Selected abstracts from the Clinical Genetics Symposium

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ADVANCES IN PHARMACOLOGICAL TREATMENT OF GENETIC DISORDERS: TUBEROUS SCLEROSIS AND RAPAMYCIN

Professor Julian Sampson, Medical Genetics, University of Cardiff, UK
Email sampson@cf.ac.uk

Inactivating mutations of *TSC1* or *TSC2* cause the inherited disorder tuberous sclerosis. They lead to loss of GTPase-activating protein (GAP) activity of the *TSC1/2* complex for the small GTPase Rheb and thereby to inappropriate activity of mammalian target of rapamycin (mTOR) signalling that appears critical to the pathogenesis of many disease manifestations. mTOR inhibitors are candidate therapies targeted to the molecular pathology of tuberous sclerosis and have been used in a number of pre-clinical and early phase clinical trials.

mTOR inhibitors have reduced the size of tuberous sclerosis complex (TSC) associated renal tumours (angiomyolipomas) and improved lung function in patients with lung manifestations due to lymphangioleiomyomatosis (LAM). A case series of TSC-associated brain tumours (sub-ependymal giant cell astrocytomas) was also reported to shrink during rapamycin therapy. Important central nervous system features of the tuberous sclerosis include learning difficulties and seizures. Recent studies in TSC mouse models have demonstrated improvement in these phenotypes following treatment with rapamycin. Trials for patients are in planning.

Tuberous sclerosis is an early example of targeting pharmacological therapy to the underlying defect in order to treat an inherited disorder. Others will follow.

Further reading

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Declaration of interests Sirolimus for the TESSTAL trial was provided without charge by Wyeth.

AORTIC ANEURYSMS: A NOVEL APPROACH TO THERAPY

Professor Bart Loeys, Center for Medical Genetics, Ghent University Hospital, Belgium
Email Bart.Loeys@ugent.be

Aortic aneurysms are an important cause of mortality in the Western world. Monogenic disorders such as Marfan's syndrome (MFS) and the vascular type of Ehlers-Danlos syndrome (EDS) are good models for the study of the pathogenesis of aortic aneurysm. In MFS, progressive dilatation of the aortic root leads to aortic aneurysm and dissection, often associated with precocious death. Early pathogenetic models for MFS focused upon structural weakness of the tissues imposed by microfibrillar deficiency.

However, recent studies of transgenic mouse models have challenged this model and demonstrated a central role for the upregulation of the TGFbeta signalling pathway. The discovery of a new aortic aneurysm syndrome, the Loeys-Dietz syndrome (LDS), confirmed the importance of the cytokine transforming growth factor (TGFbeta) in aneurysm pathogenesis. The main distinguishing features between LDS and MFS include the presence of hypertelorism, cleft palate/bifid uvula and arterial tortuosity/widespread aneurysms. LDS is caused by mutations in the genes encoding the receptors for TGFbeta (*TGFBR1/2*). Timely recognition of LDS is important in view of the different management strategies needed in this disorder.

We have recently also looked into the involvement of TGFbeta signalling in other syndromic causes of aortic aneurysms, including the genes encoding the smooth muscle contractile apparatus proteins alpha-actin and myosin heavy chain 11 encoded by the *ACTA-2* and *MYH-11* genes. In analogy to LDS, we demonstrated an upregulation of TGFbeta in arterial tortuosity syndrome. Finally, all these insights have also led to new therapeutic insights. In transgenic mouse models it was shown that losartan, an angiotensin II type 1 receptor with known inhibiting effects on TGFbeta, rescues the aortic phenotype. If these promising results are confirmed in human trials, losartan might have beneficial effects in the treatment of more common non-hereditary aortic aneurysms.

Further reading

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THERAPEUTICS AND THE ZEBRAFISH: POTENTIAL FOR TREATMENT OF HUMAN DISEASE

Dr Elizabeth Patton, MRC Human Genetics Unit, Edinburgh, UK
Email elizabeth.patton@hgu.mrc.ac.uk

Well suited to high-throughput genetic and chemical screening, zebrafish are becoming widely used in the drug-discovery process for target validation, disease modelling, toxicology and target and lead compound discovery. The Ras/mitogen-activated protein kinase (MAPK) pathway is critical for human development, and plays a central role in the formation and progression of most cancers. We are using the zebrafish system to identify small molecules that may reveal new insight, and possible therapeutic leads, into melanoma and developmental syndromes.

We have developed a zebrafish model of cancer of the melanocytes (the pigment-producing cells), called melanoma. Metastatic melanoma is aggressive and resistant to all chemotherapy, with afflicted individuals having a median life expectancy of less than one year. Clues to why melanoma is so difficult to treat may come from the nature of the melanocyte itself. Derived from highly motile neural crest cells, melanoma has high metastatic potential, and armed with enhanced survival and anti-apoptotic capabilities, melanocytes are naturally resistant to cytotoxic agents. Understanding the biology of the melanocytes – their development from precursor/stem cells, their proliferation, migration, differentiation, interaction with their environment and death – may be highly informative for new therapeutic approaches to melanoma. We study these processes using the zebrafish model system, which allows the visualisation of melanocytes in live tissue as well as their progression to melanoma. Screening 1,600 bioactive compounds has allowed us to identify a small molecule panel that alters distinct aspects of melanocyte biology in zebrafish, which may reveal new points of vulnerability for the otherwise resilient melanocyte.

Sporadic mutations in *BRAF,* a component of the MAPK pathway, lead to the development of common moles, and contribute to the progression of melanoma. On the other hand, children born with germ-line mutations in *BRAF* develop cardio-facio-cutaneous (CFC) syndrome, an autosomal dominant syndrome characterised by a distinctive facial appearance, heart defects, skin and hair abnormalities and mental retardation. CFC syndrome has a progressive phenotype, and the availability of clinically active inhibitors of the MAPK pathway prompts the important question as to whether such inhibitors might be therapeutically effective in the treatment of CFC syndrome. To study the developmental effects of CFC mutant alleles in vivo, we have expressed a panel of human CFC alleles in zebrafish embryos to assess the function of human disease alleles and available chemical inhibitors of this pathway. Importantly, we find a developmental window in which treatment with an MEK inhibitor can restore the normal early development of the embryo without the additional unwanted developmental effects of the drug.

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ECZEMA AND ASTHMA: GENETICS AND NEW TREATMENT THERAPIES

Professor WH Irwin McLean, Head of Human Genetics, Ninewells Hospital & Medical School, Dundee, UK
Email w.h.i.mclean@dundee.ac.uk

Atopic dermatitis ('eczema') is a common complex disease in which a combination of strong genetic predisposing factors combine with appropriate environmental stimuli and lead to inflammatory skin disease. Ichthyosis vulgaris (common dry, flaky skin) is arguably the most common Mendelian single gene disorder, where about one in 90 of the British population have a severe form and as many as one in seven have mild or sub-clinical form of the disease.

In 2006, my research group identified the first mutations in the filaggrin gene as the cause of ichthyosis vulgaris and showed that the condition is semi-dominant – homozygotes are severely affected; heterozygotes are mildly affected.[1] Profilaggrin is the main protein component of keratohyalin granules in the epidermis and its processed product, filaggrin, is involved in the biogenesis and subsequent hydration of the stratum corneum.[2] The nonsense or frameshift mutations identified in the filaggrin gene lead to complete loss of profilaggrin/filaggrin protein production in the epidermis. Surprisingly, these mutations are very common in the population and are carried by 5–14% of people of white European ancestry.

Since many individuals with ichthyosis vulgaris also have eczema and associated allergies, and the filaggrin gene is located within a known eczema susceptibility locus, we went on to investigate these mutations in atopic disease. This revealed that filaggrin loss-of-function mutations are a major risk factor for atopic eczema and associated allergic conditions, importantly including atopic asthma and allergic rhinitis.[3,4] These studies have been very widely replicated, including in non-white populations.

In light of recent genome-wide association analysis, filaggrin is now recognised as the major gene for eczema. Recently, we have reported a mouse mutant that is deficient in filaggrin expression due to a frameshift mutation highly analogous to the common human mutations.[5] These mice have allowed us to prove experimentally that filaggrin deficiency causes enhanced percutaneous antigen transfer, leading to an allergic immune response; i.e. this confirms the hypothesis that filaggrin is essential for normal skin barrier function and that disruption of the skin barrier is a major pathomechanism underlying atopic eczema. We are currently pursuing small molecule drug discovery aimed at restoring or enhancing filaggrin expression in the skin. These mice, in which we have identified a range of clinically relevant biomarkers, will be instrumental in validating new therapeutic approaches for atopic eczema that target the skin barrier rather than treating the subsequent allergic immune response.

References

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Declaration of interests None declared.

Discussion:

FAMILIAL HYPERCHOLESTEROLAEMIA: DO WE CARE AND WHO SHOULD DO THE CARING?

Position statement 1:

Professor Steve Humphries, Chief Executive Officer, UCL Genetics Institute, London, UK
Email rmhaseh@ucl.ac.uk

In the UK it is estimated that there are 120,000 individuals with familial hypercholesterolaemia (FH) and that currently only about 15% of these have been identified and are being treated appropriately. In 2008, NICE published a thorough evidence-based review of the management and treatment of FH patients, with 109 recommendations covering all aspects of the social, medical and familial needs of these patients. The recommended care pathway has the consultant-led lipid clinic in the central role, with the use of specially trained 'FH nurses' to perform cascade testing. Such a system has been successfully used in the Netherlands for the last ten years, and small research pilot studies in the UK, and most recently a multi-centre study funded by the Department of Health, have confirmed the feasibility, acceptability and cost-effectiveness of such an approach.

One of the key recommendations of NICE is to offer all FH patients a DNA test to confirm their diagnosis, and to be used as a basis for the cascade testing of their relatives. Once a mutation has been found in an index case, testing for it in relatives gives an unambiguous diagnosis and can guide management and treatment, while diagnosis based solely on the measurement of plasma lipids inevitably leads to a degree of uncertainty. Modelling suggests that implementation of cascade testing from the currently known index cases could, over the next five years, result in the identification of an additional 30,000 FH patients. Other index cases can be identified through general practice by electronic note searching and by interrogation of databases of myocardial infarction. Based on the current situation and for the foreseeable future, cascade testing centred on the lipidologist-run network of clinics is the most effective setting for the management of families with FH.

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Position statement 2:

Dr David Wald, Senior Lecturer and Consultant Cardiologist, Wolfson Institute of Preventive Medicine, London, UK
Email d.s.wald@qmul.ac.uk

In the UK about 15,000 of the approximate 100,000 individuals with familial hypercholesterolaemia (FH) have been identified.[1] People aged 20–40 with the disorder have about a 100-fold higher risk of a coronary heart disease event in the absence of preventive lipid-lowering treatment.[2]

Cascade testing has been proposed to identify new FH cases. This involves tracing and testing relatives of known cases (first-degree relatives have a 50% chance of being affected). If each known case identified one new case (UK feasibility studies indicate it is about half this) then the 15,000 known cases would yield only 15,000 new cases, leaving about 70,000 cases of FH undetected. No existing method would identify these 70,000. An alternative method of population screening is required.

Recent research has indicated that a serum cholesterol test between one and nine years of age would detect about 90% of FH cases with a false positive rate (unaffected children with positive results) of only about 0.1%.[3] Screening at birth or above the age of nine is less effective. Children could have a cholesterol test when they undergo immunisation at one to two years of age. Each affected child would have an affected parent,

identifiable as the parent with the higher cholesterol, so screening children provides a means of identifying and treating both FH children and their affected parent in a systematic manner. A pilot study implementing this method of screening (termed 'child-parent screening') in east London general practice child immunisation clinics shows that it is feasible and acceptable.

A national electronic FH register, documenting whether a person has been tested and whether the result is positive or negative, would be needed. Such child parent screening would not need to continue indefinitely. Once most affected families were known (which could take about 30 years), screening would simplify to looking up each parent's FH status at child immunisation clinics and then only testing the child if a parent's FH status is positive or unknown – a simple and low-cost method of screening, capable of benefiting one in 500 people in the population.

Co-authors
A Kasturiratne, L Ma, J Bestwick, JK Morris, NJ Wald

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Position statement 3:

Dr Michael Wright, Clinical Director, Northern Genetics Service, Newcastle, UK
Email michael.wright@nuth.nhs.uk

Familial hypercholesterolaemia (FH) is one of the most common heritable diseases. It is associated with high rates of morbidity and mortality which can be prevented by appropriate treatment with cholesterol lowering drugs. The importance of this has been recognised by recently published NICE guidance. Identification of asymptomatic affected relatives is fundamental to effective management. The management and commissioning of this 'cascade screening' is the subject of much debate.

NICE guidance indicates that we should 'use a nationwide, family-based, follow-up system to enable comprehensive identification of affected people.' In reality a highly developed and effective system for doing this already exists within the network of regional genetic services (RGS) covering the entire UK population. Regional genetic services already provide cascade screening for other heritable diseases. It is argued that the number of families affected by FH are such that using the RGS network in the management of FH would overwhelm them. Current models of service provision and levels of resource allocation mean that this is indeed the case. Adopting a model of collaborative working between the Northern Genetics Service and local lipid services, we have demonstrated that a highly effective model of cascade screening can be developed.

The development of cascade screening for FH in isolation from either lipid services or regional genetic services is a mistake. This approach will inevitably lead to duplication of effort and will ignore the many lessons learned by clinical genetic services in delivering this type of care for families affected by other diseases. This in turn will inevitably lead to higher service development costs and will require a longer development phase.

Further reading

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THE GENETICS OF OBESITY

Professor Steve O'Rahilly, Department of Clinical Biochemistry, University of Cambridge, UK
Email so104@medschl.cam.ac.uk

Implicit in the justification for the enormous increase in investment in bio-molecular research that has occurred over the past 20 years has been the promise that this would provide insights relevant to the understanding of human pathophysiology and the ultimate alleviation of suffering from human disease. To this end, model organisms are enormously attractive as they provide tractable and controllable systems in which precise data can be obtained. In contrast, the study of complex human disease is fraught with difficulties related to the multifactorial nature of most human illness, and the challenges of controlling for the effects of largely immeasurable confounding factors, both genetic and environmental.

While it is critical that research on complex human phenotypes and diseases continues, an approach that focuses on extreme human phenotypes has established itself as a useful complementary strategy. Firstly, it is more likely that extreme human phenotypes are caused by tractable monogenic or oligogenic defects. Secondly, the consequences of such defects are not always identical to those seen in animal models. Thirdly, once a link between a major mutation, particular gene and a human phenotype is established it is much more likely that subtle variation in those genes is involved in influencing susceptibility to common human diseases.

Finally, discoveries in this area may lead to effective mechanism-based therapies which provide justification for the continuation of investment in basic biomedical research. I will discuss advances that have come from the studies of two cohorts of humans with extreme phenotypes, namely obesity and insulin resistance.

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EPIGENETICS AND GROWTH

Professor Eamonn Maher, Medical Genetics Department, University of Birmingham, UK
Email E.R.Maher@bham.ac.uk

Epigenetic modification is a process by which the expression (and so function) of specific genes is altered without changes in the sequence of DNA. Research into epigenetics has demonstrated that epigenetic regulation of gene expression has a critical role in normal development and cell functions, including genomic imprinting, X-inactivation and tissue-specific gene expression. In addition, disordered epigenetic gene regulation is a feature of a number of important human diseases including cancer. A number of processes have been implicated in epigenetic gene regulation including DNA methylation, chromatin structure and modification (in particular the chemical modification of 'histone tails'), and small untranslated RNAs.

Genomic imprinting is an epigenetic process whereby the expression of an imprinted gene differs between the maternal and paternal alleles (e.g. IGF2 is paternally expressed and H19 and CDKN1C are maternally expressed). Although only a minority of human genes are imprinted (<0.5%), imprinted genes appear to be preferentially involved in prenatal growth and neurodevelopment. Important information about the role of imprinted genes in human development has been derived from studies of imprinting disorders such as Beckwith-Wiedemann syndrome (BWS), Silver-Russell syndrome (SRS) and Prader-Willi and Angelman syndromes. In particular, studies of BWS have led to the identification of imprinting centres and the recognition of the role of epimutations in human disease. Both genetic and environmental factors may influence genome methylation (epimutations) in BWS, and epigenetic research is providing important insights into how epigenetic alterations might influence susceptibility to human disease.

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