European School of Genetic Medicine

27th Course in

Medical Genetics

Bertinoro, Italy, May 11-15, 2014

Bertinoro University Residential Centre
Via Frangipane, 6 – Bertinoro

Course Directors:

H. Brunner (Nijmegen, The Netherlands), G.Romeo (Bologna, Italy), B.Wirth (Cologne, Germany)

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Departure

An application has been made to the EACCME for CME accreditation of this event.
*The application for European accreditation has been granted **27 European CME credits** (ECMEC) by the European Accreditation Council for Continuing Medical Education (EACCME).*

ABSTRACTS OF LECTURES

Sunday, May 11

Medical Genetics Today

Dian Donnai
University of Manchester , Faculty of Medical & Human Sciences - Manchester, UK

Medical Genetics today is built on a distinguished history of clinical, scientific and technological contributions. Over the 60 years since the discovery of the structure of DNA and the ~ 40 years since the introduction of chromosome analysis for diagnostic purposes an increasing range of services has been available to benefit patients with genetic disorders and their families.

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

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

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

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

shown to be due to mutations in functionally related genes perhaps amenable to treatment by similar molecules.

Over the past two 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 http://www.ddduk.org/intro.html) or targeted testing using large panels of appropriate genes being offered to patients with specific disorders such as retinal dystrophy, cataract, epilepsy etc. (O'Sullivan, 2012 Rehm 2013). Interestingly results of diagnostic applications of NGS indicate that there is a much wider phenotypic spectrum associated with mutations in many genes than was suspected from initial clinical definition and Sanger sequencing. Concerns have been expressed about the ethical aspects of NGS but as experience deepens most centres are finding ways of addressing these in conjunction with patient groups (Bredenoord et al 2013).

Some may argue that Medical Genetics as a clinical/laboratory 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, other professionals in health and social and in education.

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

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

The role of clinicians and scientists in Medical Genetics departments are likely to change. Certainly we will be called upon to educate our colleagues in other specialties and engage more with patient groups and the public. However our clinical roles are also likely to change; we may for example have treatments for some of the conditions we already see, we may be involved in disease stratification as part of

multidisciplinary teams involved in clinical trials The time has certainly come to change the name of our specialty from Medical Genetics to Genetic or Genomic Medicine.

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Genotypes and phenotypes

Han G. Brunner

Department of Human Genetics University Hospital Nijmegen, the Netherlands

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

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

I shall discuss the following categories:

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

       Similar allelic variation is present for cystic fibrosis, for spinal muscular atrophy, for hemophilia, and for many other genetic diseases. This means that in some families who have a milder or more severe form of a genetic disease the prognosis may be very different from what the textbooks say.
    b. Opposite phenotypes may occur if some mutations activate, and others inactivate the same gene. As an example I shall discuss activating mutations of the luteinizing hormone receptor gene which cause early puberty in males, and inactivating mutations which cause Leydig cell hypoplasia. Activating mutations of the RET gene cause thyroid tumors (FMTC, and MEN2B), while inactivating mutations cause Hirschprung's disease.
    c. Sometimes, mutations affect different functional domains within a gene. If this is the case, then the resulting phenotypes may be markedly different.

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

2. Two or more genes cause the same phenotype. This is called genetic heterogeneity.It appears to be very common, and is usually due to the fact that different genes encode components of a

multiprotein complex, or a receptor and its ligand, or different components of a biochemical or cellular pathway.

a. As an example, several genes that cause Fanconi anemia encode proteins that form part of a single complex that functions in DNA repair. Many other examples exist. It is likely that all Usher syndrome genes interact with each other in the cell.
b. The Walker Warburg syndrome can be caused by mutation of either the POMT1, POMT2, FUKUTIN, or FKRP genes. All genes encode proteins that function in glycosylation of target proteins in brain and mucle such as alpha-dystroglycan. Here, the phenotypic similarity is explained by the loss of the same biochemical function in the cells.

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

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

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Introduction in Next Generation Sequencing technologies and applications

Joris A. Veltman

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

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

Recommended reading:

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2. Boyd SD. Diagnostic applications of high-throughput DNA sequencing. Annu Rev Pathol 8: 381-410 (2013). Review.
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5. 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. Diagnostic Exome Sequencing in Persons with Severe Intellectual Disability. N Engl J Med. 367: 1921-1929 (2012).

How to deal with next generation sequencing output

Christian Gilissen

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

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

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

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

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

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

Monday, May 12

Linkage and association (in a conceptual and historic perspective)

Andrew Read

University of Manchester,

Human Development and Reproductive Health Academic Group , UK

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

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

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

Arrays and CNVs

Joris Vermeesch

Katholieke Universiteit Leuven - Department of Human Genetics
Leuven, Belgium

In addition to single nucleotide variation, copy number variations (CNVs) are recognized as another major class of variants. In this session the molecular mechanisms inducing CNVs will be explained. In addition, the clinical consequences of CNVs, the clinical interpretation pipeline and the mechanisms by which CNVs can exert a phenotypic effect will be discussed. Finally, the different methodologies used to detect CNVs will be analysed.

Resources

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Basic Concepts in Dysmorphology and Syndrome classification

Dian Donnai and Jill Clayton-Smith

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

What is dysmorphology?

David Smith from the USA first used the term "dysmorphology" in the 1960's to describe the study of human congenital malformations and patterns of birth defects. The subject is broad, requiring knowledge in many areas, from embryology, through a wide range of clinical disciplines to genetic counselling. Dysmorphology is a specialty within Medical Genetics dealing with people who have congenital malformations. As well as the benefits for families the study of malformations can also help to identify mechanisms underlying normal development.

Why study dysmorphology?

Dysmorphologists are regarded by some as mere "collectors" of rare syndromes. In fact there are several good reasons for pursuing the study of dysmorphology that directly benefit patients. A syndrome diagnosis can be helpful for the individuals and families concerned because it can help to answer their questions and in some cases the individual concerned will be spared further, possibly invasive investigations to determine the cause of their problems. The parents of a baby with birth defects usually have many questions:

- What is the problem?
- Why did it happen?
- What will it mean for our baby?
- Will it happen again?

A dysmorphologist will be able to answer many of these questions for the family. Whilst most families value having a diagnosis for their child's problems, a few families find it difficult to have a "syndrome label" attached to their child. The dysmorphologist needs to be sensitive to these concerns when dealing with the family.

Benefits of Syndrome Diagnosis

- Provision of accurate information about the condition, its natural history and its prognosis to parents and professionals involved in the care of the baby.
- Often influences the management of the baby e.g. it may direct further investigations or screening for complications
- Facilitates accurate genetic counselling, especially as regards prognosis, recurrence risk and possibilities for prenatal diagnosis.
- Easier for families to access support from other sources e.g. lay support groups, social services (benefits), education system.
- Aids research into normal and abnormal morphogenesis.

How do you make a syndrome diagnosis?

The steps followed are essentially the same as for other clinical situations i.e.

- History
- Examination
- Investigations
- Synthesis (Putting it all together)

A different emphasis is placed on the above, however, compared to other clinical situations.

The **History** concentrates particularly on:

- Family history. This is usually taken in the form of a pedigree, noting such details as consanguinity or a possible Mendelian pattern of inheritance. It is usual to get details of other affected family members and where they were treated. It may be necessary to approach them to ask for consent to access their medical records.
- Past obstetric history. Multiple early miscarriages may suggest a chromosome abnormality, for example
- Maternal health. Some maternal diseases e.g. diabetes or SLE may confer a higher risk of fetal abnormality. Mothers with epilepsy also have a 2-3 times increased risk of fetal abnormality.
- Maternal vitamin supplements and drug use. Check if any of these are likely to be teratogenic
- Pregnancy history. It would be relevant to know, for examples whether abnormalities were detected on scan, whether any invasive procedures were carried out and whether there was any problem with liquor volume.

During the **Examination** of a dysmorphic child the following should be taken into account:

- Posture and tone. Some diagnoses can be suggested by observing a child prior to examination. The characteristic flexed posture of the fingers in Trisomy 18, for example, or a very hypotonic posture in Prader-Willi syndrome.
- Movements and behaviour patterns are very characteristic in some syndromes. A girl with Rett syndrome will have repetitive hand movements and individuals with Smith Magenis syndrome may hug themselves.
- Facial expressions may be typical in some syndromes. An individual with myotonic dystrophy has a mask-like face with poor facial movement. The happy, smiling face of Angelman syndrome is unmistakable.
- Characteristic personality can be observed in some syndromes such as Williams syndrome where there is a friendly and talkative manner.

Physical examination should include documentation of:

- Height and weight, which should be plotted on an appropriate growth chart. Parental height should be taken into consideration.
- Proportions, which can be altered in certain conditions e.g. achondroplasia or Marfan's syndrome
- Measurements of head circumference, facial features and other body parts where appropriate. These can be plotted onto charts for normal ranges and for specific conditions (Greenwood Genetic Centre publish some of these)
- Major and minor abnormalities. Document carefully all abnormalities. Where minor anomalies are concerned, be aware of what is abnormal and what is just part of normal variation e.g. with minor 2/3 toe syndactyly.
- Photography. It is often useful to document major and minor anomalies by taking photographs if the patient/parents permit. One has to be sensitive about removing clothes for photographs, especially in older children, as this is not always necessary. It is useful to remove as much "clutter" as possible from the background and avoid patterned backgrounds. Sequential photos of children at different ages are especially helpful in studying the evolution of phenotypes
- Parents. Some of the distinctive features may just be family characteristics. Taking a look at the rest of the family in person or from a family photograph is helpful.

Terminology used in dysmorphology

Malformation: A morphologic abnormality that arises because of an abnormal developmental process. (A primary error in morphogenesis e.g. cleft lip).

Malformation sequence: a pattern of multiple defects resulting from a single primary malformation e.g. talipes and hydrocephalus can result from a lumbar neural tube defect.

Malformation syndrome**:** a pattern of features, often with a unifying underlying cause, that arises from several different errors in morphogenesis. ("syndrome" from the Greek "running together")

Deformation. Distortion by a physical force of an otherwise normal structure

Disruption. Destruction of a tissue which was previously normal

Dysplasia. Abnormal cellular organisation within a tissue resulting in structural changes e.g. within cartilage and bone in skeletal dysplasias

Association. The occurrence of two or more features which are seen together more frequently than would be expected by chance alone but are not known to have a common cause.

Investigations

The dysmorphologist can be aided by many different types of investigation including:

- Cytogenetics. In many laboratories a routine karyotype is the basic investigation supplemented by the appropriate FISH test where a microdeletion syndrome is suspected. However in an increasing number of centres array studies have replaced routine karyotyping. Mosaic chromosome disorders may not be detectable on lymphocyte chromosome analysis and skin chromosome tests may be needed. Chromosome breakage studies may be indicated in some patients, particularly in those who are small, have microcephaly and other features such as radial aplasia and café au lait patches.
- Molecular genetic tests are now available for many different conditions. There are databases which can be used to identify laboratories offering testing for specific disorders e.g. Orphanet (www.orpha.net) Unfortunately testing may not be available on a service basis at the present time for many rare conditions. However the rapid application of next generation sequencing for gene discovery and translation to service diagnostics means that testing for large panels of genes and whole exome sequencing is becoming feasible in many centres now.
- Metabolic testing. E.g. amino acids, organic acids, peroxisomal disorders, disorders of cholesterol metabolism. There may be diagnostic pointers to metabolic disease such as hepatosplenomegaly, skeletal features or seizures occurring soon after birth.
- Infection screen is helpful where congenital infection is suspected from the history or from clinical signs.

- Imaging studies. X-rays are of paramount importance in the diagnosis of skeletal dysplasias. They must be of good quality and you must make sure to request the necessary X-rays as some departments do only limited skeletal surveys, for example. Radiographs of the hands and feet can be particularly useful. CT scans are useful to look for intracranial calcification; otherwise MRI scans provide more information and do not expose patients to radiation.
- Pathology/Autopsy. Pathology investigations are useful in the diagnosis of syndromes with specific pathological features and for defining the full extent of abnormalities. With fetal pathology it is important to take into account the gestation of the fetus and the possibility of traumatic abnormalities sustained during delivery.
- Other miscellaneous investigations may be needed e.g. Hb electrophoresis in ATR-X, white cell count in Cohen syndrome etc.

Expert opinions

Although dysmorphic conditions can involve all body systems it is impossible for a dysmorphologist to be an expert in all areas, and it is often necessary to refer for a specialist opinion. A detailed ophthalmological or dermatological examination is often needed, for instance and skeletal dysplasias are notoriously difficult to diagnose unless you are a specialist in this area.

How do you put all this information together to make a syndrome diagnosis? (Synthesis)

1. **Ask some basic questions:**

- Are you dealing with a single malformation or multiple malformations?
- Is the child likely to have a multiple anomaly syndrome?
- Are there deformations that might tie in with the pregnancy history?
- Does the family history help?

2. Think about the various mechanisms by which birth defects come about:

- Chromosomal abnormalities
- Single gene defects (consider different types of genes e.g. genes encoding structural proteins, transcription factors, chromatin modelling etc) Also consider disturbances in gene expression e.g. imprinted genes
- Effects of multiple gene mutations/polymorphisms e.g. as in Hirschsprung Disease
- Multifactorial disorder (a combination of genetic predisposition and environmental factors e.g. NTD)
- Mainly environmental e.g. mechanical compression and teratogens (although in the latter genetic predisposition may play a part)
- Mosaicism – chromosomal, single gene mutation or in gene expression

3. When chromosomal syndromes have been ruled out and a single gene cause is strongly suspected

- Consider possible syndrome diagnoses in broad categories or 'syndrome families' where there may be mutation of genes in the same pathways including;
    - Skeletal dysplasias
    - Overgrowth syndromes
    - Low birth weight and proportionate dwarfism syndromes
    - Prader Willi-like and obesity syndromes
    - Angelman/Rett-like syndromes
    - Noonan-like syndromes
    - Neurocutaneous and Vascular syndromes
    - Ectodermal dysplasias and other skin disorders
    - Distinct MCA/MR syndromes with a 'gestalt'

  For many of these groups of disorders diagnostic NGS panels are now available

4. Think whether you have seen this before.

Personal experience is helpful and people get better and more experienced at dysmorphology over time. You may be able to recognise a "gestalt" which is familiar to you from a previous presentation or from literature you have read.

5. Seek help from the literature

There are numerous textbooks and journals that can be of help to the dysmorphologist. Gorlin's "Syndromes of the Head and Neck" is particularly useful and is not confined to the head and neck, covering chromosomal and other disorders too.

6. Search the Dysmorphology Databases

There are several available including the London Dysmorphology Database and POSSUM. You get most help from databases if you search on features which are very distinctive ("hard" diagnostic handles) and if you know something about dysmorphology already so as to be able to sift out which syndromes are least likely matches with your patient.

7. Seek help from colleagues.

Share information and photographs/images with other colleagues within your department and specialists in the field. It's hard to be a good dysmorphologist in isolation. Present distinctive cases at dysmorphology meetings. The ability to send images by e-mail (if you have parent's permission) makes getting this type of help even easier. Increasingly 'Networks of Experts' are being established to assist diagnosis of rare distinct conditions. These include ESDN (European Skeletal Dysplasia Network) (www.esdn.org).

Following the Diagnosis

- Clinical diagnosis should be confirmed with a diagnostic test if available
- Even experienced dysmorphologists should consult with colleagues to see if they agree with the diagnosis
- Further consultation with parents to explain child's problems and full discussion of the implications
- Arrange appropriate screening investigations if the condition is associated with complications.
- Make sure parents have support e.g. from local services/family doctor/parent support group/follow-up by genetic services.

New dilemmas for dysmorphology

Whereas previously the model for diagnosis was clinical examination – differential diagnosis – relevant investigations, now, with NGS testing new dilemmas are arising from the detection of mutations, considered pathogenic, in genes known to be associated with distinct syndromes where the features in the patient don't fit the distinct syndrome. There are a number of explanations for this situation but in many instances it is that the phenotypic spectrum for mutations in many genes is far wider than previously recognized. This poses problems for counselling and is confusing and often worrying for families. In time and with greater knowledge matters may become clearer and patient focused literature can be amended.

What if the diagnosis remains unknown?

A child should not be labelled as having a particular dysmorphic syndrome unless the dysmorphologist is absolutely sure about this. It is far more difficult to remove an incorrect diagnosis than to attach one in the first place. Where a syndromic diagnosis is still likely but not apparent at the first consultation it is important for the child to be followed up and re-evaluated at a later stage. A few years later new syndromes may have been delineated or more investigations might be available. This is particularly relevant now with the advent of NGS testing and early results have demonstrated a much wider phenotypic spectrum than suspected associated with mutations in many genes.

Where patients have very distinctive features, either representing a 'new' syndrome or showing unusual features of one already described, it is sometimes useful to document the findings in the form of a case report for the literature. Someone else may have seen a similar child before, or may come across the report when searching the literature for one of their own patients. This type of case report serves a useful purpose in the delineating new syndromes.

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Gorlin's Syndromes of the Head and Neck 5th Edition 2010
Raoul C.M. Hennekam, Ian D. Krantz and Judith Allanson. Oxford University Press

Human Malformations and Related Anomalies. Eds Stephenson, Hall, Goodman, Oxford University Press (2nd edition published 2005)

Management of Genetic Syndromes. (3rd edition 2011) Eds SB Cassidy, JE Allanson

Databases

LDDB, London Dysmorphology Database (www.lmdatabases.com)

POSSSUM (Pictures of Standard Syndromes and Undiagnosed Malformations)
Melbourne: The Murdoch Research Institute, 2001(www.possum.net.au)

Molecular syndromology in the NGS-era: which phenotype, which family, which strategy?

Bernd Wollnik
Center For Molecular Medicine, University of Cologne , Germany

Novel sequencing technologies as well as adopted conceptual strategies can dramatically speed up gene identification in medical genetics. There was little doubt that massive parallel sequencing would have a great impact on studying causative genes for rare syndrome in the future, and the last year has impressively shown that this future has already started. We currently do see a huge wave of gene identification studies using these novel sequencing technologies. It is important to note that only together with subsequent functional work on identified proteins and pathways these novel technologies will elucidate underlying pathogenic mechanisms. This talk will present our recent experiences in using whole-exome-based approaches in medical genetics and show successful examples, which shed light into the pathogenesis of selected syndromes.

Tuesday, May 13

Marfan syndrome and related disorders: from gene to therapy

Bart Loeys

Center for Medical Genetics,
Antwerp University Hospital, Edegem
Belgium

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

Most recently, mutations in other components of the TGFbeta signaling pathway, including SMAD3, TGFB2 and SKI have been associated with LDS-like phenotypes and Shprintzen-Goldberg syndrome.

As such, these human diseases and different mouse models have offered the opportunity to unravel the complex interaction between aortic integrity and extracellular matrix regulation of TGFbeta activity. There is increasing evidence indicating that misregulation of TGFbeta signaling owing to defects in extracellular proteins is centrally important to the development of aortic aneurysms. This view has now replaced the previous idea that aortic aneurysms were simply due to a structural deficiency of the elastin matrix in the aorta. Moreover, this new view offers excellent targets for therapeutic interventions

Aging phenotypes

Bernd Wollnik

Center For Molecular Medicine, University of Cologne , Germany

Research into aging and age-related diseases is of very high social relevance. It is not simply about extending lifespan, but mainly about ensuring high quality of life in the elderly. Understanding the molecular processes of aging and aging-associated diseases is essential to identify key points for therapeutic interventions across the whole spectrum of aging-associated disease. Progeria syndromes, such as the well-known Hutchinson-Gilford or the Wiedemann-Rautenstrauch progeria syndromes, are rare congenital disorders, which share an overlapping premature-ageing phenotype including among others alopecia, wrinkled skin, lipoatrophy, and cardiovascular abnormalities. The diverse progeria syndromes differ with regards to their time of manifestation, the severity of the symptoms, and the life expectancy of the affected patients. The genetic cause has been identified for several progeria syndromes, e.g. *de novo* dominant mutations in the *LMNA* gene cause Hutchinson-Gilford progeria syndrome. Our strategy is to find genes causing human congenital disorders associated with premature aging phenotypes and to investigate mechanisms responsible for premature aging. Disturbance of genomic integrity and accumulation of DNA damage seems to have an important impact on accelerated aging processes in these patients. Examples will be given and molecular mechanisms discussed.

Complex genetics

Cisca Wijmenga

University Medical Center Groningen
Department of Human Genetics
GRONINGEN
The Netherlands

The lecture on complex disease genetics will discuss the genetic architecture of common complex diseases, how genetic factors can be identified by genetica association studies (GWAS), and also concentrate on the post-GWAS era. it is well established that nearly all human disease has a genetic component and GWAS studies have identified genetic risk factors for many of the diseases. It is much less clear how these genetic risk factors contribute to the disease pathology. Potential ways to zoom in into causative risk factors and causative genes will be discussed and how population-based resources can assist in this. How the filed has learned that genetic risk variants for common complex traits often function through gene regulation changes will be shown. The potential to use genetic risk factors for diagnosis, patient management and in the development of new therapies will briefly outlined.

During the workshop the students will become familiar with searching and interpreting GWAS results. In the workshop we will also compare differences in genetic architecture between different categories of diseases.

Wednesday, May 14

Inherited cancer and prospects for therapy

John Burn
Newcastle University
Genetics Chair, National Institute of Health Research
Biomedicine West, Centre for Life, Newcastle UK

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

As molecular pathways become better understood, therapeutic and preventive drug treatments become feasible. The most exciting recent development is the emergence of the PARP inhibitors which block single strand DNA repair forcing cells to rely on homologous recombination. This pathway requires functional BRCA1 and 2. Where gene carriers have lost the second gene copy and developed a cancer, HR is compromised and PARP inhibitors become lethal. The fierst of these agents is approaching market.

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

A review of early trials of aspirin to prevent cardiovascular disease has revealed fewer cancers in the following decade among those randomised to aspirin. Two trials examined the effects of aspirin on cancer prevention. The women's Health Study gave alternate day low very dose (100mg) aspirin or placebo to 18,000 women and found after 10 years that the incidence of colorectal cancer fell by 18% in those on aspirin (Cook NR et al Ann Int Med 2013; 159:77-85.). The CAPP2 trial randomized 1009 carriers of a mismatch repair gene defect, at risk of Lynch syndrome or hereditary non-polyposis colorectal cancer, to daily 600mg aspirin or placebo for 2-4 years. Analysis in those who completed the target of 2 years treatment revealed a 63% reduction in colorectal cancer at 5 years and a similar fall in other cancers such as endometrial cancer.(Burn et al Lancet 2011;378:2081-87). Several lines of evidence suggest part of the effect

is attributable to suppression of inflammation. Aspirin may also enhance apoptosis of pre malignant cells, analogous to effects of salicylates in plants. CaPP3 will test different doses of aspirin in 3000 MMR gene defect carriers commencing in 2014. Aspirin may be combined with other lifestyle interventions to reduce the burden of hereditary cancers, even in the presence of a highly penetrant gene defect.

Spinal muscular atrophy: from gene to therapy

Brunhilde Wirth
Institute of Human Genetics
University Hospital of Cologne, Germany

Proximal spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder that represents the leading genetic cause of death in childhood. Homozygous mutation of the *survival motor neuron* gene *1* (*SMN1*) causes SMA, while the number of nearly identical *SMN2* copies determines disease severity. *SMN1* almost exclusively produces full-length (FL) transcripts. Due to a silent mutation, *SMN2* undergoes alternative splicing and generates only 10% of FL-*SMN2* transcripts but 90% of transcripts lacking exon 7 (Δ7-*SMN2*). The latter encode a biochemically defective, truncated protein. However overexpression of the splicing factor Htra2-beta1 that binds to an ESE in exon 7 restores the correct splicing to almost 80%. Therefore, activation of the *SMN2* transcription or modulation of its splicing pattern is likely to be clinically beneficial (1).

Several inhibitors of histone deacetylases (HDACs) have been identified as potential drugs for SMA treatment (2). Valproic acid (VPA), a short-chain fatty acid and histone deacetylase inhibitor, is able to significantly increase the protein level of SMN2 in fibroblast cell lines from SMA patients as well as in neuronal tissue, such as cultured rat and human hippocampus brain slices (3). Since VPA is an FDA approved drug and used since more than three decades in long-term epilepsy treatments, a first clinical trial in parents of SMA patients was carried out in order to verify the finding *in vivo*. Ten SMA carriers were enrolled in a VPA pilot trial. Drug treatment revealed increased FL-*SMN* mRNA/protein levels in blood from 7/10 probands. In a subsequent investigation of peripheral whole blood from 20 SMA type I-III patients treated with VPA in individual experimental curative approaches, FL-*SMN2* mRNA levels were found to be increased in 7 patients, whereas 13 presented unchanged or decreased transcript levels (4). This provided a first proof of principle of an *in-vivo* activation of SMN2 by VPA in SMA. But is this happening also in the CNS, the main target tissue of SMA? We therefore generated induced pluripotent stem cells from fibroblasts of responders and non-responders to VPA and differentiated these into neurons, and showed that these react in the same way. By using whole transcriptome differential expression analysis, we identified CD36, a fatty-acid translocase, as the most likely gene causing VPA non-responsiveness (5). Individual therapies of type I-III SMA patients with VPA/L-carnitine showed an improvement of the clinical picture or stabilization after 5-6 months of treatment in about half of the patients. Finally, a first fully protective modifying gene for

SMA, named plastin 3 has been identified and will be briefly presented (6, 7) and discussed in detail within the workshop.

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Epigenetics and disease – lessons from imprinting disorders

Karen Temple
Faculty of Medicine,
University of Southampton, UK

Epigenetics

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

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

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

Imprinting Disorders

Several well- known disorders of imprinting are known including Beckwith Wiedemann syndrome, Transient Neonatal Diabetes, Temple syndrome, Wang syndrome, Russell Silver syndrome, Angelman syndrome Prader Willi syndrome and Pseudohypoparathyroidism type 1B. Only a proportion of people with these syndromes have a true epigenetic error, as uniparental disomy (inheritance of both chromosome homologues from one parent with no contribution from the other) and copy number variation are more common underlying causes. Studies to determine the cause of seemingly 'true' epigenetic aberrations, identified in imprinting disorders, may provide helpful insights into the causes of epigenetic mutations in general. For example the work on imprinting disorders has led to the identification of *ZFP57*, as a gene essential for DNA methylation maintenance.

Disease	**Prevalence**	**Main diagnostic clinical features**	**Additional clinical features (may develop with time)**	**Frequency of 'epigenetic' aberration**	**Reference**
Prader Willi syndrome	1 in 17,500	Low birth weight Hypotonia, Hyperphagia Developmental delay	Hypogonadism Diabetes Obesity	Approximately 1%	(Williams, Driscoll, and Dagli)
Angelman syndrome	1 in 16,000	Severe developmental delay No speech Epilepsy Ataxia	Microcephaly	4%	(Cassidy and Driscoll)
Beckwith Wiedemann syndrome	1 in 13,700	Macrosomia/overgrowth Macroglossia Umbilical defect	Increased risk of Wilms tumour Hypoglycaemia	60%	(Weksberg, Shuman, and Beckwith)
Silver Russell syndrome	1 in 100,000 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	unknown	Intrauterine growth retardation Hypotonia, Scoliosis	Hydrocephalus Cleft palate	uncertain	(Kotzot)

UPD 14 associated syndrome)		Developmental delay Early puberty ,Short stature			
Wang syndrome (Paternal UPD 14 associated syndrome)	unknown	Bell shaped chest Hypotonia Developmental delay	Umbilical defects Larger birth weight	uncertain	(Kagami et al.)
Pseudohypo parathyroidism 1B	unknown	Hypocalcaemia due to Parathryoid resistance (tetany/parasthesia)	Obesity	>90%+	(Bastepe et al.)

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Long distance regulation in human disorders

Eva Klopocki

Universität Würzburg
Institut für Humangenetik
Biozentrum Am Hubland,Würzburg Germany

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

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

Besides CNVs in non-coding sequences structural aberrations such as inversions and translocations may disturb gene regulation 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.

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

Thursday, May 15

Mitochondrial inheritance and disease

Elena Rugarli

University of Cologne, Germany

Mitochondrial disorders represent one of the most common inborn errors of metabolism with a frequency of about 1 in 5,000. Traditionally, we define as “mitochondrial disorder” a disease caused by defects in mitochondrial oxidative phosphorylation (OXPHOS), however it is increasingly clear that other aspects of mitochondrial function, for example mitochondrial dynamics, are highly relevant for human pathology. Mitochondrial disorders are very heterogeneous from a clinical, genetic, biochemical, and molecular point of view. Mitochondrial disorders can affect almost any organ and tissue, can arise at any age, can be sporadic or inherited. Inheritance of mitochondrial disorders can follow both Mendelian and non-Mendelian rules of transmission. The clinical heterogeneity of mitochondrial disorders can be in part explained by the unique rules of mitochondrial genetics, and by the dependence of mitochondrial function on protein products encoded by the nuclear genome. However, the reason for tissue-specificity and clinical presentation of mitochondrial diseases elude in most case our comprehension. Due to their complexity, mitochondrial disorders are usually classified by their genetic defect rather than clinical manifestation.

Mitochondria are the only organelles in animal cells, besides the nucleus, that contain their own DNA. Mitochondrial DNA is a 16,569 bp circular, double-stranded molecule that encodes 13 protein subunits of the respiratory chain, and the 22 tRNAs and 2 rRNAs required for mitochondrial protein synthesis. The strands of the DNA duplex are distinguished as “heavy” and “light” based on their G/T composition. Most of the protein encoding genes (12 out of 13), as well as the two rRNA genes and 14 of the tRNA genes are encoded by the heavy strand, whereas the light strand codes for eight tRNAs and a single polypeptide. A small region of about 1kb, called the displacement loop (D-loop), is the only non-coding region of mammalian mtDNA, and contains promoters for the light and heavy strand as well as the origin of heavy strand replication.

Transmission of mtDNA does not follow the Mendelian rules of inheritance, since mtDNA is exclusively maternally inherited. As a result, a mother carrying an mtDNA mutation can transmit it to her children, but only her daughters can further transmit it to the next generation. As each cell contains between 1,000 to 10,000 copies of mtDNA, a pathogenic mutation could be present in all or just some mtDNA molecules. Existence of two or more different populations of mtDNA in a single cell is called heteroplasmy in contrast to homoplasmy where all mtDNA molecules are identical. As a consequence, an important concept in mitochondrial genetics is the so called “threshold effect”, which is defined as the minimal critical level of a pathogenic mutation in mtDNA that should be present in the cell or tissue to have a deleterious effect. Since this deleterious effect is at the level of the respiratory chain, tissues with high oxidative capacity, such as

muscle, heart, and brain are preferential targets. Another important characteristics of mtDNA genetics is mitotic segregation. Random distribution of mtDNA molecules during cell division can lead to very different amounts of mutant mtDNA molecules in daughter cells. For instance, a cell carrying low levels of mutated mtDNA molecules can in principle give rise to a daughter cell with relatively high levels of mutated mtDNA copies, which in turn will affect oxidative phosphorylation in that cell. This principle is at the basis of the extreme phenotypic variability among individuals carrying the same mtDNA mutation, and underlies the uneven distribution of mutated mtDNA among siblings. A mammalian oocyte contains ~100,000 copies of mtDNA and experimental evidence suggest that all of these copies are derived from replication of just a few mtDNA copies of a precursor cell. This "bottleneck" phenomenon occurs at very early stages of oogenesis with a yet unclear mechanism. All of the above-mentioned aspects contribute to the high complexity of mitochondrially-inherited diseases, which are often characterized by variable expressivity and incomplete penetrance. Mitochondrial diseases due to mutations in the mtDNA can be further distinguished in diseases caused by point mutations or by rearrengements of the mtDNA.

The majority of the mitochondrial proteins, estimated to be 1,500 in total, are encoded by the nuclear DNA, translated in the cytoplasm and transported into mitochondria. Mitochondrial disorders caused by mutation in these nuclear-encoded genes are a very heterogeneous group. In fact these mutations can affect genes encoding: 1) subunits of the respiratory chain; 2) factors required for mitochondrial protein complex assembly; 2) proteins involved in mtDNA replication, stability, and translation; 3) proteins affecting the phospholipid composition of the mitochondrial membrane; 4) proteases and chaperons involved in quality control of the mitochondrial proteome; 5) molecules mediating mitochondrial dynamics.

In conclusion, about 50 years after the first description of a mitochondrial disease, the field of mitochondrial medicine is in rapid expansion, and is moving from the elucidation of the genetic basis of these disorders to the dissection of pathogenic mechanisms and the development of therapies.

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Detection and functional impact of mitochondrial DNA mutations in cancer progression

Giuseppe Gasparre

Dept. of Medical and Surgical Sciences, Unit of Medical Genetics, University of Bologna, Italy

Mutations in the mitochondrial DNA (mtDNA) have been implicated in tumorigenesis because of their multifaceted role in reactive oxygen species generation, apoptosis regulation and, particularly, in affecting the metabolic remodeling cancers undergo during progression[1]. Recently, a debated has sparked on the pro- versus anti-tumorigenic role of mutations hitting subunits of respiratory complexes encoded by the mtDNA, which seldom takes into account the genetic and genomic features of the polyplasmic mitochondrial chromosome. In the detection of mtDNA mutations/variants in cancer, as well as in other biological systems, contamination by nuclear mitochondrial sequences (NumtS), the occurrence of false heteroplasmies[2] and that of false mutations due to the sequencing techniques used[3], and the nucleotide variability[4] ought to be taken into account to infer pathogenicity or neutrality.

From the functional point of view, also the type of mutations need be considered to evaluate the impact on neoplastic progression. MtDNA mutations may hit *de facto* metabolic enzymes, impinging on metabolic plasticity and on the adaptation to hypoxia, one of the main features of solid neoplasms. Hypoxia drives progression to malignancy via the stabilization of the hypoxia inducible factor 1□ (HIF1□), which transcribes a large set of target pro-glycolytic, pro-metastatic and pro-angiogenetic genes. According to whether mtDNA mutations, especially those hitting complex I of the respiratory chain, contribute to hamper function or both function and assembly of the complex, they may represent pro- or anti-tumorigenic modifier events during progression, as they may differently affect adaptation to hypoxia and metabolic reprogramming.

The issues inherent to mtDNA mutations detection and their potential roles in determining the cancer cell's fate will be discussed, along with the lessons taken from oncocytic tumors, a usually bening, indolent subtype of epithelial cancer whose genetic hallmark is the high frequency of mtDNA mutations disassembling respiratory complexes[5].

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Population genetics in the genome era: the Sardinia project

Francesco Cucca

Institute for Genetics & Biomedical Research (IRGB),
University of Sassari, Italy

Comprehensive Genome-Wide Association Scans (GWAS) have greatly expanded our understanding of the genetic component of complex traits, identifying numerous genomic regions unequivocally associated with the risk for multifactorial diseases and quantitative variables. However, there are only a few examples of successful reduction of these associations to the causal DNA changes: those variants whose protein products are directly involved in the assessed traits. Moreover, many of these associations remain biologically cryptic. Functional experiments can be useful to overcome these limitations but we must be confident that the most plausible causal variants and pathways -- and not secondary epiphenomena, and hence the wrong pathway of biological interrogation -- are being studied. Major challenges are therefore to establish which variants are directly associated with disease risk and to relate this information to function.

To meet these challenges, we have run 3 integrated initiatives in the founder Sardinian population. Because of genetic drift, following the early peopling and colonization of the island and, to a lesser extent, natural selection, a large number of variants that are rare elsewhere in Europe have drifted to higher frequency in Sardinia, enlarging the spectrum of genetic variation that can be reliably assessed for association with phenotypes of biomedical interest. In the first initiative, large-scale DNA sequencing in ~3,500 individuals, combined with extensive genotyping and powerful imputation methods, has yielded essentially complete genetic variation data (~24 million SNPs) available for the analysis and has provided an unprecedentedly powerful tool to detect casual associations with any trait and disease. In the second initiative, we profiled extensively the levels of over 800 selected quantitative variables and extensively genotyped with a combination of high density arrays ~6,600 individuals from a cohort study, the Sardinia project, of four clustered Sardinian villages. This allowed us to perform sequencing based GWAS to dissect the genetic bases of the genetic component of the assessed quantitative traits. In the third initiative, we performed sequencing based GWAS in a case –control collection of ~3,000 Sardinian multiple sclerosis cases, ~2,000 type 1 diabetes cases, and ~4,000 controls.

I will show some examples of new associations with a special emphasis placed on the variants associated with quantitative traits and fully overlapping with new and known disease risk variants. Such coincident associations identify specific cells and molecules that are unbalanced in disease status and also suggest mechanisms by which specific risk alleles might lead to disease susceptibility, providing a solid entrée to ongoing functional studies of steps in pathogenesis.

Posters of Students

Differences in frequency and type of the *SCN1A* mutations between Dravet Syndrome and Dravet Borderline Syndrome – are they?

Górka P.[1,2], Terczynska I.[2], Duszyc K.[1.3], Tataj R.[1], Szczepanik E.[2], Hoffman-Zacharska D.[1,3]

[1]Dept. of Medical Genetics, [2]Clinic of Neurology of Children and Adolescents, Institute of Mother and Child, Warsaw Poland, [3]Institue of Genetics and Biotechnology, University of Warsaw, Poland

Objective and background: Mutations in the *SCN1A* gene, encoding the neuronal voltage-gated sodium channel alpha 1 subunit (Nav1.1) have been associated with various types of epilepsy. The *SCN1A*-associated phenotypic spectrum ranges from more benign genetic epilepsy with febrile seizures plus (GEFS+) to the severe epileptic encephalopathies; Dravet Syndrome / Dravet Syndrome Borderline (DS/DBS) . *SCN1A* mutations have been also found in single cases with less common phenotypes. All identified mutations are dominant and approximately 95% of them arise *de novo*. The remaining 5% are familial cases with milder phenotypes often consistent with GEFS+ spectrum.

The aim of this study was to characterize and compare mutations (type, frequency) of the *SCN1A* gene in two group of patients with epileptic encephalopathies diagnosed as Dravet or Dravet Borderline Syndrome.

Patients and methods: The investigated group consisted of 123 Polish patients clinically diagnosed with DS (82) or DSB (41). The *SCN1A* gene analysis involved identification of point mutations (direct gene sequencing) and rearrangement analysis (MLPA, aCGH). Sequencing was performed for all patients, and rearrangement analysis only for individuals without an identified point mutation.

Depending on the type of identified mutation the appropriate method was involved in order to investigate its possible inheritance.

Results and discussion: The *SCN1A* mutations were identified in 84 of 123 investigated individuals (70 out of 82 patients clinically diagnosed with DS (85%) and 14 out of 41 with DSB (34%)). In the both groups the point mutations were the most common (85%) however low frequency (4,7%) of the gene rearrangements was identified as well. Most of the patients had no relevant familial history of disease and the majority of the mutations arose *de novo*. We were able to perform a parental DNA analysis for 44 DS and 8 DBS patients.

In DS group all mutations were de novo, whereas among DBS patients we observed 2 cases of familial history. In both cases mutations were inherited form affected parents, whose form of epilepsy was milder comparing to patients' phenotype.

Conclusions: Analysis of the frequency and types of mutations of the *SCN1A* gene among DS and DBS patients showed that point mutations were the most frequent mutation type in both groups of patients. The

frequency of the various types of SCN1A point mutations in the analyzed groups of DS and DSB patients were similar.

However, that the prevalence of SCN1A mutations was more common in DS group than DBS.

The overall frequency of gene rearrangements was 4,7%. Due to the rarity of SCN1A gene deletions and the difference in the number of DS and DSB patients we could not compare the rearrangements frequency between the two groups.

Our results indicates that the SCN1A recurrent mutations were very rare, and that some mutations may show patients' clinical variability, which indicates an impact of modifying factors.

TBC1D7 mutations are associated with intellectual disability, megalencephaly, patellar dislocation and celiac disease

Barbara Mandriani1, Ali Abdullah Alfaiz2, Lucia Micale1, Bartolomeo Augello1, Maria Teresa Pellico1, Carmela Fusco1, Jacqueline Chrast2, Ioannis Xenarios2,3, Pasquelena De Nittis1, Carmela Rinaldi1, Alessandra Di Lauro1, Leopoldo Zelante1, Alexandre Reymond2, and Giuseppe Merla1,4

1Medical Genetics Unit, IRCCS Casa Sollievo Della Sofferenza Hospital, San Giovanni Rotondo, Italy.
2Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland
3Swiss Institute of Bioinformatics (SIB), Lausanne, Switzerland
4PhD Program, Scienze della Riproduzione e dello Sviluppo, University of Trieste, Italy

Mutations in both *TSC1* and *TSC2* cause the tuberous sclerosis complex (TSC), a multisystemic disorder characterized by the development of hamartomas or benign tumors in various organs as well as epilepsy, intellectual disability (ID) and autism. Whereas the binding of TBC1D7, the third constitutive subunit of the TSC1-TSC2 complex, is required to maintain its integrity, sequencing of TSC patients with no *TSC1-TSC2* mutations indicated that *TBC1D7* is unlikely to represent a "TSC3" gene. Loss of function of *TBC1D7* results in an increase in mTORC1 signaling, and consequently a delay in the induction of autophagy.

Mutations in *TBC1D7* were recently reported in a family with ID and macrocrania. Using exome sequencing we identified two sisters homozygote for a novel *TBC1D7* truncating mutation. In addition to the already described macrocephaly and mild ID, they share osteo-articular defects, patella dislocation, behavioral abnormalities, psychosis, learning difficulties, celiac disease, prognathism, myopia and astigmatism. Consistent with a loss-of-function of *TBC1D7* the proband's cell lines show an increase in the phosphorylation of 4EBP1, a direct downstream target of mTORC1 and a delay in the initiation of the autophagy process.

This second family allows enlarging the phenotypic spectrum associated with *TBC1D7* mutations and defining a *TBC1D7* syndrome. Our work reinforces the involvement of TBC1D7 in the regulation of mTORC1 pathways and suggests an altered control of autophagy as possible cause of this disease.

Whole Exome Sequencing identifies a novel candidate gene for non-syndromic autosomal recessive intellectual disability in a consanguineous Finnish family.

Anju K Philips1, Michele Pinelli2,3, Aki Mustonen4, Tuomo Määttä5, Shaffaq Raza1, Christian Gillisen2, Irma Järvelä1

1Department of Medical Genetics, Haartman Institute, University of Helsinki
2Department of Human Genetics, Nijmegen Centre for Molecular Life Sciences and Institute for Genetic and Metabolic Disorders, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands
3Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università Degli Studi di Napoli "Federico II"
4Department of Clinical Genetics, Oulu University Hospital, Oulu , Finland
5Service Centre of Kuusanmäki, Kajaani, Finland

Nonsyndromic intellectual disability is a heterogenous disorder. Clinical diagnosis is challenging as intellectual disability is the sole trait present. Here we report a Finnish family with four affected sons with non-syndromic intellectual disability. In order to find the disease causing gene in this family, we perfomed whole exome sequencing on two affected sons, father and the mother. We identified a homozygous missense mutation in a novel candidate gene *C12orf4* in chromosome 12 resulting in the amino acid substitution L328P.

Whole blood DNA methylation changes are associated to Malignant Pleural Mesothelioma

E. Casalone[1,2], S. Guarrera[1,2], G. Fiorito[1,2], M. Betti[3], D. Ferrante[4], C. Di Gaetano[1,2], F. Rosa[1,2], A. Russo[1,2], S. Tunesi[4], M. Padoan[4], A. Aspesi[3], C. Casadio[5], F. Ardissone[6], E. Ruffini[7], P.G. Betta[8], R. Libener[8], R. Guaschino[9], E. Piccolini[10], D. Mirabelli[11,12], C. Magnani[4,12], I. Dianzani[3,12], G. Matullo[1,2]

[1] Human Genetics Foundation, HuGeF, I-10126 Turin, Italy
[2] Department of Medical Sciences, University of Turin, I-10126, Turin, Italy
[3] Laboratory of Genetic Pathology, Department Health Sciences, University of Piemonte Orientale, I-28100, Novara, Italy
[4]CPO-Piemonte and Unit of Medical Statistics and Epidemiology, Department Translational Medicine, University of Piemonte Orientale, I-28100, Novara, Italy
[5]Thoracic Surgery Unit, University of Piemonte Orientale, I-28100, Novara, Italy
[6] Chest Surgery, Department of Clinical and Biological Sciences, University of Turin, I-10043, Orbassano, Italy
[7] Thoracic Surgery Unit, University of Turin, I-10126, Turin, Italy
[8]Pathology Unit, Azienda Ospedaliera Nazionale SS, Antonio e Biagio e Cesare Arrigo, I-15121, Alessandria, Italy
[9]Transfusion Centre, Azienda Ospedaliera Nazionale SS, Antonio e Biagio e Cesare Arrigo, I-15121, Alessandria, Italy
[10] Pneumology Unit, Santo Spirito Hospital, I-15033, Casale Monferrato, Italy
[11]Unit of Cancer Epidemiology, CPO-Piemonte and University of Turin, I-10126, Turin, Italy

[12] Interdepartmental Center for Studies on Asbestos and other Toxic Particulates “G. Scansetti”, University of Turin, I-10125,Turin, Italy

Malignant pleural mesothelioma (MPM) is a rare and aggressive tumor strongly associated with asbestos exposure. Its onset is usually 30-40 years after the first exposure, and it is characterized by a poor prognosis with a median survival of 12 months.
Alterations in DNA methylation have been reported in several cancers, and are becoming an established hallmark of tumor. MPM is frequently associated with genetic mutations but also epigenetic changes leading to gene expression modifications.
The identification of MPM-specific epigenetic markers in peripheral blood might be a useful methodology for defining biomarkers for potential early detection and may define methylation changes due to asbestos exposure.
We conducted an epigenome-wide analysis (>450K CpG sites) on DNA from whole blood cells of 129 MPM cases and 127 controls to evaluate differences in methylation profiles. The sample population was randomly split into two sets: training and test set. In the training set 60 differentially methylated regions (DMRs) between cases and controls, adjusting for gender, age, asbestos exposure, and white blood cells percentage were found (FDR adjusted $p< 0.01$). Using a cluster algorithm we validated the DMRs prediction performance in the test set (AUC=0.7625). We found significant enrichment for genes involved in leukocyte trans-endothelial migration, natural killer cell mediated cytotoxicity and cell adhesion molecules pathways. Moreover we identified several genes belonging to the inflammation pathways and related to the cancer progression.
Our results suggested that methylation status in whole blood DNA might provide a useful biomarker for potential MPM early detection.

Partial trisomy 5p associated with sagittal craniosynostosis

Schrom E.-M.[1], Engmann L.[1], Schweitzer T.[2], Kress W.[1], Klopocki E.[1]

[1]Institute of Human Genetics University of Würzburg, Würzburg, Germany;
[2]Department of Pediatric Neurosurgery University of Würzburg, Würzburg, Germany

Craniosynostosis is defined as the premature fusion of one or more cranial sutures resulting in characteristic skull deformities. An increased intracranial pressure may cause neurological impairments and mid-face hypoplasia leads to eye and respiratory problems. Craniosynostosis is a frequent craniofacial malformation and estimated to affect 1 in 2100-2500 newborns. 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.

Here we present a patient with premature closure of the sagittal suture. He underwent cranial surgery during the first year of life. Furthermore, the patient showed additional clinical features like posteriorly rotated ear, pectus carinatum, mild scoliosis, and mild intellectual disability. Initially, FGFR associated craniosynostosis was excluded by sequencing of *FGFR1*, *FGFR2* and *FGFR3*. Because of the additional clinical features we performed an array CGH analysis using a 1M array (Agilent, Santa Clara, USA) to screen for submicroscopic copy number variations. Using standard analysis setting we detected a 13.1 Mb duplication on chromosome 5p [arr[hg19] 5p15.1p13.3(17,686,734-30,849,372)x3]. This region includes 6 OMIM genes. After visual inspection of the profile and changing of the analysis settings a considerably larger duplication extending further proximal on the short arm was detected [arr[hg19] 5p15.1p12(17,452,895-46,115,086)x3]. This 28.6 Mb duplication encompasses more than 40 OMIM genes.

Investigation of the parents to determine the origin of the aberration is on-going. We hypothesize that the partial trisomy 5p might be caused by a small supernumerary marker chromosome. To proof our hypothesis we intend to perform a conventional chromosome analysis in combination with FISH using probes located within the aberrant regions of chromosome 5p.

A review of published cases with partial trisomy 5p indicates that distal duplications (5p13.3-pter) are associated with a milder phenotype, whereas patients with proximal duplications (5p11-p13.2) show more severe phenotypes. Common clinical features are facial and limb mal-formations, cardiac defects, renal and intestinal malformations as well as mental retardation. In line with these data our patient with a proximal duplication presents with a relatively mild phenotype. A precise genotype-phenotype correlation remains difficult due to small sample size.

Interestingly, the duplication encompasses the gene encoding for *FGF10*, a ligand which has been shown to interact with *FGFR2*. Since *FGFR2* mutations have been associated with craniosynostosis it is conceivable that an over- or misexpression of *FGF10* causes disturbed *FGFR2* signaling resulting in premature fusion of the sagittal suture in our patient. To investigate the influence of *FGF10* overexpression on cranial suture patterning we are going to use zebrafish *Danio rerio* as *in vivo* model.

Molecular characterization of WFS1 in an Iranian family with Wolframsyndrome reveals a novel frameshift mutation associated withearly symptoms

Maryam Sobhani, Mohammad Amin Tabatabaiefar, Asadollah Rajab,Abdol-Mohammad Kajbafzadeh, Mohammad Reza Noori-Daloii

Wolfram syndrome (WS) is a rare autosomal recessive neurodegenerative disorder that represents a likely source of childhood diabetes especially among countries in the consanguinity belt. The main responsible gene is WFS1 for which over one hundred mutations have been reported from different ethnic groups. The aim of this study was to identify the molecular etiology of WS and to perform a possible genotype–phenotype correlation in Iranian kindred.

Material and Method: An Iranian family with two patients was clinically studied and WS was suspected. Genetic linkage analysis via5 STR markers were carried out. For identification of mutations, DNA sequencing of WFS1 including all the exons, exon–intron boundaries and the promoter was performed.

Result: Linkage analysis indicated linkage to the WFS1 region. After DNA sequencing of WFS1, one novel pathogenic mutation, which causes frameshift alteration c.2177_2178insTCTTC (or c.2173_2177dupTCTTC) in exon eight, was found.

Conclusion: The genotype–phenotype correlation analysis suggests that the presence of the homozygous mutation may be associated with early onset of disease symptoms. This study stresses the necessity of considering the molecular analysis of WFS1 in childhood diabetes with some symptoms of WS.

Keywords: Genetic linkage analysis, Iran, Novel mutation, Wolfram syndrome

Child with a Homozygous Mutation in the Treacher Collins-Franceschetti Syndrome 1 gene (TCOF1) Presenting with Ambiguous Genitalia, Cranio-facial Dysmorphism and Multiple Congenital Malformations

Nirmala D. Sirisena1, Kenneth McElreavey2, Anu Bashamboo2, K.S.H. de Silva3, Rohan W. Jayasekara1, Vajira H. W. Dissanayake1

1Human Genetics Unit, Faculty of Medicine, University of Colombo, Sri Lanka, 2Human Developmental Genetics, Institut Pasteur, Paris, France, 3Department of Paediatrics, Faculty of Medicine, University of Colombo, Sri Lanka

Background: This study was undertaken to identify disease-causing mutations in an eighth month old Sri Lankan male child with ambiguous genitalia, cranio-facial dysmorphism and multiple congenital malformations using the exome sequencing approach.

Methods: Clinical information and peripheral blood samples were collected from the proband and his parents after obtaining their written informed consent. Genomic deoxyribonucleic acid (DNA) was prepared

from peripheral blood lymphocytes using the PAXgene system (Qiagen) following the manufacturer's instructions. Exon enrichment was performed using Agilent SureSelect Human All Exon V4. Paired-end sequencing was performed on the Illumina HiSeq2000 platform using TruSeq v3 chemistry. Novel variants were analyzed by a range of web-based bioinformatics tools. All variants were screened manually against the Human Gene Mutation Database Professional [Biobase] (http://www.biobase-international.com/product/hgmd). *In silico* analysis was performed to determine the potential pathogenicity of the variants. Potentially pathogenic mutations were verified using classic Sanger sequencing.

Results: The proband was the first child born to healthy, consanguineous parents. All the antenatal scans were reported to be normal. Family history was unremarkable. Ambiguous genitalia and cranio-facial dysmorphism were recorded at birth. Genital examination at the age of 8 months showed evidence of micropenis (<2cm), peno-scrotal hypospadias, bifid scrotum with bilateral palpable testes in the scrotum. He had multiple cranio-facial dysmorphic features such as microcephaly, narrow forehead, low set ears, down slanting palpebral fissures, strabismus, bilateral epicanthal folds, broad nasal tip with anteverted nostrils and micrognathia. Other associated congenital anomalies included anteriorly placed anus, osteum secundum atrial septal defect and ventricular septal defect, dysplastic, multicystic pelvic kidney and asymmetrically dilated right lateral and third ventricles. His karyotype was 46,XY. An analyses of the exon dataset revealed a mutation in the Treacher Collins-Franceschetti Syndrome 1 gene (*TCOF1*) gene. A homozygous nucleotide substitution in exon 7 was identified [NM_000356.3 (*TCOF1*): c.889G>T; 2 p.A297S; ENST00000323668]. This mutation was previously reported (rs11203991) in 9 of 4395 African American Alleles and in 2 of 192 Luhya from Webuye, Kenya (1000 Genomes Project). However, it has never been observed in the homozygous state. This change causes a non-synonymous substitution within the TCOF1 protein, which is predicted to be deleterious. Both parents were carriers of the mutation.

Discussion: Treacher Collins Syndrome (TCS) [OMIM number 154500], also known as Mandibulofacial dysostosis, is a rare genetic disorder of craniofacial morphogenesis with a high degree of penetrance and variable phenotypic expression due to mutations in genes involved in ribosome biogenesis and synthesis. Mutation of one of three genes is known to be causative: *TCOF1* (78%-93% of individuals with TCS) and *POLR1C* or *POLR1D* (8%) [Shaefer *et al.*, 2014]. In the case of *TCOF1* or *POLR1D*, the mode of inheritance is autosomal dominant, while in the case of *POLR1C*, it is autosomal recessive. The *TCOF1* gene mutation identified in this child has not previously been observed in the homozygous state in TCS patients. According to Franceschetti, TCS has been classified into five categories i.e., complete, incomplete, unilateral, abortive and atypical forms [Kasat and Baldawa, 2011]. The phenotypic features observed in this child fit into the atypical form of TCS due to the multiple congenital anomalies, which are not usually part of the typical syndrome reported in scientific literature.

Conclusions: The phenotypic features observed in this child add to the spectrum of clinical features seen in patients with TCS. These findings demonstrate the phenotypic variability associated with mutations in the *TCOF1* gene and sheds more light on the molecular pathogenetic basis of TCS. These results have

significant implications for clinical genetic counseling, screening and management strategies for affected families.

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A NOVEL MUTATION IN PERICENTRIN (PCNT) GENE CAUSED MICROCEPHALIC OSTEODYSPLASTIC PRIMORDIAL DWARFISM

Ozlem Oz

Dokuz Eylul University Medicine Faculty, Department of Medical Genetics

Microcephalic primordial dwarfisms (MOPDs) are autosomal recessive disorders characterized by prenatal and postnatal growth retardation, microcephaly and distinct facial features with prominent beaked nose, receding forehead, and micrognathia. The most common type is MOPD type II and it's characteristic features are specific growth pattern with very severe IUGR, adult height below 110 cm, neonatal proportioned head size later progressing to microcephaly, absent or mild mental retardation, typical bone dysplasia and small loose secondary dentition. Here we report a 6 months old boy with MOPD II syndrome diagnosed by clinical and radiographic findings and confirmed by mutation analysis. As far as we know ten genes have been identified for microcephalic primordial dwarfism, encoding proteins involved in fundamental cellular processes including genome replication, DNA damage response, mRNA splicing and centrosome function. We find a novel premature stop codon mutation in the PCNT gene. Hence, the diagnosis of MOPD by mutation analysis can be a valuable method for patients. Furthermore, in the near future, other genes could be identified in patients with MOPD.

SOS1 ASSOCIATED NOONAN SYNDROME PRESENTING WITH SUPERNUMARY TEETH; A CASE REPORT

Gulser Kılınc[1], Ozge Aksel[2], Mujdet Cetin[1], Hüseyin Onay[3], Ozlem Giray Bozkaya[4]

1.Dokuz Eylul University Faculty Of Medicine, Pediatric Dental Clinic.
2. Dokuz Eylul University Faculty Of Medicine, Department of Medical Genetics.
3. Ege University Faculty Of Medicine, Department of Medical Genetics.
4. Dokuz Eylul University Faculty of Medicine, Department of Pediatrics, Division of Genetics and Department of Medical Genetics.

Noonan syndrome (NS) is a common autosomal dominant disorder which is characterized by chest deformation, congenital heart disease, short stature and distinctive facial features. Severe gingivitis and supernumerary teeth are rarely seen in connection with NS. In this report, a 12-year-old boy with Noonan syndrome whose oral examination revealed an anterior open bite, supernumerary teeth, severe anterior gingival enlargement, dental crowding associated with diestema, tapered incisors, narrow high-arched palate and prominent rugae was presented.

DNA diagnostics of MIDD and MELAS syndromes in Slovakia

Skopkova M.[1], Masindova I.[1], Valentinova L.[1], Stanik J.[1,2], Varga L.[1,3], Huckova M.[1], Danis D.[1], Slovak MIDD/MELAS Study Group, Profant M.[3], Klimes I.[1] and Gasperikova D.[1]

[1]DIABGENE & Diabetes Laboratory, Institute of Experimental Endocrinology, SAS, Bratislava, Slovakia, [2]First Department of Pediatrics, School of Medicine, Comenius University, Bratislava, Slovakia; [3]First Department of Otorhinolaryngology, School of Medicine, Comenius University, Bratislava, Slovakia

Background. The two syndromes, MIDD (Maternally Inherited Diabetes and Deafness) and MELAS (Mitochondrial Myopathy, Encephalopathy, Lactic Acidosis and Stroke-like episodes) arise on a common genetic cause – a mutation in mtDNA, most often the m.3243A>G transition in the gene *MT-TL1* for $tRNA^{Leu(UUR)}$. This mutation leads to different clinical symptoms according to the heteroplasmy levels in different tissues. Usually, the first presentation of the MIDD syndrome is a progressive bilateral sensorineural hearing loss emerging in adolescence, meanwhile, diabetes develops mostly between 30 – 40 years of life. The MELAS syndrome has a more severe progression with further neurological and metabolic symptoms.

Patients and methods. The aim of our study was to search for the m.3243A>G mutation among patients fulfilling at least one of the following criteria: matrilineal inheritance, conjoint diabetes and hearing impairment, diabetes development after 25th year of life, and progressive hearing loss. DNA of 255 unrelated probands was extracted from peripheral blood and/or buccal mucosa and analyzed for presence of m.3243A>G variant using RFLP and/or Real-Time PCR. DNA testing was also extended to the family members of probands carrying the mutation.

Results. The m.3243A>G mutation was found in 8 probands and 10 of their family members. Probands´ phenotypes varied from diabetes as the sole symptom to a complex picture of the MELAS syndrome (in one proband). All of the probands (100%), but only in 3 of 10 relatives with the mutation (30%), developed diabetes or impaired glucose tolerance (with onset age from 21 to 54 years). Five probands (62.5%), and 4 (40%) of the relatives with the mutation had hearing impairment (with onset age from 16 to 59 years). Three family members, aged 18, 28 and 33 years (buccal heteroplasmy levels 58, 33 and 15%, respectively), were

asymptomatic in the time of blood withdrawal. The heteroplasmy was higher in DNA samples from buccal swabs compared to blood DNA samples. In one proband (age 54), suffering from diabetes, hearing impairment and retinopathy, the heteroplasmy was detected in his buccal DNA only, while the DNA sample gave negative results repeatedly.

Among 255 probands with the clinical suspicion on mitochondrial diabetes or hearing impairment, 8 (3%) had the m.3243A>G mutation. Other 10 mutation carriers were identified in their families. At the time of testing, only 5 (62.5%) of the probands were diagnosed with typical combination of symptoms, i.e. diabetes and hearing loss. Therefore, DNA testing for MIDD seems to be reasonable also in carefully selected diabetes patients without hearing impairment, and vice versa, particularly using DNA from the buccal mucosa. DNA testing permitted correct patient management and intensified surveillance of yet healthy mutation carriers.

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Spectrum of glucokinase (*GCK*) mutations with prevailing promotor mutation (c.-87G>C) and prevalence of monogenic diabetes type GCK-MODY in Slovakia

Lucia Valentínová[1], Juraj Staník[1,2], Miroslava Hučková[1,3], Martina Sůrová, Slovak MODY Collaborative Study Group, Iwar Klimeš[1,3] and Daniela Gašperíková[1,3]

[1]DIABGENE & Diabetes Laboratory, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia
[2]First Department of Paediatrics, Comenius University, Bratislava, Slovakia
[3]Centre for Molecular Medicine, Slovak Academy of Sciences, Bratislava, Slovakia

Heterozygous inactivating glucokinase (GCK) mutations cause a subtype of maturity-onset diabetes of the young (GCK-MODY) characterised by familial mild stable fasting hyperglycaemia. Over 600 GCK mutations have been reported, nevertheless none of them prevails between GCK-carriers. The aim of the study was 1) to determine the GCK mutation spectrum and 2) to disclose the prevalence of GCK-carriers among the Slovakian population.

Patients and methods: 409 unrelated probands with clinical suspicion on MODY were referred for genetic testing to our laboratory between 2004 – 2013 from >100 clinical diabetologists and endocrinologists across Slovakia. Following the proband's clinical phenotype consistent with GCK-MODY, direct sequencing and MLPA analyses of pancreatic GCK gene were carried out.

Results: DNA analyses identified 37 different GCK mutations in 67 probands and 104 relatives located in promoter, coding and intronic regions. Missense mutations occurred most frequently – 78% (29/37), followed by deletions 8% (3/37), splice-site substitution 5% (2/37) and nonsense substitution 3% (1/37) promoter substitution 3% (1/37) and intronic variant 3% (1/37); no exon deletion was detected. Different exon mutations were scattered along the entire GCK length, no hotspot region was found. The most frequent

GCK mutation in Slovakia (promoter c.-87G>C) occurred in 27% (18/67) of unrelated families. In Slovakia with population of 5,4 millions the minimum prevalence of GCK-MODY was estimated to 32 cases/million inhabitants.

Conclusion: We have determined the GCK mutation profile with the Slovakian prevailing mutation (-c.87G>C). The minimum prevalence of GCK-MODY in Slovakia is 32 cases/million inhabitants, which is higher than studies in UK (Shields et al., Diabetologia 2010, Kropff et al, Diabetologia, 2011) and our previously published data.

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Replication of GWAS identified loci in the Tunisian population: Susceptibility and prognostic implications in Breast Cancer

Wijden Mahfoudh[1], Jingxuan Shan[2], Elham Hassen[1], Noureddine Bouaouina [1] and Lotfi Chouchane [2]

[1] *Laboratory of Immuno-Oncology, Faculty of Medicine of Monastir, University of Monastir, Tunisia.*

[2] *Genetic Medicine and Immunology Laboratory, Weill Cornell Medical College in Qatar, Qatar Foundation, Education City, P.O. Box 24144, Doha, Qatar.*

Recent genome-wide association studies (GWAS) have lead to the identification of multiple new genetic variants associated with breast cancer risk. Most of these breast cancer GWAS and replication studies have been conducted in European populations and to a lesser extent in Asians. Therefore, we designed a broad study to investigate the susceptibility and prognostic implications of the GWAS breast cancer loci in the Tunisian population.

In a cohort of 640 unrelated patients with breast cancer and 371 healthy control subjects, we characterized the variation of 9 single nucleotide polymorphisms (SNPs) using the TaqMan® SNP genotyping assays. The chi-square test was used for statistical analysis.

Only 5 (rs1219648, rs2981582, rs8051542, rs889312, rs13281615) out of 9 GWAS breast cancer loci were found to be significantly associated with breast cancer in Tunisians. The strongest associations were found for rs2981582 in the *FGFR2* gene and rs8051542 in the *TNRC9* gene (OR = 1.55, P = 3 × 10(-6); OR = 1.40, P = 4 × 10(-4), respectively). Homozygous variant genotypes of rs2981582 were strongly related to lymph node negative breast cancer (OR = 3.33, P = 6 × 10(-7)) and the minor allele of rs2981582 was associated with increased risk of ER+ tumors (OR = 2.15, P = 0.001) and increased risk of distant metastasis development (OR = 3.57, P = 6 × 10(-5)). The association for rs8051542 was stronger for high-grade SBR tumors (OR = 2.54, P = 2 × 10(-4)). GG genotype of rs13387042 on 2q35 showed a significant association with the risk of developing distant metastasis (OR = 1.94, P = 0.02).

In conclusion, GWAS breast cancer FGFR2, TNRC9, MAP3K1, and 8q24 loci are associated with an increased risk of breast cancer and genetic variation in FGFR2 gene may predict the aggressiveness of breast cancer in Tunisians.

Investigation of DNA methylation differences in the canine oxytocin receptor gene promoter between differently raised dogs and wolves

Zsofia Banlaki[1], Giulia Cimarelli[2,3], Zsofia Viranyi[2,3], Jozsef Topal [5], Dora Koller[1,4], Maria Sasvari-Szekely[1], Zsolt Ronai[1]

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

Oxytocin receptor (OXTR) acts as a behavioral modulator within the central nervous system (CNS), affecting stress, aggression, affiliation and cognitive functions. Genetic polymorphisms in the OXTR gene of both human and animal are known to influence behavior, but little is known about the presence and effect of epigenetic variations. DNA methylation pattern is generally stable and has the ability of being transmitted during cell division; however, it can also change presumably due to environmental factors. Hypermethylation of CpG dinucleotides within promoter regions leads to transcriptional repression, and methylation level differences tend to accumulate in regions called CpG shores. Our aim was to elucidate whether species-specific differences exist in DNA methylation patterns within CpG shores near the canine OXTR promoter between dog and wolf, and also if keeping conditions affect these patterns. We developed novel pyrosequencing based assays and prepared completely unmethylated and totally methylated control DNA samples *in vitro* to assess the reliability of the results. As a pilot study, methylation levels were investigated in small groups of differently treated animals of both species (trained wolves, non-trained human-raised wolves, pet dogs and pack dogs), in order to identify regions of potential interest. Plans for the near future include measurement of DNA methylation at the selected candidate loci in 200 pet border collies raised and trained by distinct styles and already tested for a variety of behavioral markers. These results will hopefully contribute to the understanding of the biological background of behavioral aspects such as friendliness, reliance and aggression in dogs.

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Students Who's Who

SURNAME - NAME	AFFILIATION	NATIONALITY	CITY	MAIL
AL NABHANI MARYAM	Qaboos University Hospital	OMAN	MUSCAT	alnabhani_mr@hotmail.co
AKSEL OZGE	Eylul University	TURKEY	IZMIR	ozge.aksel@deu.edu.tr
BANLAKI ZSOFIA	Semmelweis University	HUNGARY	BUDAPEST	banlaki.zsofia@med.semmelweis-univ.hu
BASART HANNEKE	Academic Medical Centre	THE NETHERLANDS	AMSTERDAM	h.basart@amc.nl
CARVALHO ANA	Centro Hospitalar e Universitario de Coimbra	PORTUGAL	COIMBRA	aluisa.dcarvalho@gmail.com
CASALONE ELISABETTA	HUGEF - Human Gentics Foundation Torini	ITALY	TORINO	barbara.bertetto@csp-st.it
CELADA VERA	Caja Costarricense Seguro Social	SPAIN	SAN RAFAEL	veracelada@gmail.com
CRAPANZANO MIRELLA IRENE STELLA	University of Messina	ITALY	MESSINA	mirella.crapanzano@hotmail.it
DE BIE CHARLOTTE	UMC Utrecht	THE NETHERLANDS	AMSTERDAM	c.i.debie-2@umcutrecht.nl
DENNIS CATHERINE	Northern Genetics Service	UK	NEWSCASTLE	catherine.dennis@nuth.nhs.uk
ELINE OVERWATER	Academic Medical Centre	THE NETHERLANDS	AMSTERDAM	e.overwater@amc.nl
FEBEN CANDICE	NHLS and the University of Witwatersrand	SOUTH AFRICA	JOHANNESBURG	candice.feben@nhls.ac.za
FLOOR DUIJKERS		THE NETHERLANDS	UTRECHT	f.a.duijkers@amc.nl
GERMAINE DOMINIQUE	University of Versailles	FRANCE	BOULOGNE BILLANCOURT	dominique.germain@rpc.aphp.fr
GOHRINGER CAROLINE	Institute of Clinical Genetics Heidelberg	GERMANY	HEIDELBERG	c_goehringer@web.de
GORKA PAULINA	Clinic of Neurology of Child and Adolescents, Institute of Mother and Child, Warsaw, Poland	POLAND	WARSAW	paulina.gorka19@wp.pl
HANKEL MARGOT	VU Medical Centre	THE NETHERLANDS	AMSTERDAM	m.hankel@vumc.nl
HOLLINK IRIS	Erasmus MC	THE NETHERLANDS	ROTTERDAM	i.hollink@erasmusmc.nl
HOPMAN SASKIA	Utrecht University	THE NETHERLANDS	AMSTERDAM	shopman2@umcutrecht.nl
OZLEM OZ	Dokuz Eylul Medicine faculty, Dept. Of Medical Genetics	TURKEY	IZMIR	ozlemdroz@hotmail.com
IVANOV OFVERHOLM INGEGERD	Karolinska Institutet	SWEDEN	STOCKHOLM	ingegerd.ofverholm@ki.se
KAISER ANN-SOPHIE	Institute of Human Genetics, Heidelberg University	GERMANY	HEIDELBERG	a.kaiser@med.uni-heidelberg.de
KHAZANEHDARI KAMAL	MGB Laboratory	EMIRATI ARABI	DUBAI	kamalk@mbg.ae
KLANCAR GASPER	University Medical Center Ljubljana	SLOVENIA	LJUBLJANA	gasper.klancar@kclj.si
KOROLEVA IULIIA	Research Institute of Medical Genetics	RUSSIA	TOMSK	korolevajua@gmail.com
KOUDIJS MARCO	UMC Utrecht	THE NETHERLANDS	UTRECHT	m.j.koudijs@umcutrecht.nl
KRAUS SARAH	University of Cape Town	SOUTH AFRICA	CAPE TOWN	s.kraus@uct.ac.za

LIU YICHENG	Center for Molecular Medicine	GERMANY	COLOGNE	liuyicheng84427@163.com
LODDO ITALIA	University of Messina	ITALY	REGGIO CALABRIA	italia.loddo@gmail.com
LOPES DE ALMEIDA MARIA	Centro Hospitalar e Universitario de Coimbra	PORTUGAL	COIMBRA	marialopesdealmeida@hotmail.com
LOURO PEDRO	Centro Hospitalar e Universitario de Coimbra	PORTUGAL	COVILHA	pjlouro@gmail.com
MEIER STEPHANIE	University Hospital Basel	SWITZERLAND	BASEL	stephanie.meier@usb.ch
MENDOZA FERREIRA NATALIA	Institute of Human Genetics	GERMANY	COLOGNE	nmendoza@smail.uni-koeln.de
OTAIFY GHADA	National Research Centre	EGYPT	CAIRO	ghadaotaify@yahoo.com
PETERS MIRIAM	Institute of Human Genetics	GERMANY	COLOGNE	miriam.peters@uk-koeln.de
PHILIPS ANJU	University of Helsinki	FINLAND	HELSINKI	anju.philips@helsinki.fi
POPP BERNT	Humangenetisches Institut Friedrich Alexander Universitat	GERMANY	NURNBERG	bernt.popp@uk-erlangen.de
PORRET NAOMI	Inselspital Bern	SWITZERLAND	BERN	naomi.porret@insel.ch
POUROVA RADKA	University Hospital Motol	CZECH REPUBLIC	PRAGUE	radka.pourova@fnmotol.cz
REIJNDERS MARGOT	Radboudumc	THE NETHERLANDS	ARNHEM	margot.reijnders@radboudumc.nl
SAHLIN ELLIKA	Karolinska Institutet	SWEDEN	STOCKHOLM	ellika.sahlin@ki.se
SAHNI JAYASHREE	Royal Liverpool University Hospital NHS Trust	UNITED KINGDOM	LIVERPOOL	jayashreesahni@yahoo.co.uk
SCHOTIK MARIA	Institute of Human Genetics	GERMANY	DUSSELDORF	maria.schotik@googlemail.com
SCHROM EVA MARIA	Institute of Human Genetics, Biozentrum	GERMANY	WURZBURG	eva-maria.schrom@uni-wuerburg.de
SHAUL LOTAN NAVA	Hadassh Hebrew University Medical Center	ISRAEL	JERUSALEM	navashaul34@gmail.com
SINNEMA MARGJE	Dept. Of Clinical Genetics MUMC	THE NETHERLANDS	MAASTRICHT	margje.sinnema@mumc.nl
SIRISENA NIRMALA	University of Colombo	SRI LANKA	COLOMBO	nirmalasirisena@yahoo.com
SKOPOVA MARTINA	Institute of Experimental Endocrinology SAS	SLOVAKIA	BRATISLAVA	martina.skopkova@savba.sk
SNIJDERS BLOK LOT	Radboud University Medical Center	THE NETHERLANDS	NIJMEGEN	lot.snijdersblok@radboudumc.nl
STALMAN SUSANNE	Tergooi Hospitals	THE NETHERLANDS	AMSTERDAM	se.stalman@gmail.com
STERNA OLGA	University Children's Hospital	LETTONIA	RIGA	olga.sterna@bkus.lv
VALENTINOVA LUCIA	Institute of Experimental Endocrinology SAS	SLOVAKIA	BRATISLAVA	lucia.valentinova@savba.sk
VAN KAAM KIM	Maastricht University Medical Center	THE NETHERLANDS	MAASTRICHT	kim.van.kaam@mumc.nl
VANHOUTTE ELS	Department of Clinical Genetics MUMC	THE NETHERLANDS	MAASTRICHT	e.vanhoutte@mumc.nl
WIJDEN MAHFOUDH	Faculty of Medicine of Monastir	TUNISA	MONASTIR	wijdene_mahfoudh@yahoo.fr
ZEIDLER SHIMRIET	Erasmus MC	THE NETHERLANDS	ROTTERDAM	s.zeidler@erasmusmc.nl

FACULTY Who is Who

Family Name	Name	Affiliation	Work e-mail
Brunner	**Han**	Radboud University Nijmegen Medical Center Department of Human Genetics, Nijmegen the Netherlands	h.brunner@gen.umcn.nl
Burn	**John**	Newcastle University Genetics Chair, National Institute of Health Research Biomedicine West, Centre for Life, Newcastle UK	john.burn@newcastle.ac.uk
Cucca	**Francesco**	nstitute for Genetics & Biomedical Research (IRGB), University of Sassari, Italy	fcucca@uniss.it
Donnai	**Dian**	University of Manchester , Faculty of Medical & Human Sciences - Manchester, UK	dian.donnai@cmft.nhs.uk
Gasparre	**Giuseppe**	Dept. of Medical and Surgical Sciences, Unit of Medical Genetics, University of Bologna, Italy	giuseppe.gasparre3@unibo.it
Gilissen	**Christian**	Nijmegen Centre for Molecular Life Sciences Radboud University Nijmegen Medical Centre, The Netherlands	Christian.Gilissen@radboudumc.nl
Klopocky	**Eva**	Universität Würzburg Institut für Humangenetik Biozentrum Am Hubland,Würzburg Germany	eva.klopocki@uni-wuerzburg.de
Loeys	**Bart**	Center for Medical Genetics, Antwerp University Hospital, Edegem Belgium	bart.loeys@uantwerpen.be
Katsanis	**Nicholas**	Duke University Medical Center Durham,USA	nicholas.katsanis@duke.edu

Read	**Andrew**	University of Manchester, Human Development and Reproductive Health Academic Group , UK	Andrew.Read@manchester.ac.uk
Romeo	**Giovanni**	University of Bologna, European Genetics Foundation, Bologna, Italy	romeo@eurogene.org
Rugarli	**Elena**	University of Cologne, Germany	elena.rugarli@uni-koeln.de
Temple	**Karen**	Faculty of Medicine, University of Southampton, UK	I.K.Temple@soton.ac.uk
Veltman	**Joris**	Department of Human Genetics - Nijmegen Centre for Molecular Life Sciences Radboud University Nijmegen Medical Centre, The Netherlands	Joris.Veltman@radboudumc.nl
Vermeesch	**Joris**	Katholieke Universiteit Leuven - Department of Human Genetics Leuven, Belgium	joris.vermeesch@uzleuven.be
Wirth	**Brunhilde**	Institute of Human Genetics University Hospital of Cologne, Germany	brunhilde.wirth@uk-koeln.de
Wijmenga	**Cisca**	University Medical Center Groningen Department of Human Genetics GRONINGEN The Netherlands	cisca.wijmenga@med.umcg.nl
Wollnik	**Bernd**	Center For Molecular Medicine, University of Cologne , Germany	bwollnik@uni-koeln.de