV) is the most common valvular, congenital heart defect. BAV affects around 2% of general population and shows strong familial clustering, with heritability calculated at 89%. BAV commonly associates with other congenital cardiovascular defects like aortic coarctation, ventricular septal defect or hypoplastic left heart syndrome. Despite its usually benign presentation, approximately 30% of BAV individuals develop complications and need surgical interventions within their lifetime. BAV is thought to be inherited as an oligogenic, autosomal dominant trait, but so far only limited number of causative mutations have been identified, leaving large proportion of the overall heritability unexplained.

The aim of the BRAVE study is identification and classification of genetic variants associated with predisposition to BAV.

The research team will identify and recruit patients with BAV. All first degree relatives, who agree to participate, will be screened for the presence of bicuspid aortic valve and associated congenital cardiovascular defects. Families, with at least 2 individuals affected will be enrolled in the study. A group of unrelated individuals with normal aortic valves will be recruited to enable case control analysis. In all participants blood samples will be obtained for the purpose of genetic and other molecular analyses. The main analyses will consist of identifying and cataloguing genetic variants using exome sequencing. This will be followed by application of function prediction software and statistical analyses in case-control and family-based design. Putative causative variants will be validated in-vitro by assessing their influence on gene and pathway expression.

Identification of the genes and pathways involved in the predisposition to BAV and related phenotypes will allow for more accurate clinical classification of these phenotypes. This should translate into better prediction of complications risk, allow for familial screening and genetic counselling of family members.

Best practice for use of splice variant prediction tools

Hugues Fontenelle1*, Morten C. Eike 1, Tony Håndstad 1, Espen Skorve 2, Svein Tore Koksrud Seljebotn 1, Thomas Grünfeld 1 and Dag E. Undlien 1

1Department of Medical Genetics, Oslo University Hospital, University of Oslo, Norway.
*2Department of Informatics, University of Oslo,Norway. *Correspondence: hugues.fontenelle@medisin.uio.no*

Mutations affecting pre-mRNA splicing are strong candidates when hunting for disease causing variants. A number of computational prediction tools have been developed that predict whether a variant in the splice region is deleterious.
Five of these (MaxEntScan, SpliceSiteFinder, NNSplice, GeneSplicer and HumanSpliceFinder) are included in the commercial package Alamut.
A number of guidelines have been published, but no clear consensus has been reached yet as of how to best use and combine these tools. Moreover, these recommendations have not been validated on a large set of known variants, so their accuracy is unknown.
We collected true positives (473 variants in splice region and disease causing) and true negatives (374 variants in splice region but benign) in order to determine the specificity and sensitivity of several published and unpublished strategies.
Using Machine Learning, we computed the best possible combination of these five tools. We show that, in fact, using a single algorithm is sufficient, and that previously suggested methods offer worse accuracy. Our validated recommendation is to use MaxEntScan with a -20% threshold between the scores for the ancestral allele and the variant allele (88% Sensitivity and 90% Specificity).

PERSPECTIVES OF HEALTH care and INSIghtS INTO IMPROVEMENT OF lives of children SUFFERING from Duchenne muscular dystrophy IN ARMENIA

Kristine Hovhannesyan, Artsruni Hakobyan, Artem Gasparyan, Tamara Sargsyan

The Center of Medical Genetics and Primary Health Care

The recent developments in the research of Duchenne muscular dystrophy (DMD) related to the aspects of early identification, diagnostics and management of different stages of disease manifestaions are discussed in the article. The experience in the management of 53 children with progressive muscular dystrophies, including 19 boys with DMD, stresses an important role of consolidadation of local resources for diagnostics and rehabilitation, and also need for collaboration with leading research centers in the field. Thus, precise identification of mutations at the molecular level in frames of collaborative investigation gives a big chance to potential DMD patients (after meeting inclusion criteria) to be participants in the ongoing clinical trials. Herein we emphasize the importance of early identification of primary manifesations and secondary complicaions of DMD by medical services by coordinating local multidisciplinary team to introduce modern standards of treatment, rehabilitation and care in perspective. It will allow to prolong twice the life expectancy of affected children with confirmed DMD diagnosis and to improve quality of their life as well as that of their families.

Finding eQTLs from RNA sequencing data of placental transcriptome

Kikas T 1, Sõber S 1, Rull K 1, 2, Laan M 1.

1. Human Molecular Genetics Group, Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia
2. Department of Obstetrics and Gynaecology, Tartu University Hospital, Tartu, Estonia

Background: Expression quantitative trait loci (eQTL) are SNPs, both in cis and trans positions, that can determine or alter the expression of certain genes, both in cis and trans positions; often the effect can be tissue-specific. As the gene expression changes, it can also affect the phenotype of an individual. It has been shown that in preeclampsia and other pregnancy complications, there are several changes in gene expression of placenta [1, 2]. Therefore it would be very important to determine the eQTLs in placenta which has not been done yet.

Aims of this study: Firstly, to establish a pipeline for finding eQTLs in placental RNAseq data by focusing on cis-eQTLs of genes that were differentially expressed in term pregnancy complications (preeclampsia, gestational diabetes, small and large for gestational age babies). Secondly, to conduct the whole genome screen with an aim to identify placental cis- and trans-eQTLs.

Methods: We utilized two datasets generated from term placentas: a) RNA transcriptome data sequenced in cooperation with Institute for Molecular Medicine Finland, University of Helsinki, on a Illumina Hiseq 2000 platform [1], b) SNP genotyping data using Illumina HumanOmniExpress-12-v1, genotyped by in-house institutional core facility (n=40). Cis-eQTLs were defined by us as SNPs 10kB up- or downstream from the

gene. For validating chosen SNPs we used Taqman RT-qPCR with expanded samples (n=72). For analysing cis-eQTLs and screening whole genome for eQTLs we have used linear regression with PLINK 1.07 software [3]

Results: From first part of our study, I have determined 29 SNPs with a p-value <0.05 before FDR correction. For the downstream analysis we five SNPs were selected, considering p-values and genomic context of the SNP. We were able to validate two SNPs with Taqman RT-qPCR: rs11697869 (p-value = 2.76E-06) and rs216259 (0.001743) were significantly associated with gene expression of TMEM74B and FAM65B, respectively. From the pilot whole genome analysis we have determined 125 SNPs (FDR p-values range 1.73·10-14-2.69·10-4) that are associated with the placental expression of 23 genes.

Conclusions: For the first time we have conducted a placental eQTL study and we were able to observe significally associated SNPs across genome. In perspective these eQTLs may be developed further as molecular diagnostics markers for predicting risks of pregnancy complications.

References:
1. S Sõber, M Reiman, T Kikas, K Rull, R Inno, P Vaas, P Teesalu, JM Lopez Marti, P Mattila, M Laan. Profile of placental transcriptome in normal and complicated pregnancies. Submitted

2. V Sitras, C Fenton, G Acharya, 2014; Gene expression profile in cardiovascular disease and preeclampsia: A meta-analysis of the transcriptome based on raw data from human studies deposited in Gene Expression Omnibus. Placenta vol. 36 (2) p. 170-8

3. S Purcell, B Neale, K Todd-Brown, L Thomas, MAR Ferreira, D Bender, J Maller, P Sklar, PIW de Bakker, MJ Daly, PC Sham, 2007; PLINK: a toolset for whole-genome association and population-based linkage analysis. American Journal of Human Genetics, 81.

Targeted NGS for analysis of craniosynostosis identifies a novel mutation in MEGF8

König E.-M. 1, Spranger S. 2, Schweitzer T. 3, Kress W. 1, Klopocki E. 1

1Institute of Human Genetics University of Wuerzburg, Germany
2Praxis fuer Humangenetik, Bremen, Germany
3Department of Pediatric Neurosurgery University of Wuerzburg, Germany

Craniosynostosis is a frequent craniofacial malformation affecting 1 in 2500 newborns and is defined as the premature fusion of one or more cranial sutures. Premature fusion of the cranial sutures can occur either as isolated malformation in non-syndromic craniosynostoses or as part of a syndrome. So far genetic causes have been identified mainly for syndromic craniosynostoses, i.e. mutations in *FGFR2, FGFR3, TWIST1,* and *EFNB1*. However, in more than 50% of cases the underlying genetic cause remains unknown.

We compiled a next generation sequencing (NGS) gene panel comprising 68 genes. In addition to known and candidate craniosynostosis genes of the syndromic and isolated type, the panel includes downstream targets of participating signaling pathways and genes associated with bone development. Target enrichment was performed by the Nextera Rapid Capture Enrichment kit of Illumina and sequencing was done on the

Illumina MiSeq platform. Sequencing data were analysed with the NextGENe software. Performance of the NGS gene panel was validated by sequencing 5 control patients with known mutations. All of these mutations were detected correctly. Subsequently, we sequenced DNA of 25 patients with syndromic as well as isolated craniosynostosis. Two patients are siblings and children of consanguineous parents. Both patients show an a-typical Carpenter phenotype with sagittal craniosynostosis. We identified in both a novel homozygous splice site mutation (c.828G>A) in *MEGF8* leading to a predicted loss of the splice donor of exon 5. The mutation was confirmed by Sanger sequencing. Mutations in *MEGF8* were shown to be associated with Carpenter syndrome 2 (MIM 614976).

Application of Next-generation Sequencing for Molecular Diagnosis of Hereditary Breast Cancer Associated with BRCA 1 & 2 genes

Barathy Logendra 1, Shruti Bhagat 1**,** Ramin Badii 1, Ronald G Crystal 2, Ajayeb Al-Nabe t1

BRCA1 and BRCA2 are the best known breast cancer genes with high incidences in several ethnic groups including Qatari population. So far we have used Sanger DNA sequencing for mutation analysis of these genes, which involves bidirectional sequencing of 57 exons. Here we present our sequencing results by using the BRCA1 and BRCA2 Ion AmpliSeq™ panel on the Ion Torrent™ personal genome machine (PGM). For validation purposes we have used 40 samples including 16 positive controls from Coriell Institute with confirmed unique pathogenic mutations. Eight samples at a time were sequenced in parallel on a 316 chip resulting in 100% coverage at 100X. Five samples at random were further chosen to establish reproducibility and all variants were found to be reproducible. The resulting next-generation sequencing test is a highly efficient platform producing reliable data at a significantly lower cost and reduced turnaround time.

[1]Department of Laboratory Medicine and Pathology, Hamad Medical Corporation, Doha, Qatar
[2]Department of Genetic Medicine, Weill Cornell Medical College, New York, USA

Rapid and low cost next generation sequencing approach for routine diagnosis of cystic fibrosis

Barathy Logendra 1, Shruti Bhagat 1**,** Ramin Badii 1, Ronald G Crystal 2, Ajayeb Al-Nabet 1

Qatari population is highly endogamous with the consanguinity rate of 54%, resulting in a higher frequency of autosomal recessive disorders including Cystic Fibrosis (CF). CF is a chronic, life-shortening disease that occurs as a result of genetic variations in *CFTR*. Currently, our approach to CF diagnosis is by full CFTR exon sequencing by using the Sanger method, which is laborious and expensive with a turnaround time (TAT) of 4 weeks. This study aims to develop and validate a robust and cost-effective next generation sequencing (NGS) method which will help reduce the TAT to 1 week. 56 samples that were previously

sequenced by the Sanger method were re-sequenced by using the Ion Torrent™ personal genome machine (PGM) on the 316 chip. All the variants were accurately identified with 100X coverage except the homo polymer region (IVS8). Alternate software's will be used for further verifications of the bioinformatics data. The higher throughput data and efficiency of Ion Torrent PGM sequencing and other NGS platforms has begun to replace the traditional Sanger sequencing which will allow clinicians to treat the patients in a timely manner.

[1]Department of Laboratory Medicine and Pathology, Hamad Medical Corporation, Doha, Qatar
[2]Department of Genetic Medicine, Weill Cornell Medical College, New York, USA

Maintenance of the methylation pattern on fresh and cultured Chorionic Villi (CV) in normal and Beckwith Wiedemann Syndrome (BWS)-suspected pregnancies

Leda Paganini 1, Nicole Carlessi 1, Sara Giangiobbe 1, Rosamaria Silipigni 2, Silvana Guerneri 2, Faustina Lalatta 3, Anna Cereda 4, Silvia Sirchia 5, Monica Miozzo 1, Silvia Tabano 1

[1] *Division of Pathology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico; Dept of Pathophysiology & Transplantation, Università degli Studi di Milano, Milano, Italy.*
[2] *Medical Genetics Laboratory, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy.*
[3] *Clinical Genetics Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy.*
[4] *Pediatric Unit, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy.*
[5] *Medical Genetics, Department of Health Sciences, Università degli Studi di Milano, Milano, Italy.*

Presenting author: Leda Paganini (leda.paganini@unimi.it)

Corresponding author: Silvia Tabano (silvia.tabano@unimi.it)

BWS is an imprinting-related disorder that can be prenatally suspected following established clinical guidelines. Molecular confirmation is commonly performed on amniocytes and the possibility to use fresh (CVF) and cultured (CVC) CV should be proved since embryonic and extra-embryonic compartments usually have different methylation profiles.

To verify whether CVF and CVC are eligible sources of DNA, we tested by pyrosequencing in normal pregnancies the methylation percentage at: ICR1, ICR2, H19 promoter (imprinted locus 11p15.5), PWS/AS-ICR (imprinted locus 15q11-13), MGMT and RASSF1A (not imprinted gene). We highlighted stable methylation levels at the imprinting-driving regions ICR1 (CVF: 45.38% ± 1.77; CVC: 45.04% ± 1.81), ICR2 (CVF: 44.32% ± 1.84; CVC: 43.67% ± 2.10) and PWS/AS-ICR (CVF: 43.70% ± 5.60; CVC, 43.15% ± 3.41). Conversely, H19 promoter was severely hypomethylated at both CVF (11.33% ± 1.92) and CVC (19.30% ± 4.30), and showed a significantly increased methylation after culture. In two unrelated and

biallelic genes, the methylation remained stable at MGMT promoter (CVF: 2.06% ± 0.48; CVC: 2.16% ± 0.53) and changed at RASSF1A (CVF: 52.50% ± 7.33; CVC: 28.00 ± 11.10).

As second step, we investigated ICR1 and ICR2 methylation level on both fresh (CVF) and cultured (CVC) chorionic villi of two BWS-suspected fetuses (P1 and P2). P1 showed hypomethylation at ICR2 both in CVF and CVC (CVF: 17.63% ± 0.88; CVC: 16,13% ± 0.18); P2 showed normal methylation profiles.

Taken together these findings suggest that: i) ICR1 and ICR2, but not H19, are reliable targets for BWS prenatal methylation test in CV also after culture; ii) similarly, PWS/AS-ICR is steadily hemimethylated in CV from healthy pregnancies, independently from culture. Thus, methylation analysis of these regions represents a very useful tool for prenatal diagnosis of imprinting related syndromes.

Funding: this work was supported by intramural grant "Ricerca Corrente 2014-2015", Foundation IRCCS Policlinico, Mangiagalli, Regina Elena.

Various bioinformatics pipelines used for data from singleplex-PCR enrichment method

Martin Reboun 1, Josef Vcelak 2, Sarka Dvorakova 2, Vlasta Sykorova 2, Lenka Dvorakova 1

1 Institute of Interited Metabolic Disorders Ke Karlovu 455/2 128 08 Prague 2, Czech Republic
2 Institute of Endocrinology Narodni 8 116 94 Prague 1, Czech Republic

We would like to introduce molecular diagnostic perfotrmed in three pacients with different diagnoses. Gene defect of these patients was determined by other methods and NGS was used for detailed description of these cases. We used a flexible workflow of singleplex-PCR (PCR, Long PCR, RT-PCR) for highly efficient target enrichment powered by Nextera XT kits and MiSeq instrument (Illumina). This approach allows cheap and fast simultaneous detection of SNPs and other variants in DNA as well as in cDNA.

In the first patient we have detected a large deletion covering both exons of TIMM8A and exon 19 of BTK gene. This deletion was detected by qPCR method. Determination of deletion boundaries by Sanger sequencing failed. To solve this problem we used NGS analysis. The results were evaluated by NextGENe (Softgenetis) and TopHat Fusion software.

The second case is a pediatric patient with papillary thyroid cancer (PTC). PTC is often caused by the formation of RET/PTC rearrangements. Primary variant detection was performed by qPCR. Using NGS analysis we have discovered a novel RET/PTC1 variant: a fusion of exon 1 of CCDC6 with exon 9 of extracellular domain of RET directly followed by exon 12 of RET was revealed by TopHat Fusion and NextGENe software.

NGS was used of analysis of the X chromosome inactivation in three heterozygotes for X-linked disorders. Exonic SNPs in LAMP2 and IDS were used for transcription-based assay. Percentages of alleles in genomic DNA and cDNA were evaluated by MiSeq Reporter and TopHat, respectively. The results of X-inactivation skewing obtained by this method were compared with methylation status analysis in androgen receptor gene.

Bringing NGS to NeuroGeneticS

Ribeiro C 1, Silva P 1, Sequeiros J 1,2, Alonso I 1,2

1CGPP, Instituto de Biologia Molecular e Celular (IBMC) and Instituto de Investigação e Inovação em Saúde (i3S), and 2Instituto de Ciências Biomédicas Abel Salazar (ICBAS); Universidade do Porto, Portugal

The worldwide success of next-generation sequencing (NGS) in research and clinical laboratories, the advent of affordable bench-top NGS machines and an increased knowledge on the genetics of disease, have revolutionized the molecular diagnosis field. The validation process of NGS protocols comprises several technically challenging steps. So far, few guidelines have been established in an effort to harmonize the validation methodology; however, a general consensus has not been achieved yet. Therefore, each laboratory must tailor their approach to tackle their specific challenges.

CGPP is a leading expert laboratory in the field of genetic diagnosis of neurological disorders, and the only one accredited by ISO15189 in Portugal. In this context, we are dedicated to upgrade our methodologies to NGS. Here, we describe the strategy chosen to assess the validity of this technology to be implemented in the diagnostics routine.

Currently our standardized approaches to diagnostics integrate homemade and Ion Ampliseq DNA custom panels (Life Technologies). Our first line approach was to select and sequence amplicons harbouring disease-causing mutations previously identified by Sanger sequencing, representing the most common disorders diagnosed at our lab. A panel of 71 unique mutations, from 40 different genes, was screened. All amplicons were amplified with conventional primers, pooled and sheared during library preparation, to be sequenced on Ion-PGM, with a minimum coverage of 40x. Sequencing data was analysed from FASTQ files, screened using three software packages (JSI SeqNext, DNASTAR Lasergene and Torrent Suite Server/ Ion Reporter) and compared in terms of their capability to detect the selected mutations. More recently, in order to improve the performance of sequencing and/or bioinformatics pipeline, our second line approach to validate panels was to sequence a DNA reference material from Coriell (NA12878 DNA).

The analysis with JSI proved to be the most reliable in identifying all the mutations previously found and the most effective in integrating all the steps for data analysis. Our results showed that NGS is a better methodology to identify large deletions/insertions and has a higher sensitivity to detect somatic mutations. Small indels in homopolymeric regions and strand bias are challenging aspects to be taken in consideration for the design of an analysis pipeline.

We have tested and established a workflow to implement NGS into our molecular diagnostic routine and designed a pipeline for bioinformatics analysis that may be constantly improved.

Significant expressivity of Wolfram syndrome: report of a novel mutation and phenotypic assessment of two known mutations in the WFS1 gene in the Iranian patients

Maryam Sobhani[1], Mohammad Amin Tabatabaiefar[2], Asadollah Rajab[4], Abdol-Mohammad Kajbafzadeh[5], Mohammad Reza Noori-Daloii[1]

1- *Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
2- *Department of Medical Genetics, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
3- *Iranian Diabetes Society ,Tehran, Iran*
4- *Pediatric Urology Research Center, Department of Pediatric Urology, Children's Hospital Medical Center, Tehran University of Medical Sciences, Tehran, Iran*

Purpose: Wolfram syndrome also known as DIDMOAD (Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, and Deafness) is a rare neurodegenerative autosomal recessive disorder. There is evidence of variable expressivity both in patients and heterozygous carriers. In this study we describe three Persian Wolfram syndrome families with differences in the age of onset, signs and symptoms of the disease.

Method: We clinically evaluated affected families for verifying WS clinical diagnosis. After linkage analysis via 5 STR markers, molecular analysis for WFS1 was performed by direct sequencing for patients and available family members.

Results: Three homozygous mutations were identified including c.1885 C >T, c. 2205C>A both in exon 8 and c. 460+1 G>A in intron 4. The mutation c. 2205C>A was found to be novel. We report interesting phenotype-genotype correlation: homozygous c.1885 C >T and c. 2205C>A variants were correlated with quite different disease severity and onset in the siblings. We report a rare case of WS with homozygous c.1885 C >T who is married and has a healthy child. c.460+1 G>A showed a possible partial dominant inheritance put forth by a heterozygous parent showing partial WS symptoms while her daughter displayed typical WS symptoms.

Conclusion: Due to variable expressivity, molecular diagnostics should be used to confirm WS and a more exact recurrence risk data.

NEFROCHUS: FIRST DIAGNOSTIC/PROGNOSTIC TESTS FOR ALL HEREDITARY KIDNEY DISEASES

Lisbeth Sousa Silva, Lara Besada Cerecedo, Olaya Lamas Gonzalez, Beatriz Sobrino, Patricia Regueiro Casuso, Jorge Amigo Lechuga, Angel Carracedo, Cándido Díaz Rodriguez, Miguel A. García-González, et al.

1 Group of Genetics and Developmental Biology of Renal Diseases. Health Research Institute of Santiago de Compostela (IDIS), Spain.
2 Galician Public Foundation of Genomic Medicine, Santiago de Compostela, Spain.
3 Department of Nephrology, University Hospital Complex of Santiago de Compostela, Spain

Our group is mainly focused on the study of hereditary kidney diseases. These diseases are associated with mutations in a large number of genes. Sanger is a traditional and reliable method for sequencing, but new generation sequencing (NGS) has improved this method in a time and cost effective manner. Recently, we have developed new rapid genetic tests for all renal hereditary disorders in three main groups, polycystic kidney disease (PKD), tubulophaties and glomerulophaties. The Haloplex technology allows the preparation of libraries with barcodes that can be mixed and loaded into a single Ion chip to streamline workflow and enable an effective comparison sample to sample. We can screen in a single Ion chip multiple genes and several patients reducing, even more, the price and the time of genetic diagnosis.

We have developed a strategy of four panels/tests have, including all known genes related to hereditary renal disorders:

- Panel for Common Cystic Diseases: This panel includes 8 genes associated with the most prevalent form in the population of PKD, and therefore more likely to be responsible for the disease. This can be applied for patients with family history of cystic disease.
- Diagnostic/prognostic Panel for all Cystic Diseases: This panel includes the complete list of 72 genes associated with cystic disease. This can be applied for patients lacking family history or when diagnosis/prognosis is needed.
- Diagnosis/prognosis Panel for all glomerular kidney diseases: Includes the sequencing of 26 genes associated with hereditary glomerular disease.
- Diagnostic/prognostic Panel for all tubular kidney diseases: Includes the sequencing of 36 genes associated with hereditary tubulo/interstitial disease.

The in silico design of these tests predict a coverage of 98.50% of the target region (coding and flanking exons) for the panel of common cystic disease, 99.06% for the panel including all cystic diseases, 99.97% for the panel including all glomerular kidney diseases, 99.70% for the diagnostic/prognostic panel for all tubular kidney disease. We have tested the optimal real depth coverage for each individual panel, obtaining an optimal real coverage of 96.4% (n=64), 96.8% (n=22), 98.47% (n=27), 98.13% (n=26), respectively for each panel at >30X of depth read. We have also developed an algorithm that allow us to reduce the false positives associated to this technology, and abolish possible false negatives, critical for clinical decision. In addition, this algorithm helps us to dramatically reduce the costs for mutation validation by Sanger.

Here we present the first genetic strategy for the diagnosis/prognosis of all hereditary renal disorders in cost/efficient manner.

Next-Generation Sequencing based investigation of a family with unknown cancer predisposition

Julia Vogt, Christine Fauth, Katharina Wimmer
Medical University Innsbruck, Department of Human Genetics, Innsbruck, Austria

Germline mutations that occur in tumor suppressor genes or oncogenes, are responsible for familial cancer syndromes which account for about 5-10% of all types of cancer. Clustering of a specific tumor spectrum within a family and/or early onset of cancer in an individual or several relatives within the family may be indicative for a cancer pre-disposition syndrome. A family with a high index of suspicion for a cancer pre-disposition syndrome was counselled and investigated in our department. Three siblings were affected by a wide spectrum of tumor entities including an embryonal germ cell carcinoma of the testis, a Hodgkin's lymphoma and an osteosarcoma. All patients were diagnosed before or at the age of 20 years. The observed combination of tumor entities is very unusual and if at all overlapped best with the tumor spectrum described in Li-Fraumeni syndrome patients. Intensive target gene mutation analysis in this family was however unable to uncover a TP53 mutation, which is causative of Li-Fraumeni syndrome. Therefore, we propose to perform a Next-Generation Sequencing based approach, starting with panel sequencing of 94 genes and 284 SNPs known to be associated with cancer pre-disposition in order to identify a candidate gene that is responsible for the clustering of this unusual combination of tumors in the brothers. If we do not identify a candidate gene by this approach, we would continue the search with whole exome sequencing that has a larger target region. The data will be analyzed under two different hypotheses basedon the suspected mode of inheritance. The aim of this explorative study is to identify a (novel) gene with a pathogenic variant that might cause the tumor pre-disposition in the family and it will set the basis for further studies aiming at the confirmation of the role of the identified gene as a cancer pre-disposition gene.

STUDENTS WHO IS WHO

SURNAME & NAME	AFFILIATION	NATIONALITY	CITY	E-MAIL
ALAQEEL AHMAD	Kuwait Medical Genetic Center	KUWAIT	KUWAIT CITY	aaqeel1976@yahoo.com
ALBALOOL HAYA	Kuwait Medical Genetic Center	KUWAIT	KUWAIT CITY	hhalbalool@hotmail.com
ALESI VIOLA	Osp. S. Pietro Fatebenefratelli Roma	ITALY	ROME	violaalesi@gmail.com
ALEXANDER JOHN	Democritus Univ. of Thrace	GREECE	ALEXANDROUPOLIS	jalexand@mbg.duth.gr
ALSAEDI ATIF	University of Birmingham	UK	BIRMINGHAM	asa195@student.bham.ac.uk
AL-SHARSHANI MOHAMMED	Hamad Medical Corp	QATAR	DOHA	malsharshani@hamad.qa
BADICEAN DUMITRU	Institute of Mother and Child	REP. OF MOLDOVA	CHISINAU	dbadicean@yahoo.com
BANLAKI ZSOFIA	Semmelweis University	UNGHERIA	BUDAPEST	banlaki.zsofia@med.semmelweis-univ.hu
BARESIC ANNA	Institute of Anthropological Research	CROATIA	ZAGREB	ana.baresic@inantro.hr
BARREDA SANCHEZ MARIA	Univ. Catolica de Murcia - UCAM	SPAIN	MURCIA	mbarreda@ucam.edu
BHAGAT SHRUTI	Hamad Medical Corp	QATAR	DOHA	shruti.b1491@gmail.com
BIRKAN MARIA	Tel Aviv University	ISRAEL	BEIT SHEMESH	birkan.maria@gmail.com
BORG CARBOTT FRANCESCA	Univ. of Malta	MALTA	SLIEMA	francesca.borgcarbott@gmail.com
BUENA ATIENZA ELENA	Institute of Ophthalmic Research	GERMANY	TUEBINGEN	elenabuena3@gmail.com
CAMELI CINZIA	Univ. of Bologna	ITALY	CARASSAI	cinzia.cameli3@unibo.it
CASTRO FERNANDEZ CRISTINA	Galician Foundation of Genomic Medicine	SPAIN	SANTIAGO DE COMPOSTELA	cristinacastrofdez@gmail.com
CHOUEIRY ELIANE	Universite Saint Joseph	LEBANON	BEIRUT	eliane.chouery@usj.edu.lb
COHEN LIOR	Rabin Medical Centre	ISRAL	TIKVA	liorco1@clalit.org.il
DARMANI ELEANNA	Eurogenetica sa	GREECE	THESSALONIKI	ddarmanii@yahoo.gr
DEBIEC RADOSLAW	Univ. of Leicester	UK	LEICESTER	rmd24@le.ac.uk
FONTENELLE HUGUES	Oslo University Hospital	NORWAY	OSLO	hugues.fontenelle@medisin.uio.no
FRANCISCO DAVID	Instituto Medicina Molecular	PORTUGAL	LISBOA	dfrancisco@medicina.ulisboa.pt

SURNAME & NAME	AFFILIATION	NATIONALITY	CITY	E-MAIL
FRIDMAN HILA	Shaare Zedek Medical Center	ISRAEL	LOD	hilaf@szmc.org.il
GILDE BODIL	St. Olavs Hospital HF	NORWAY	TRONDHEIM	bodil.gilde@stolav.no
GULLANDER JACOB	Univ. and Regional laboratories Dept. Of Clinical Genetics	SWEDEN	LUND	jacob.gullander@skane.se
HOFVANDER JAKOB	Dept. of clinical genetics	SWEDEN	LUND	jakob.hofvander@med.lu.se
Hovhannesyan Kristine	Centre of Medical Genetics and Primary health care	ARMENIA	YEREVAN	kristgen@yahoo.com
JALKH NADINE	Universite Saint Joseph	LEBANON	BEIRUT	nadine.jalkh@usj.edu.lb
KERAMATIPOUR MOHAMMAD	Tehran Univ. of Medical Science	IRAN	TEHRAN	keramatipour@sina.tums.ac.ir
KIKAS TRIIN	University of Tartu	ESTONIA	TARTU	triinc7@gmail.com
KIRKEVOLD MARTE	Oslo University	NORWAY	OSLO	kirkma@ous-hf.no
KONIG EVA-MARIA	Univ. Wurzburg	GERMANY	WURZBURG	eva-maria.koenig@uni-wuerzburg.de
LITWIN MONIKA	Wroclaw Specialist Hospital	POLAND	WROCLAW	litwin@wssk.wroc.pl
LLAVONA JUEZ PABLO	Institute of Ophthalmic Research	GERMANY	TUEBINGEN	pablo.llavona-juez@med.uni-tuebingen.de
LOGENDRA BARATHY	Hamad Medical Corp	QATAR	DOHA	blogendra@hmc.org.qa
MARTINEZ ROMERO CARMEN	Hospital Clinico Universitario Virgen de la Arrixaca	SPAIN	MURCIA	mcarmen.martinez16@carm.es
MAURUS LOCHER	Univ. of Geneva	SWITZERLAND	GENEVE	maurus.locher@unige.ch
MICHALOWSKA DAGMARA	Wroclaw Specialist Hospital	POLAND	WROCLAW	michalowska@wssk.wroc.pl
NAOUFAL RANIA	CHU de PoitIers	AUSTRIA	POITIERS	rania.naoufal@gmail.com
NASRALLA HANAN	Hamad Medical Corp	QATAR	DOHA	hnasralla@hmc.org.qa
NOUSPIKEL THIERRY	Geneva Univ. Hospital	SWITZERLAND	GENEVA	thierry.nouspikel@hcuge.ch
OSTERN RUNE	St. Olav Univ. Hospital	NORWAY	TRONDHEIM	rune.ostern@stolav.no
PAGANINI LEDA	University of Milan	ITALY	MILAN	leda.paganini@unimi.it
PECA LOREDANA	Univ. of Medicine and Pharmacy Iuliu Hatieganu	ROMANIA	CLUJ NAPOCA	danapeca@gmail.com
PESSINA CHIARA	Hospital of Varese	ITALY	MILANO	chiara.pessina22@yahoo.it
PIRIM DILEK	University of Pittsburgh	TURKEY	YALOVA	dip16@pitt.edu
PONTEN ANNICA	Dept. Of Clinical Pathology	SWEDEN	HALMSTAD	annica.ponten@regionhalland.se
PORENTA GEROLD		AUSTRIA	WIEN	porenta@cardiocare.at
REBOUN MARTIN	Univ. of Prague	CZECH REP.	PRAGUE	martin.reboun@lf1.cuni.cz

SURNAME & NAME	AFFILIATION	NATIONALITY	CITY	E-MAIL
RIBEIRO CHEILA	Wide Travel	PORTUGAL	LISBOA	Cheila.Ribeiro@ibmc.up.pt
SILVA PAULO FILIPE	Instituto de Biologia Molecular e Celular	PORTUGAL	PORTO	paulo.silva@ibmc.up.pt
SOBHANI MARYAM	Iranian Blood Tranfusion org.	IRAN	TEHRAN	msobhani@alumnus.tums.ac.ir
SOUSA SILVA LISBETH ELENA	Group of Genetics and Dev. Biology of Renal Diseases	SPAIN	AMES	lisbeth.silva.88@gmail.com
STARCEVIC CIZMAREVIC NADA	Faculty of Medicine, Dept. Of Biology and Medical genetics	CROATIA	RIJEKA	nadasc@medri.uniri.hr
VALO SATU	University of Helsinki	FINLAND	HELSINKI	satu.valo@helsinki.fi
VAN GHELUE MARIJKE	Univ. Hospital North Norway	NORWAY	KVALLOYSLETTA	marijke.van.ghelue@unn.no
VOGT JULIA	Institute of Human Genetics	AUSTRIA	INNSBRUCK	julia.vogt@i-med.ac.at
VOLD TRINE	St. Olavs Hospital HF	NORWAY	TRONDHEIM	trine.vold@stolav.no
VRANEKOVIC JADRANKA	Faculty of Medicine, Dept of Biology and Medical genetics	CROATIA	RIJEKA	jadranka.vranekovic@medri.uniri.hr
WERNSTEDT ANNEKATRIN	Humangenetics Innsbruck	AUSTRIA	INNSBRUCK	annekatrin.wernstedt@i-med.ac.at
WHITE SUE	Victorian Clinical Genetics Services	AUSTRALIA	MELBOURNE	sue.white@vcgs.org.au
ZEINEB BAKEY	Human Genetics Dept	THE NETHERLAND	NIJMEGEN	zeineb.bakey@radboudumc.nl

FACULTY WHO IS WHO

Family Name	Name	Affiliation	e-mail
De Franco	Elisa	University of Exeter Medical School, UK	E.De-Franco@exeter.ac.uk
Gerstung	Moritz	Cancer Genome Project Wellcome Trust Sanger Institute Wellcome Trust Genome Campus Hinxton, Cambridgeshire, UK	mg14@sanger.ac.uk
Gilissen	Christian	Department of Human Genetics - Nijmegen Centre for Molecular Life Sciences- Radboud University Nijmegen Medical Centre, the Netherlands	c.gilissen@antrg.umcn.nl
O'Halloran	Jonathan	QUANTUMDx GROUP LIMITED, Newcastle, U.K	Jonathan.OHalloran@quantumdx.com
Parker	Matthew	Genomics England - Queen Mary's University London, UK	matthewparker@genomicsengland.co.uk
Pippucci	Tommaso	University of Bologna, Italy	tommaso.pippucci@unibo.it
Rauch	Anita	University of Zurich, Institute of Medical Genetics, Schlieren-Zurich, Switzerland	anita.rauch@medgen.uzh.ch

Family Name	Name	Affiliation	e-mail
Robinson	Peter	Freie Universität Berlin Institut für Medizinische Genetik und Humangenetik Charité - Universitätsmedizin Berlin Augustenburger, Germany	peter.robinson@charite.de
Romeo	Giovanni	University of Bologna, Italy & European School of Genetic Medicine	egf.giovanni.romeo@gmail.com
Sidore	Carlo	CNR, Institute of Genetics Research, Sassari, Italy	scarlino81@gmail.com
Spielmann	Malte	Institut für Medizinische Genetik und Humangenetik Charité - Universitätsmedizin Berlin- Max Planck Institute for Molecular Genetics Research Group, Germany	spielman@molgen.mpg.de
Veltman	Joris	Department of Human Genetics- Nijmegen Centre for Molecular Life Sciences Radboud University Nijmegen Medical Centre, the Netherlands	J.Veltman@antrg.umcn.nl
Voet	Thierry	Department of Human Genetics, Leuven, Belgium & Sanger Institute- EBI Single-Cell Genomics Centre, Wellcome Trust Sanger Institute, Hinxton, UK.	Thierry.Voet@med.kuleuven.be