



# European School of Genetic Medicine

## 26<sup>th</sup> Course in Medical Genetics

**Bertinoro, Italy, May 12-16, 2013**

Bertinoro University Residential Centre  
Via Frangipane, 6 – Bertinoro

### **Course Directors:**

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# 26<sup>th</sup> COURSE IN MEDICAL GENETICS

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Bertinoro (Italy), May 12-16, 2013**

**Arrival day: Saturday May 11**

**Sunday, May 12**

**Morning Session: Introduction to Human Genome Analysis**

9.00 – 9.30	Introduction to the course <b>G. Romeo</b>
9.30 – 10.10	Medical Genetics Today <b>D. Donnai</b>
10.10 – 10.50	Genotypes & phenotypes <b>H. Brunner</b>
10.50 – 11.20	<b>Coffee Break</b>
11.20 – 12.00	Arrays & CNVs <b>J. Vermeesch</b>
12.00 – 12.40	Molecular syndromology in the NGS-era: which phenotype, which family, which strategy? <b>B. Wollnik</b>
12.40 – 14.00	<b>Lunch Break</b>

**Afternoon Session:**

14.00 – 14.30	Poster Viewing Session
14.30 – 16.00	Concurrent Workshops
16.00-16.30	<b>Coffee Break</b>
16.30 – 18.00	Concurrent Workshops

## Monday, May 13

### Morning Session: Approaches to Clinical and Molecular Genetics

- 9.00 – 9.50 Mitochondrial inheritance and disease  
**P. Chinnery**
- 9.50 – 10.40 Linkage and association in a conceptual and historic perspective  
**A. Read**
- 10.40 – 11.10 Coffee Break
- 11.10- 12.00 Complex disease genetics: GWAS and beyond  
**C. van Duijn**
- 12.00 – 12.50 Basic Concepts in Dysmorphology and Syndrome classification  
**D. Donnai**
- 12.50 – 14.00 **Lunch Break**

### Afternoon Session:

- 14.00 –14.30 Poster Viewing Session
- 14.30 – 16.00 Concurrent Workshops
- 16.00-16.30 **Coffee Break**
- 16.30 – 18.00 Concurrent Workshops

## Tuesday, May 14

### Morning Session: From Monogenic to Complex Genetic Disorders

- 9.00 – 9.50 Aging phenotypes  
**B. Wollnik**
- 9.50 – 10.40 The ciliopathies: model disorders to study epistasis and total mutational load  
Oligogenic inheritance  
**E. Davis**
- 10.40 – 11.10 **Coffee Break**
- 11.10 – 12.00 Marfan syndrome, related diseases and therapy  
**B. Loeys**
- 12.00 – 12:5 Epigenetics and disease – lessons from imprinting disorders  
**K. Temple**

13:00 – 14.00      **Lunch Break**

**Afternoon Session:**

14.00 –14.30      Poster Viewing Session

14.30 – 16.00      Concurrent Workshops

16.00-16.30      Coffee Break

16.30 – 18.00      Concurrent Workshops

**Wednesday, May 15**

**Morning Session: From Gene Identification and Gene Regulation to Therapy and Ethical Implications**

9.00 – 9.50      Gene identification for Intellectual Deficiencies (I.D.)  
**H. Brunner**

9.50 – 10.40      SMA: From gene and modifiers to therapy  
**B. Wirth**

10.40 – 11.10      Coffee Break

11.10 – 12.00      Long distance regulation in skeletal disorders  
**E. Klopocki**

12.00 – 12.50      Ethical Issues  
**A. Read**

13:10 - 14.00      **Lunch Break**

**Afternoon Session:**

14.00 –14.30      Poster Viewing Session

14.30 – 16.00      Concurrent Workshops

16.00 – 16.30      Coffee Break

16.30 – 18.00      Concurrent Workshops

**Thursday, May 16**

**Morning Session: Next Generation Sequencing**

9.00 – 9.50 Introduction in Next Generation Sequencing technologies and applications  
**J. Veltman**

9.50 – 10.40 How to deal with next generation sequencing output.  
**C. Gilissen**

10.40 – 11.00 **Coffee Break**

11.00 – 11.50 Best Posters Presentations by students

11.50 Wrapping up of the course

12.00 **Lunch**

**Departure**

# ABSTRACTS OF LECTURES

## Sunday, May 12

### Medical Genetics Today

**Dian Donnai**

*University of Manchester,  
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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.

Careful clinical observation is at the heart of medical genetic practice. Many important observations were made before the technology to explore them further existed (see review by Harper 2005). Examples include:

- Those that predated the recognition of the mutational mechanism in three relatively common genetic disorders. Myotonic dystrophy was long known to manifest the phenomenon called anticipation, where the offspring of mild or moderately affected women tended to be much more severely affected. Similarly in Huntington's Disease, children of classically affected fathers sometimes had onset in childhood, and in Fragile X families there were apparently unaffected transmitting males in early generations and the risk of an affected child seemed to increase down the generations. Eventually it turned out that all of these disorders shared a novel mutational mechanism, namely unstable trinucleotide repeats within the affected gene that tend to increase in number between generations. (La Spada AR, Taylor JP 2010).
- Clinical observations have suggested that many conditions with asymmetry and localized overgrowth or skin lesions are likely mosaic disorders and over the past two years this has been confirmed in Proteus syndrome, melanocytic nevus, linear sebaceous nevus, hemimegalencephaly syndromes, Ollier and Maffucci syndromes.

Similarly the concept of syndrome families (now known to closely match developmental pathways) was based largely on clinical observation (Spranger 1985, see review by Brunner and van Driel 2004). The examples usually given are the disorders associated with FGFR mutations (achondroplasia group of skeletal dysplasias) and disorders of the RAS-MAPK pathway (Noonan syndrome disorders) (Denayer et al. 2008). Interestingly the mosaic conditions mentioned in the paragraph above involve mutations in genes affecting pathways such as RAS-MAPK, PI3K-AKT-mTOR and IDH1/IDH2 which are also well described in common cancers.

The new technologies enabling targeted capture and massively parallel sequencing of individual genomes/exomes have resulted in major discoveries 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. Whilst there is still an emphasis on discovery, in some centres diagnostic applications of these new technologies are being rapidly introduced. Families of individuals with unknown disorders are being offered exome sequencing of trios (mother, father, child) (Veltman, Brunner 2012) or targeted testing using large panels of appropriate genes being offered to patients with specific disorders such as retinal dystrophy, cataract, epilepsy etc. (Rehm 2013). Interestingly early 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 research. 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 only group which our current services can 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.



Genetics is set to influence greatly the practice of medicine in the future. 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 and genetic laboratories may have a role in pharmacogenetic testing. Maybe the time has come to change the name of our specialty from Medical Genetics to Genetic Medicine.

## References

Bredenoord AL, de Vries MC, van Delden JJM Next generation sequencing: does the next generation still have a right to an open future? *Nature Reviews Genetics AOP*, published online 26 March 2013; doi:10.1038/nrg3459

Brunner HG, van Driel MA. From syndrome families to functional genomics. *Nat Rev Genet.* 2004;5:545-51

Denayer E, de Ravel T, Legius E. Clinical and molecular aspects of RAS related disorders. *J Med Genet* 45. 695-703. 2008

Harper PS. Julia Bell and the Treasury of Human Inheritance. *Hum Genet* 2005;116:422-32

Hood RL et al, Mutations in SRCAP, Encoding SNF2-Related CREBBP Activator Protein, Cause Floating-Harbor Syndrome *AJHG* 90, 1–6, February 10, 2012

La Spada AR, Taylor JP. Repeat expansion disease: progress and puzzles in disease pathogenesis. *Nature Genetics Reviews* 11. 247-258.2010

Mitchell K et al, Exome Sequence Identifies RIPK4 as the Bartsocas-Papas Syndrome Locus *AJHG* 90, 69–75, January 13, 2012

Ng SB et al. exome sequencing identifies the cause of a mendelian disorder. *Nature Genetics* 42. 30-36. 2010

Rehm HL Disease-targeted sequencing: a cornerstone in the clinic. *Nature Reviews Genetics* 14: (April) 295. 2013

Spranger J. Pattern recognition in bone dysplasias. *Prog Clin Biol Res* 1985; 200:315-42

Veltman JA, Brunner HG De novo mutations in human genetic disease. *Nature Rev Genet* 13: ( Aug 2012) 565

# Genotypes and Phenotypes

**Han Brunner**

*Human Genetics Radboud University, Nijmegen Medical Centre, 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.

Finally, FGFR2 mutations can cause Apert and Crouzon syndrome when activating but a completely different disease Lacrimo-Auriculo-Dento-Digital syndrome when inactivating

- 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 situation 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 muscle 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 form a heterotrimeric triple helix collagen protein.

3b. Similarly, all genes causing Usher syndrome appear to be involved in a single molecular complex of interacting proteins.

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.

#### References:

- Brunner HG, van Driel MA. From syndrome families to functional genomics. *Nat Rev Genet.* 5:545-551,2004.
- Oti M, Brunner HG. The modular nature of disease genetics. *Clin Genet* 71:1-11, 2007
- 

Kremer H, van Wijk E, Marker T, Wolfrum U, Roepman R. Usher syndrome: molecular links of pathogenesis, proteins and pathways. *Hum Mol Genet.* 2006 Oct 15;15 Spec No 2:R262-70.

Rinne T, Hamel B, Bokhoven HV, Brunner HG. Pattern of p63 mutations and their phenotypes-update. *Am J Med Genet A.* 140:1396-1406, 2006

van Reeuwijk J, Brunner HG, van Bokhoven H. Glyc-O-genetics of Walker-Warburg syndrome. *Clin Genet* 67:281-9, 2005.

Rohmann E, Brunner HG, Kayserili H, Uyguner O, Nürnberg G, Lew ED, Dobbie A, Eswarakumar VP, Uzumcu A, Ulubil-Emeroglu M, Leroy JG, Li Y, Becker C, Lehnerdt K, Cremers CWRJ, Yuksel-Apak M, Nürnberg P, Kubisch C, Schlessinger J, van Bokhoven H, Wollnik B. Mutations in different components of FGF-signalling in LADD syndrome. *Nature Genetics.* 38:414 – 417, 2006

# Arrays and CNVs

Joris Vermeesch

K.U.Leuven, Belgium

In addition to single nucleotide variation, copy number variations (CNVs) are recognized as another major class of variants. In this session the mechanisms leading to and the different mechanisms by which CNVs can cause genetic variation will be illustrated. 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

Vermeesch, J. (2009). *Mendelian CNV mutations*. In: Copy Number Variation. London: Henry Stewart Talks Ltd.

Van Vooren, S., Coessens, B., De Moor, B., Moreau, Y., Vermeesch, J. (2007). Array comparative genomic hybridization and computational genome annotation in constitutional cytogenetics: suggesting candidate genes for novel submicroscopic chromosomal imbalance syndromes. *Genetics in Medicine*, 9(9), 642-649

Vermeesch, J., Fiegler, H., de Leeuw, N., Szuhai, K., Schoumans, J., Ciccone, R., Speleman, F., Rauch, A., Clayton-Smith, J., Van Ravenswaaij, C., Sanlaville, D., Patsalis, P., Firth, H., Devriendt, K., Zuffardi, O. (2007). Guidelines for molecular karyotyping in constitutional genetic diagnosis. *European Journal of Human Genetics*, 15(11), 1105-1114

Miller, D., Adam, M., Aradhya, S., Biesecker, L., Brothman, A., Carter, N., Church, D., Crolla, J., Eichler, E., Epstein, C., Faucett, W., Feuk, L., Friedman, J., Hamosh, A., Jackson, L., Kaminsky, E., Kok, K., Krantz, I., Kuhn, R., Lee, C., Ostell, J., Rosenberg, C., Scherer, S., Spinner, N., Stavropoulos, D., Tepperberg, J., Thorland, E., Vermeesch, J., Waggoner, D., Watson, M., Martin, C., Ledbetter, D. (2010). Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *American Journal of Human Genetics*, 86(5), 749-764

Robberecht, C., Vanneste, E., Pexsters, A., D'Hooghe, T., Voet, T., Vermeesch, J. (2010). Somatic genomic variations in early human prenatal development. *Current Genomics*, 11(6), 397-401

Brady, P., Vermeesch, J. (2012). Genomic microarrays: a technology overview. *Prenatal Diagnosis*, 32(4), art.nr. 10.1002/pd.2933, 336-43

Vermeesch, J., Brady, P., Sanlaville, D., Kok, K., Hastings, R. (2012). Genome-wide arrays: Quality criteria and platforms to be used in routine diagnostics. *Human Mutation*, 33(6),

Lee C, Iafrate AJ, Brothman AR. (2007) Copy number variations and clinical cytogenetic diagnosis of constitutional disorders. *Nat Genet*. Jul;39(7 Suppl):S48-54.

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

**Bernd Wollnik**

*Institute of Human Genetics 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 are now expecting 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.

# Monday, May 13

## Mitochondrial inheritance and disease

**Patrick Chinnery**

*University of Manchester, Genetic 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, and to be a dysmorphologist one needs to be knowledgeable in many areas, from embryology, through a wide range of clinical disciplines to genetic counselling. Currently, the term “dysmorphology” is most commonly used to refer to 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. There is no doubt, however, that some families find it difficult at first to have a “syndrome label” attached to their child, and perceive some disadvantages of having such a diagnosis. A dysmorphologist needs to be sensitive to these concerns when dealing with the family.

### **Benefits of Syndrome Diagnosis**

- Provision of accurate information about the condition, and its natural history and its prognosis, for parents and for 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)
- **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. Routine karyotype is still offered as the first-line test in most centres and where a specific microdeletion syndrome is suspected the appropriate FISH test is done. 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. However there is now increasing use of newer techniques such as array CGH, often as a first-line test, with phasing out of traditional karyotyping. Interpretation of such tests is complex and may require parental samples and consultation of databases of CNV variants to elucidate whether a finding is clinically significant.
- Single gene molecular genetic tests are now available for many different conditions and the range of tests is widening rapidly as more genes are associated with the many hundreds of single gene syndromes. There are databases which can be used to identify laboratories offering testing for specific disorders. Unfortunately testing may not be available on a service basis at the present time for many rare conditions.
- Whole exome sequencing offers the promise of an almost ‘comprehensive’ genetic test where all coding exons of all genes are screened but it will be a few years before this is widely available in a diagnostic setting. Some laboratories are developing focused diagnostic panels enriched for exons associated with various groups of conditions such as retinal dystrophies, Noonan-like disorders etc.
- 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 or seizures occurring soon after birth.
- Infection screen is helpful where congenital infection is suspected from the history or from clinical signs.
- Imaging. 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. 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, the timing of death 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 etc) Also consider disturbances in gene expression e.g. imprinted genes. Many syndromes are now known to be due to mutations in genes involved in chromatin configuration.
- 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’ 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’
  - Etc.etc

### **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. Other databases such as REAMS and OSSUM specialize in skeletal dysplasias. 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](http://www.esdn.org)) and DYSCERNE ([www.dyscerne.org](http://www.dyscerne.org)) a new European initiative for dysmorphic syndromes which will have at least one 'node' in each EU country.

#### **Following the Diagnosis**

- Clinical diagnosis should be confirmed with a diagnostic test if available
- Even experienced doctors 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 associate.

#### **What if the diagnosis remains unknown?**

A child should not be labelled as having a particular dysmorphic syndrome unless the clinician 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.

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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## Linkage and association (in a conceptual and historic perspective)

**Andrew Read**

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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 association is in principle more powerful than linkage for detecting weak susceptibility factors. In reality, although hundreds of weak susceptibility factors have been identified by GWAS, 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.

## **Complex disease genetics: GWAS and beyond**

**Cornelia Van Duijn**

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Genome wide association studies are rapidly uncovering an increasing number of loci involved in complex diseases. With some exceptions such as age related macular degeneration, for many traits including dyslipidemia and Alzheimer's disease, it remains difficult to predict risks for the future based on these common variants. At present, the utility of genome tests is limited, predominantly because they lack predictive ability and clear benefits for disease prevention that are specific for genetic risk groups. In the near future, personal genome tests will likely be based on whole genome sequencing, but will these technological advances increase the utility of personal genome testing? The utility of testing depends on the predictive ability of the test, the likelihood of actionable test results, and the options available for the reduction of risks. For monogenic forms of disease, it will be a challenge to recognize new causal variants among all rare variants that are found using sequencing. For complex diseases, the predictive ability of genetic tests will be mainly restricted by the heritability of the disease, but also by the genetic complexity of the disease etiology, which determines the extent to which the heritability can be understood. Given that numerous genetic and non-genetic risk factors may contribute to complex diseases, the predictive ability of genetic models will likely remain modest for most of the people. However, an important exception may be those with extreme phenotypes, e.g., extremely high levels of lipids, which are clinically most relevant. Another avenue for improving predictions will be identification of biomarkers using new -omic technology. A powerful approach will be to build the search for biomarkers on genetic studies.

### ***Basic Concepts in Dysmorphology and Syndrome classification***

**Dian Donnai (and Jill Clayton-Smith)**

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#### **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](http://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 increasingly translation to service diagnostics means that testing for large panels of genes or even whole exome sequencing will be common before too long.
- 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.
- Radiological investigations. 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)

8. 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?

9. 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 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

10. 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/Retts-like syndromes
- Noonan-like syndromes
- Neurocutaneous and Vascular syndromes
- Ectodermal dysplasias and other skin disorders
- Distinct MCA/MR syndromes with a ‘gestalt’
- Etc.etc

11. 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.

12. 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.

13. Search the Dysmorphology Databases

There are several available including the London Dysmorphology Database and POSSUM. Other databases such as REAMS and OSSUM specialise in skeletal dysplasias. 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.

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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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# Tuesday, May 14

## Aging phenotypes

**Bernd Wollnik**

*Institute of Human Genetics 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. 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.

## **The ciliopathies: model disorders to study epistasis and total mutational load**

**Erica Davis**

*Center for Human Disease Modeling, Duke University Medical Center*

The overwhelming majority of human genetic disorders manifest substantial inter- and intra-familial variability, in part due to the action of second-site modifiers on the primary causal locus<sup>1</sup>. In contrast with the identification of *bona fide* causal genes and alleles, the dissection of second-site phenomena in humans is lagging significantly, in part due to the lack of genetic power and a general paucity of functional information. This, in turn, leads to the limited utility of genetic information at the primary disease locus in terms of prognosis, patient management and, potentially, therapeutic strategies. The ciliopathies, a phenotypically and genetically heterogeneous group of disorders that manifest overlapping phenotypes such as retinal degeneration; skeletal and limb defects; central and peripheral nervous system defects; and renal, pancreatic and biliary cysts and fibrosis; have emerged in recent years as a useful model to study the effect of *trans* alleles on primary disease loci<sup>2,3</sup>. This is because a) they represent a severity/pleiotropy continuum with imperfect genotype-phenotype correlations at the primary locus; b) they are caused by structural and/or functional defects at the primary cilium, a semi-closed system whose constituent protein components are largely known; and c) the effect of ciliary dysfunction can be captured quantitatively using downstream *in vivo* functional assays and *in vitro* reporters derived from an improved understanding of this organelle in morphogenetic signaling<sup>4</sup>. Although each ciliopathy is individually rare, collectively their contribution to the overall genetic disease burden in humans approaches population frequency similar to that of common defects

such as Down syndrome, with a minimal estimated collective incidence of ~1:1,000 conceptuses<sup>5</sup>. To understand how *trans* mutations in a functional system can contribute to clinical variability in this disease group, we previously conducted unbiased medical resequencing of candidate ciliary genes in a large patient cohort across the ciliopathy severity spectrum. These studies have led to the identification of both new causal genes and also a number of *trans* alleles with a potential effect on penetrance and/or expressivity. In this lecture, we will discuss several examples, including:

1) Identification of a retinal modifier of ciliary disease, a p.Ala229Thr encoding allele in *RPGRIP1L*<sup>6</sup>. *RPGRIP1L* (retinitis pigmentosa GTPase regulator interacting protein-1 like) encodes a ciliary protein known to cause both neonatal lethal Meckel-Gruber Syndrome (MKS)<sup>7</sup> and moderate Joubert Syndrome (JBTS)<sup>7-9</sup>; we identified a p.Ala229Thr change present at intermediate population frequency, but enriched in ciliopathy patients with retinal degeneration. Using *in vivo* and *in vitro* tools, we found that the Thr229 allele disrupts the direct interaction between *RPGRIP1L* and *RPGR* (Retinitis Pigmentosa GTPase regulator), the most frequent genetic cause of X-linked Retinitis Pigmentosa (XLRP)<sup>10</sup>, thereby providing a plausible mechanistic explanation. Together, this modulator represents an important step towards improving the predictive power of the genotype in the ciliary disease group.

2) Identification of *TTC21B*, a retrograde intraflagellar transport protein as not only a novel cause of phenotypically discrete ciliopathies, isolated nephronophthisis (NPHP) and Jeune asphyxiating thoracic dystrophy (JATD), but also a significant contributor to mutational load in ciliopathies<sup>11</sup>. Mutational screening of *TTC21B* was the first large-scale analysis of an axonemal protein-encoding locus across the ciliopathy spectrum ranging from mild (isolated NPHP), moderate (Bardet-Biedl syndrome (BBS), JBTS) to severe (MKS, JATD), and sequencing data combined with functional assays revealed a five-fold enrichment of pathogenic *TTC21B* variants in the ciliopathy cohort in comparison to healthy controls. Moreover, genetic interaction studies demonstrated that genetic sensitization of retrograde IFT probably exacerbates at least 13 different primary ciliopathy loci. Together, this locus contributes to the mutational burden of 5% of individuals with ciliopathies.

Despite this progress, the fundamental challenge of predicting phenotype and or clinical progression based on single locus information remains incompletely understood. We anticipate that saturated knowledge of allele quality and quantity across the ciliopathy phenotypic spectrum can be synthesized further to generate improved models that can explain both causality and also improve our resolution of the mechanisms underscoring variable penetrance and expressivity. These findings will in turn, likely be applicable toward explaining phenotypic variability in other forms of Mendelian disease.

## References

1. Badano, J.L. & Katsanis, N. Beyond Mendel: an evolving view of human genetic disease transmission. *Nat Rev Genet* **3**, 779-89 (2002).
2. Badano, J.L., Mitsuma, N., Beales, P.L. & Katsanis, N. The ciliopathies: an emerging class of human genetic disorders. *Annu Rev Genomics Hum Genet* **7**, 125-48 (2006).
3. Baker, K. & Beales, P.L. Making sense of cilia in disease: the human ciliopathies. *Am J Med Genet C Semin Med Genet.* **151C**, 281-95 (2009).
4. Gerdes, J.M., Davis, E.E. & Katsanis, N. The vertebrate primary cilium in development, homeostasis, and disease. *Cell* **137**, 32-45 (2009).
5. Davis, E.E. & Katsanis, N. The ciliopathies: a transitional model into systems biology of human genetic disease. *Curr Opin Genet Dev* **22**, 290-303 (2012).
6. Khanna, H. *et al.* A common allele in RPGRIP1L is a modifier of retinal degeneration in ciliopathies. *Nat Genet* **41**, 739-45 (2009).
7. Delous, M. *et al.* The ciliary gene RPGRIP1L is mutated in cerebello-oculo-renal syndrome (Joubert syndrome type B) and Meckel syndrome. *Nat Genet* **39**, 875-81 (2007).
8. Arts, H.H. *et al.* Mutations in the gene encoding the basal body protein RPGRIP1L, a nephrocystin-4 interactor, cause Joubert syndrome. *Nat Genet* **39**, 882-8 (2007).
9. Wolf, M.T. *et al.* Mutational analysis of the RPGRIP1L gene in patients with Joubert syndrome and nephronophthisis. *Kidney Int* **72**, 1520-6 (2007).
10. Shu, X. *et al.* RPGR mutation analysis and disease: an update. *Hum Mutat* **28**, 322-8 (2007).
11. Davis, E.E. *et al.* TTC21B contributes both causal and modifying alleles across the ciliopathy spectrum. *Nat Genet* **43**, 189-96 (2011).

## Marfan syndrome and related disorders: from gene to therapy

**Bart Loeys**

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

Most recently, mutations in other components of the TGFbeta signaling pathway, including SMAD3, TGFB2 and SKI have been associated with LDS-like phenotypes and Shprintzen-Goldberg syndrome. Finally, perhaps most intriguingly we identified domain specific *FBNI* mutations as the molecular cause of a congenital form of scleroderma, stiff skin syndrome (SSS). We demonstrated that altered cell-matrix

interactions in SSS accompany excessive microfibrillar deposition, impaired elastogenesis, and increased TGF $\beta$  concentration and signaling in the dermis.

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.

## **Epigenetics and disease – lessons from imprinting disorders**

**Karen Temple**

*Faculty of Medicine, University of Southampton, United Kingdom*

### **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. Imprinted genes are therefore candidate genes for disorders such as diabetes.

## Imprinting Disorders

Several well-known disorders of imprinting are known including Beckwith Wiedemann syndrome, Transient Neonatal Diabetes, Temple syndrome, Wang syndrome, Silver Russell 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	approx.. 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 UPD 14 associated syndrome)	unknown	Intrauterine growth retardation Hypotonia, Scoliosis Developmental delay Early puberty, Short stature	Hydrocephalus Cleft palate	uncertain	(Kotzot)
Wang syndrome (Paternal UPD 14 associated syndrome)	unknown	Bell shaped chest Hypotonia Developmental delay	Umbilical defects Larger birth weight	uncertain	(Kagami et al.)
Pseudohypoparathyroidism 1B	unknown	Hypocalcaemia due to Parathyroid resistance (tetany/parasthesia)	Obesity	>90%+	(Bastepe et al.)

Bastepe, M. "The GNAS locus and pseudohypoparathyroidism." Adv.Exp.Med.Biol. 626 (2008): 27-40

Cassidy, S. B. and D. J. Driscoll. "Prader-Willi syndrome." Eur.J.Hum.Genet. 17.1 (2009): 3-13

Docherty LE, et al. Clinical presentation of 6q24 transient neonatal diabetes mellitus (6q24 TNDM) and genotype-phenotype correlation in an international cohort of patients. [Diabetologia](#). 2013 Feb 6. [Epub ahead of print]

Kagami, M., et al. "Deletions and epimutations affecting the human 14q32.2 imprinted region in individuals with paternal and maternal upd(14)-like phenotypes." Nat.Genet. 40.2 (2008): 237-42.

Kotzot, D. "Maternal uniparental disomy 14 dissection of the phenotype with respect to rare autosomal recessively inherited traits, trisomy mosaicism, and genomic imprinting." Ann.Genet. 47.3 (2004): 251-60.

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

Wakeling, E. L., et al. "Epigenotype-phenotype correlations in Silver-Russell syndrome." J.Med.Genet. (2010)

Weksberg, R., C. Shuman, and J. B. Beckwith. "Beckwith-Wiedemann syndrome." Eur.J.Hum.Genet. 18.1 (2010): 8-14.

Williams, C. A., D. J. Driscoll, and A. I. Dagli. "Clinical and genetic aspects of Angelman syndrome." Genet.Med. 12.7 (2010): 385-95.



# Wednesday, May 15

## Gene identification for Intellectual Deficiencies (I.D.)

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Behavioral genetics is an interesting field that has both single gene and multifactorial components.

Consider the following facts:

1. All human personal attributes such as personality are highly heritable
2. All human behavioral and psychiatric phenotypes including autism and schizophrenia are highly heritable
3. Several monogenic syndromes such as Prader-Willi syndrome, Williams syndrome, Smith-Magenis syndrome, Fragile X syndrome, and Velocardiofacial syndrome have quite specific behaviors
4. But most individual behaviors are complex, and almost all psychiatric diseases show complex inheritance patterns.

One example of the interplay between a single gene mutation, a regulatory polymorphism, and the environment is provided by the MAOA gene.

Complete absence of MAOA activity is associated with a highly reproducible behavioral phenotype. This mutation is extremely rare.

In contrast, a common regulatory polymorphism near the MAOA gene has been associated with a predisposition to antisocial behaviors but only in an adverse environment.

Whether and how behavioral genetics can be used to modify guilt and punishment in the courtroom is a topic of lively debate.

## SMA: FROM GENE AND MODIFIERS TOT HERAPY

**Brunhilde Wirth**

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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 ( $\Delta 7$ -*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 with 1 *SMN1* and 1-3 *SMN2* copies 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 the same in our 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 these 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.

1. Wirth, B., Brichta, L. and Hahnen, E. (2006) Spinal muscular atrophy: from gene to therapy. *Semin Pediatr Neurol*, **13**, 121-31.
2. Wirth, B., Riessland, M. and Hahnen, E. (2007) Drug Discovery for Spinal Muscular Atrophy. *Expert Opin Drug Discov*, **2** (4), 437-451.
3. Brichta, L., Hofmann, Y., Hahnen, E., Siebzehrubl, F.A., Raschke, H., Blumcke, I., Eyupoglu, I.Y. and Wirth, B. (2003) Valproic acid increases the SMN2 protein level: a well-known drug as a potential therapy for spinal muscular atrophy. *Hum Mol Genet*, **12**, 2481-9.
4. Brichta, L., Holker, I., Haug, K., Klockgether, T. and Wirth, B. (2006) In vivo activation of SMN in spinal muscular atrophy carriers and patients treated with valproate. *Ann Neurol*, **59**, 970-5.
5. Garbes, L., Heesen, L., Holker, I., Bauer, T., Schreml, J., Zimmermann, K., Thoenes, M., Walter, M., Dimos, J., Peitz, M. *et al.* (2012) VPA response in SMA is suppressed by the fatty acid translocase CD36. *Hum Mol Genet*.
6. Oprea, G.E., Krober, S., McWhorter, M.L., Rossoll, W., Muller, S., Krawczak, M., Bassell, G.J., Beattie, C.E. and Wirth, B. (2008) Plastin 3 is a protective modifier of autosomal recessive spinal muscular atrophy. *Science*, **320**, 524-7.
7. Ackermann, B., Krober, S., Torres-Benito, L., Borgmann, A., Peters, M., Hosseini Barkooie, S.M., Tejero, R., Jakubik, M., Schreml, J., Milbradt, J. *et al.* (2013) Plastin 3 ameliorates spinal muscular atrophy via delayed axon pruning and improves neuromuscular junction functionality. *Hum Mol Genet*, **22**, 1328-47.

## LONG DISTANCE REGULATION IN SKELETAL DISORDERS

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Structural aberrations of the human genome - such as insertions, inversions, translocations, and copy-number variations (CNVs) - contribute to phenotypic variation as well as pathogenic conditions.

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 structural aberrations such as inversions and translocations have been associated with skeletal disorders.

Liebenberg syndrome, a partial homeotic transformation in humans, These changes which are expected to be higher among conditions that are due to disturbance of complex developmental processes. Integrating these data 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.

## **Ethical Issues**

**Andrew Read**

*Andrew Read, St Mary's Hospital, Manchester*

People tend to see genetics as raising uniquely sensitive ethical issues. Whether that view is right or wrong, it does mean that as working geneticists we have to be specially sensitive to the ethical implications of our work. Although we all subscribe to general principles of ethical conduct, sometimes it is not easy to apply those principles. Is the right choice always the one that leads to the greatest good for the greatest number of people? Informed consent is important – but is it really possible? What do we do if exome sequencing reveals an unexpected risk factor in a patient or a research subject? Can data ever be truly confidential – and does confidentiality really matter so very much? Hopefully 50 minutes of exercise in the moral gymnasium will leave us all with things to think about.

# Thursday, May 16

## Introduction in Next Generation Sequencing technologies and applications

Joris A. Veltman

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

In this presentation I will introduce next generation sequencing technology, 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:

1. Shendure J, Ji H. Next-generation DNA sequencing. *Nat Biotechnol* 26: 1135-45 (2008).
2. Zhou X, Ren L, Meng Q, Li Y, Yu Y, Yu J. The next-generation sequencing technology and application. *Protein Cell* 1: 520-36 (2010). Review.
3. Tucker T, Marra M, Friedman JM. Massively parallel sequencing: the next big thing in genetic medicine. *Am J Hum Genet* 85:142-54 (2009). Review.
4. Ashley EA, Butte AJ, Wheeler MT, Chen R, Klein TE, Dewey FE, Dudley JT, Ormond KE, Pavlovic A, Morgan AA, Pushkarev D, Neff NF, Hudgins L, Gong L, Hodges LM, Berlin DS, Thorn CF, Sangkuhl K, Hebert JM, Woon M, Sagreiya H, Whaley R, Knowles JW, Chou MF, Thakuria JV, Rosenbaum AM, Zaranek AW, Church GM, Greely HT, Quake SR, Altman RB. Clinical assessment incorporating a personal genome. *Lancet* 375: 1525-35 (2010).
5. Hoischen A, van Bon BW, Gilissen C, Arts P, van Lier B, Stehouwer M, de Vries P, de Reuver R, Wieskamp N, Mortier G, Devriendt K, Amorim MZ, Revencu N, Kidd A, Barbosa M, Turner A, Smith J, Oley C, Henderson A, Hayes IM, Thompson EM, Brunner HG, de Vries BB, Veltman JA. De novo mutations of SETBP1 cause Schinzel-Giedion syndrome. *Nat Genet.* 42: 483-5 (2010).
6. 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**

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Next generation sequencing technology has been rapidly adopted in medical genetics research in the last few years due to its successful application of identifying new Mendelian disease genes. Especially the unbiased sequencing of the exome has become an integral part of the toolkit for genetics researchers. In this presentation we will discuss the basic steps that are involved in analyzing data from genomic resequencing experiments, focusing especially on experiment design and the problem of identifying pathogenic mutations among the thousands of benign variants that are identified by exome sequencing

# 26<sup>th</sup> Course in Medical Genetics

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- Chairman of the Dutch Foundation for Medical Research NOW career grant committee (2006-2008)
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- Dyscerne, European Network of Centers of Expertise for Dysmorphology
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- Professor of Medical Genetics at the University of Bologna Medical School in Italy
- Director of the European School of Genetic Medicine (ESGM), founded together with Prof. V.A.McKusick in 1988.
- President of the European Genetics Foundation (EGF), which is the only non-profit institution in Europe entirely dedicated to human genetics education.
- Chief of the Unit of Genetic Cancer Susceptibility at the International Agency for Research on Cancer, Lyon , France (1994-2002)
- European Society of Human Genetics Award for Excellence in Human Genetics Education, 2005
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- Professor of Medical Genetics and Chair of the Institute of Human Genetics at the Medical Faculty of the University of Cologne in Germany
- Group leader of the Neuromuscular Research Group
- Board of the German Society of Human Genetics (2002-2006)
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- Antoine Marfan Award for dedicated commitment to improving the length and quality of life for people with Marfan syndrome and related disorders through patient care and research from the National Marfan Foundation, Boston, USA, 2008

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# Participants - ABSTRACTS

## Identification of novel genes causing intellectual disability

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Intellectual disability (ID) or mental retardation is characterized by significant limitations in intellectual functioning and adaptive behaviour which originates before the age of 18 years.<sup>1</sup> Its prevalence varies from 1% to 10% in different studies.<sup>2, 3</sup> ID is acquired in 1-2% of cases and the rest is likely due to genetic causes.<sup>4</sup> However, the genetic cause is unknown in at least 60% of the patients and there is a rational need to identify the elusive genes and to discover the underlying pathophysiology for developing diagnostic and therapeutic approaches.

Current accessibility of molecular karyotyping and whole exome sequencing have rapidly increased our knowledge of causal genes for ID. In this project, 728 patients with idiopathic neurodevelopmental disorders have been evaluated by molecular karyotyping and/or whole exome sequencing for gene discovery. Using molecular karyotyping, 9.2% of our patients were shown to harbour micro-aberrations which are unequivocally pathological on the basis of their size and/or recognized association with known conditions. In addition, many patients that we tested demonstrate smaller deletions/duplications where the pathological significance remains uncertain. These small aberrations usually contain only few genes or even a part of a gene. So far, we have selected 94 of these small aberrations according to defined criteria such as their frequency and further analyzed by customized multiplex ligation-dependent probe amplification (MLPA). 42 of them (44.6%) were not confirmed by MLPA, 25 (26.6%) were paternally inherited, 20 (21.3%) were maternally inherited, one was homozygous (1.1%) and six (6.4%) were *de novo*. Of these *de novo* events, one was an intragenic deletion within the gene *MED13L* and exome sequencing in the patient showed no evidence for a non-allelic second hit. Subsequently, we identified 2 further patients with aberrations in this gene which delineated a recognizable *MED13L* haploinsufficiency syndrome characterized by ID, conotruncal heart defect, facial dysmorphism and hypotonia.<sup>5</sup> This phenotype overlaps with DiGeorge syndrome and implies a possible neurocristopathy. Therefore, evaluating the role of *MED13L* in development of chicken embryos, as the best model to study neural crest cells, was initiated. Other *de novo* and inherited events are under further assessment to verify their possible causal roles.

## References

1. AAIDD, Intellectual Disability: Definition, Classification, and Systems of Supports 11th ed. 2010, Washington.
2. Curry, C.J., et al. Evaluation of mental retardation: recommendations of a Consensus Conference: American College of Medical Genetics. *Am J Med Genet*, 1997. 72(4): p. 468-77.
3. Leonard, H. and X. Wen. The epidemiology of mental retardation: challenges and opportunities in the new millennium. *Ment Retard Dev Disabil Res Rev*, 2002. 8(3): p. 117-34.
4. Rauch, A., et al. Diagnostic yield of various genetic approaches in patients with unexplained developmental delay or mental retardation. *Am J Med Genet A*, 2006. 140(19): p. 2063-74.
5. Asadollahi, R., et al. Dosage changes of MED13L further delineate its role in congenital heart defects and intellectual disability. *Eur J Hum Genet*. February 2013.

## **A novel, generic, preimplantation genetic diagnosis (PGD) protocol applied to Cystic Fibrosis involving mutation detection through High Resolution Melting (HRM) analysis and simultaneous haplotype analysis through QF-PCR.**

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Cystic Fibrosis (CF) is the most common, autosomal recessive severe monogenic disease in Caucasians and a common indication for PGD. It is characterized by an extremely heterogeneous mutation spectrum (>1800 mutations <http://www.genet.sickkids.on.ca/>). Most PGD protocols to preclude CF described to date apply single-cell multiplex fluorescent PCR for analysis of linked polymorphic short tandem repeats (STR-linkage approach), including testing for the commonest *CFTR* mutation, p.Phe508del, when indicated, potentially limiting application for cases with other mutations when phase analysis is unavailable. Here we report the development and successful clinical application of a novel, flexible, generic PCR PGD protocol to facilitate direct detection of any *CFTR* nucleotide variation(s) by HRM, and simultaneous confirmation of diagnosis through STR-haplotype analysis. The primers used for *CFTR* HRM analysis were previously described (Montgomery *et al.* 2007). A touch-down, multiplex PCR was optimized supporting co-amplification of any *CFTR* exon-regions, along with 6 STRs (4 intragenic and 2 extragenic). Diluted (1/1500) 1<sup>st</sup> PCR products were used for a nested PCR to amplify the *CFTR* regions for HRM analysis (Idaho's LightScanner). *CFTR* genotypes were confirmed by STR haplotype analysis of the 1<sup>st</sup> PCR products sequencer. The protocol was validated pre-clinically according to PGD guidelines, by testing single lymphocytes, isolated (by

micromanipulation) from whole blood samples of candidate PGD patients. Four clinical PGD cycles were performed, for four CF carrier couples with the following *CFTR* genotypes: p.Phe508del heterozygous; p.Phe508del and c.489+1G>T; p.Arg334Gln and c.489+3A>G; p.Phe508del and p.Leu732X, (HGVS *CFTR* reference sequences NM000492.3 and NG016465.1). Thirty four embryos were biopsied and a total of 34 blastomeres were analyzed. Five samples failed to amplify at all loci. Genotypes were achieved in 29/29 amplified samples, of which 17 were suitable for embryo transfer (unaffected *CFTR* genotypes). Three pregnancies were achieved (2 twin and 1 singleton). PGD genotypes were confirmed following conventional amniocentesis or trophoblast PND testing. The reported PGD method is a flexible and robust tool which facilitates direct CF genotype analysis, genotype confirmation and contamination detection in single cells, with minimal family work-up prior to PGD (generic),

## **A balanced *de novo* inv(7)(p14.3q22.3) disrupting *PDEIC1* and *ATXN7L1* in a 14-year old developmentally delayed boy**

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We report a 14 year old male patient ascertained for developmental delay, carrying a *de novo* pericentric inversion on chr(7)(p14.3q22.3). Sequencing revealed that the breakpoints overlap a LTR sequence on 7q22.3 and a LINE on 7p14.3. A TTTAAA motif was found in proximity of the breakpoints on both arms. In addition the sequencing detected several small micro-rearrangements, deletion, duplication, insertion, at the breakpoints. No significant sequence identity exists between the 7p14.3 and 7q22.3 breakpoints. These features at the breakpoint junctions suggest that the inversion was triggered by TTTAAA motif, LTR and LINE and healed by a Non Homologous End Joining (NHEJ) mechanism. The genes *ATXN7L1* and *PDEIC* are disrupted by the inversion. *PDEIC* is responsible for the hydrolysis of the second messenger molecules cAMP and cGMP and is highly expressed in the human heart and certain brain regions. In mice, *Pde1c* is expressed in migrating neuronal cells within the central nervous system during early embryo development. Although neuronal migration disorder was not seen in our patient, this is the first patient described with haploinsufficiency of *PDEIC* possibly causing developmental delay.

## **SHFM Causing Missense Mutation in *FNDC3A***

**Sinje Geuer**

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Split hand/split foot malformation (SHFM) is a congenital limb malformation characterized by truncation or loss of central rays of hands and/or feet. It occurs in syndromic- and nonsyndromic forms; dominant, recessive and in rare cases x-linked inheritance have been reported. So far, six different loci (SHFM1-6) have been associated with SHFM: point mutations in *TP63* and *WNT10B*, copy number changes in 10q24, 17p13.3 and 7q21 as well as translocations in 7q21; but in a large number of cases the underlying cause remains unresolved. We studied a large consanguineous family from Syria with four affected individuals affected by non-syndromic SHFM. Mutations in the known loci had been excluded previously. We identified a homozygous mutation in the *FNDC3A* gene (*Fibronectin Domain Containing Protein 3A*) by whole exome sequencing in a homozygous run of 4 MB on chromosome 13q14.2 and subsequent confirmation by Sanger sequencing. All affected family members share the homozygous missense mutation in *FNDC3A* while the nine analyzed non-affected members carry either only a heterozygous mutation or wildtype.

*FNDC3A* is required for cell-cell adhesion (Obholz et al. 2006) which is critical for the development of different tissues. For the limb, the adhesiveness of mesenchymal cells undergoes spatiotemporal changes during cartilage formation (Wada et al., 2011) and alterations in cell adhesion have been shown to lead to limb malformations such as distal truncations (Yamaguchi et al., 1999). The mutation is located in a highly conserved region of *FNDC3A*, in the last of nine fibronectin domains. We could show by whole mount *RNA in situ* staining mouse embryos that *FNDC3A* is expressed in the Apical Ectodermal Ridge of the developing limb bud. This region is known to be essential for outgrowth and patterning of the limb and, if misregulated, to cause malformations such as SHFM.

In summary, we suggest *FNDC3A* as a candidate gene for autosomal recessive SHFM.

## **Trisomy 21 Revealed in a Linkage Scan with Whole Genome SNP Genotyping**

**Yesim Kesim**



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Whole genome SNP genotyping is an effective tool for mapping genes in families that can contribute to human diseases and phenotypes. This tool offers further advance in detecting copy number variations (CNVs), since allele frequencies and signal intensities can be analyzed simultaneously using well-established softwares with graphical outputs. Herein, we present CNV analysis in a sibling who was previously reported as unaffected in a consanguineous pedigree afflicted with a recessive form of a neurological condition. We set out to perform linkage analysis in this pedigree with 10 children (6 affected and 4 unaffected) and their unaffected parents using HumanCytoSNP-12 BeadChip kit. Interestingly, we have revealed trisomy of chromosome 21 who was reported to be unaffected for the neurological condition. This patient was later confirmed as having Down Syndrome clinically.

## **Genome-wide DNA methylation profiling in myocardial infarction**

**Alessia Russo**

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Methylation of CpG islands is an important epigenetic regulation mechanism in organ development and differentiation, aging and several diseases. To investigate the role of differential methylation on myocardial infarction (MI) risk, we examined the methylation levels of more than 450K CpG sites in 206 cases and 206 matched controls belonging to the Italian section of the EPIC cohort. EPIC healthy volunteers were recruited between 1994-98 and followed up for MI and other diseases. For the CpG methylation level assessment on blood DNA we used the Illumina HumanMethylation450 BeadChip. Data were analyzed according to standard procedures (MethyLumi, Bioconductor). To account for sex specific methylation and risk profiles, logistic regression analyses were conducted separately for males and females. All analyses were corrected for matching variables (age, season, center of recruitment) and cardiovascular risk factors when significantly different between cases and controls (smoke, BMI, waist/hip ratio). No significant association of single CpG methylation change has been found at the genome-wide significant threshold ( $p < 10^{-7}$ ) with MI risk. However, in

a genome-wide “regional” association analysis, we found multiple significant signals ( $p < 10^{-7}$ ) of differential methylation between cases and controls in 2 genomic regions for females (Chr5, Chr1) and in 4 regions for males with borderline significance (Chr6, Chr7, Chr11, Chr17). QTLs associated to MI, blood pressure regulation and metabolic disorders have been described in these regions. These results suggest that different methylation profiles between cases and controls can be involved in the regulation of these regions and in the modulation of MI risk.

## **Effectiveness of prenatal diagnosis methods.**

**Yevheniya Sharhorodska**

**Introduction.** The health of the fetus monitoring is a complicated and not solved issue both on the medical and technical aspects, as well as for ethical reasons [1,2]. At the same time scientific researches aimed at the fetus prenatal protection and at the development of measures, which ensure the birth of a healthy child.

**Objective.** To assess the implementation effectiveness of prenatal diagnosis methods of women with risk of congenital and hereditary pathology.

**Results.** It was observed 36 pregnant women with risk of congenital and hereditary pathology. In studying of observed women age range it was determined that the majority of them – 16 women (44,4 %) were 25-29 years old, the significant part – 9 women (25,0 %) were 30-34 years old, 7 women (19,4 %) were 35-39 years old, 3 women (8,3 %) were 40 years old and more, and the only one (2,9%) at the age of 20-24 years.

The data analysis revealed that among 8 women with risk of birth defects and hereditary pathology, which gave birth to healthy babies and had a deviation of biochemical markers in the first trimester, two women (5,6%) were  $\downarrow$ PAPP+ $\downarrow$ hCG, two women (5,6%) had only  $\downarrow$ hCG reduced, only one woman (2,8%) had so-called “scissors” -  $\downarrow$ PAPP+ $\uparrow$ hCG, and three women (8,4%) had isolated increase or reduction of other blood markets.

Based on the executed researches, it was revealed that 8 women with birth defects and hereditary pathology risk, which gave birth to healthy children and had biochemical markers of the second trimester changes, more frequently had the reduction of  $\downarrow$  $\alpha$ FP level – 4 women (11,1%), two other women had isolated deviation of only one indicator, two more women had the simultaneously reduction of two indicators.

The aforementioned data testify that the significant changes of biochemical markers both of the first and the second trimesters may quite often (2,8-5,6%) happen in physiological pregnancy that ends with the birth of a healthy child, which occurred in 16 cases and are pseudopositive.

It was conducted an invasive prenatal diagnosis (IPD) for four pregnant women with altered double test markers,  $\downarrow$ PAPP+ $\downarrow$ hCG, two of which had also neck edema. In all the cases it was diagnosed a trisomy for chromosome 18 (Edwards syndrome).

During the ultrasound execution in the second trimester it was revealed that two women had birth defects of the cardiovascular system in fetuses (AV-communication). In order to avoid chromosomal aberrations they were suggested to undergo invasive prenatal diagnosis. One of women, which refused to conduct a biochemical screening and invasive prenatal diagnosis gave birth to a child with Down Syndrome. It was prenatal diagnosed Down Syndrome of another pregnant woman, the triple biochemical tests revealed just isolated  $\uparrow$  hCG.

In order to predict postnatal surgical correction of six women with ultrasound detected congenital fetus malformations, it was performed IPA, set the normal fetal karyotype. Among them, 6 pregnant women (16.7%) were diagnosed with birth defects that required surgical correction: omphalocele - 2 cases (5.6%), and 1 case (2.8%) GS, BD cardiovascular systems (IBE defect, hypoplastic left ventricle, mitral atresia, aortic stenosis), cleft lip and palate; multiple congenital malformations (intestines atresia, bilateral clubfoot). One pregnant woman with fetal omphalocele had  $\downarrow$  PAPP, however it was invasive prenatal diagnosed a normal fetus karyotype.

Conclusions.

1. Biochemical blood serum markers of pregnant women, particularly performed in the first trimester, provide a possibility to reveal women with high risk of chromosomal fetus aberrations.

2. In 2,8-5,6% of cases – pseudopositive results.

3. The changes in one of the indicators in triple test do not have any diagnosis value.

4. The revealed data (particularly defects of the cardiovascular system in the second trimester) during ultrasound increase the risk of having baby with chromosomal disorders, even with normal biochemical markers of second trimester.

5. The conducted invasive prenatal diagnosis provided a possibility to 5 women (13,9%) with alterations in biochemical blood markers and birth defects revealed by ultrasound (AV-Communication) to diagnose in all cases a chromosomal fetus abnormalities (4 trisomy for chromosome 18 and trisomy 1 in 21).

Literature

1. Changes in birth defects structure and prenatal genetic load for 2003 – 2010 years period / A.A. Dubchenko, V.K. Likhachev, N.I. Mityunina [and others] // Proceedings of the Association of Obstetricians and Gynecologists Ukraine. - K.: Intermed. - 2011. - pages 311-314.

2. Psychological condition of women, that have lost a child or a disabled child birth / V. Davydov, V.V. Baranov, C.G. Apresova // Proceedings of the Association of Obstetricians and Gynecologists Ukraine. - K.: Intermed. - 2011. - pages 238 -244.

## **Dystonic cerebral palsy in monozygotic twins with 10p15.3 microdeletion syndrome**

**Athina Ververi**

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Submicroscopic deletion of 10p15.3 is a rare genetic disorder, currently reported in 21 unrelated patients. It is mainly associated with cognitive/developmental deviations, speech delay/language disorder, motor delay, craniofacial dysmorphism, hypotonia, brain anomalies and seizures. The size of the deleted region ranges between 0.15 and 4 Mb and does not generally correlate with patients' phenotype. A monozygotic female twin pair with a de novo 2.7 Mb deletion of 10p15.3 is herein reported. The girls presented at the age of 8 months with severe developmental delay and failure to thrive since the first month of life. Their perinatal and family history was unremarkable. On admission they both exhibited generalized dystonia with increased muscle tone and excessive deep tendon reflexes, microcephaly, progressive swallowing dysfunction, laryngomalacia, small omphalocele, mild dysmorphic features and complete absence of head control, voluntary movements and visual/auditory responsiveness. Both patients' brain MRIs demonstrated dilatation of ventricles, subarachnoid spaces, anterior interhemispheric fissure and sylvian fissures bilaterally. Cranial radiography revealed partial fusion of both coronal sutures. Visual and brainstem auditory evoked potentials were markedly abnormal, indicating severe visual and sensorineural hearing impairment. The electroencephalogram, as well as a screening for inborn errors of metabolism, were unremarkable. Both patients required gastrostomy and tracheostomy before the age of 1 year. They were, additionally, managed with physical therapy, as well as baclofen and low-dose haloperidol. Their current state at the age of 2 years is relatively stable. The index patients' phenotype includes features, such as dystonic cerebral palsy, visual and sensorineural hearing impairment, laryngomalacia, craniosynostosis and omphalocele, which have not been previously reported in individuals with 10p15.3 deletion. It is necessary to consider these novel clinical features and investigate their possible relationship with the recently recognized 10p15.3 microdeletion syndrome.

**Autophagy-related gene ATG7 is a genetic modifier of AAO specific in the Italian population**

## Caroline Walter

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**Background:** The only known cause of Huntington's disease (HD) is a polyglutamine repeat expansion of more than 36 units in the huntingtin protein, which is inversely correlated with the age-at-onset (AAO) of the disease. However, additional genetic factors apart from the expanded CAG repeat length can modify the course and the AAO in HD. Since autophagy, the major way for the degradation of mutant huntingtin, is thought to exert influence on the pathogenesis of HD, we hypothesized that autophagy-related (*ATG*) genes might contribute to the variation in the AAO.

**Aim/Method:** In this regard, we analysed the association of the V471A polymorphism in the autophagy-related gene *ATG7* gene in two large cohorts (1st study group: 952 patients; 2nd study group (EHDN REGISTRY): 1464 patients), composed of HD patients descending from different European countries.

**Results:** Although a polymorphism in the *ATG7* gene that substitutes alanine for valine (V471A) showed a significant effect on the AAO in the first European patient cohort mainly consisting of Italian and German HD patients, the significant effect of the *ATG7* V471A polymorphism could not be confirmed in the second independent study group that was composed of European patients other than Italian and German patients. A more detailed analysis revealed a significant effect of the *ATG7* V471A polymorphism on the AAO especially in the Italian population (327 patients) and was associated with a 4-years earlier disease onset.

**Conclusion:** Therefore, we identified a genetic modifier for HD in relationship to the autophagic pathway, but with a specific effect in a single population. This result affirms the influence of genetic modifiers on the course of HD, but also suggests population-specific modifying mechanisms in the HD pathogenesis.