

# Cardiovascular Genetics Symposium 2013 – Submitted Abstracts

## A Dutch founder mutation in the cardiac regulatory light chain (MYL2).

**Keywords:** HCM; MYL2; Myosin light chain

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**Abstract:** Hypertrophic cardiomyopathy (HCM) is caused by mutations in multiple genes. Most of the reported mutations are distinct and unique for each family. In addition, clinical heterogeneity and incomplete penetrance are observed in families with a mutation. We describe the mutation p.Glu22Lys (p.E22K, c.64G>A) in MYL2, a gene that is rarely mutated in HCM as it accounts for less than 1% of identified HCM mutations. We identified the p.E22K mutation in 11 HCM families; it is a Dutch founder mutation that probably originated about 425 years ago around Eindhoven in the south of The Netherlands. These families carrying the same founder mutation provide the opportunity to investigate phenotypic differences influenced by external or genetic factors (other than p.E22K). Mutation carriers generally have a benign disease manifestation with variable phenotype expression, reduced penetrance and late onset. However, when a comorbidity factor is present (hypertension, hypothyroidism, coronary artery disease, atrial fibrillation or a second gene mutation), disease penetrance is higher with an earlier onset and sometimes cardiac arrest requiring an implantable cardioverter defibrillator in survivors.

## **Development of a next generation sequencing assay using a panel of 74 genes associated with infantile dilated cardiomyopathy (IDCM).**

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Infantile DCM has multiple causes and can present in infancy with cardiac failure and sudden death. BGL has offered testing of fetal and infantile male patients for one of these, the multisystem X-linked disorder Barth Syndrome, since 2001.

The NHS/UKGTN Barth Syndrome Service uses an abnormal cardiolipin ratio as the principal gatekeeper to trigger sequencing of the causative *TAZ* gene. However only 7% of cases referred for cardiolipin analysis are found to be positive for Barth Syndrome suggesting the need to look for other causes of DCM in these patients. Rapid analysis of a panel of genes associated with IDCM has been developed for these and other IDCM patients. 74 genes were included in this panel following literature search and clinical advice. An Agilent SureSelect custom enrichment kit was designed and includes the housekeeping gene *HBMS*, to enable copy number variants to be detected. Validation on a panel of patients with known variants included analysis of gene coverage and read-depth using an in-house bioinformatics pipeline and use of Geneticist Assistant (SoftGenetics).

Considerable clinical and genetic heterogeneity exists within and between families with DCM. Introduction of a gene panel for IDCM using a one off cost-effective test will aid diagnosis and further understanding of the complex clinical presentations of DCM and Barth syndrome phenotypes. For example a Barth patient hemizygous for a novel *TAZ* intron 3 splice variant c.284+3G>T was simultaneously found to be heterozygous for a likely pathogenic variant in the *MYH7* gene (c.2945T>C; p.Met982Thr). Mutations in more than one gene may explain phenotypic variability.

Results will provide information for both affected, presymptomatic and carrier family members.

## **Genetic diagnosis of inherited cardiac conditions using next generation sequencing.**

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Many cardiac disorders, which include cardiomyopathies associated with sudden cardiac death, have a significant heritable component. These inherited cardiac conditions have clinical variability, reduced penetrance and locus allelic heterogeneity, which means that molecular genetic testing has previously been a time consuming and costly process. The introduction of next generation sequencing into diagnostic laboratories has the potential to contribute to a clinically useful service for the improved diagnosis and genetic analysis of patients with cardiovascular disorders.

Following the successful introduction of other next generation sequencing services within our laboratory, we have designed and validated a diagnostic service for screening a number of inherited cardiac conditions using this technology. A panel of 64 genes causative for seven cardiac disorders, Brugada syndrome, hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), Long QT, CPVT and aortic aneurysms are included in the screen. Our novel approach targets all genes in the enrichment for sequencing, but only analyses the genes relevant to the patient's cardiac condition. This will enable us to focus on the most relevant genes and reduce the number of variants of uncertain clinical significance reported.

Using next generation sequencing technology we have validated and introduced a service offering genetic analysis of a range of inherited cardiac conditions.

## **Evidence that sarcomere mutations account for a significant proportion of 'idiopathic' cardiomyopathy in a large pediatric cohort.**

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Pediatric cardiomyopathy is recognised to have a genetic cause in a subgroup of patients. Commercial clinical testing is available for mutations in over 50 genes. To date, cardiologists and geneticists at our institution have ordered genetic testing for cardiac sarcomere mutations in over 100 cardiomyopathy patients. The specific aims of our study were:- 1.To determine the proportion of patients in whom the etiology is primarily genetic 2.To correlate genetic results with the outcome measures of remodeling or death/transplant. We performed a retrospective cohort study of patients diagnosed with hypertrophic, dilated, restrictive cardiomyopathy (HCM, DCM,RCM respectively) and left ventricular noncompaction (LVNC)in our institution over a 9 year period, excluding patients with acquired disease. Our final cohort numbered 196 patients, of which 23% had a mutation in a cardiac sarcomere gene (SAR), 21% had metabolic disease (MET), 12% had neuromuscular disease (NM), 14% had a known diagnosis not classified above and 40% had no known diagnosis ('idiopathic',IDI). The sarcomere genes accounting for the greatest number of cases were MYBPC3, MYH7 and TNNI3. In the cohort of patients with HCM, 35% had a sarcomere mutation. In those with DCM, this figure was 7%, and in LVNC 24% had a sarcomere mutation. Freedom from death or transplantation up to age 16 in the SAR group was 80%, in the MET group 35% and in the NM group was 85%. We conclude that commercially available testing for sarcomere mutations in children with idiopathic cardiomyopathy allows a diagnosis to be made in a significant proportion of those with HCM and LVNC, and to a lesser degree in those with DCM. The outcome is significantly better in the SAR group compared to the MET group, suggesting that genetic testing could provide clinicians with some evidence upon which to base prognosis in this group. The limitation of this study is that it is a retrospective, observational study. There was no systematic genetic testing in the idiopathic group, therefore the percentage of cases that we report as being explained by sarcomere mutations is likely to be an underestimate.

## **Atrial fibrillation, long QT syndrome and sudden cardiac death found in an extended family with KCNQ1 c.686G>A (p.G229D) mutation.**

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Recent reports suggest some KCNQ1 mutations can cause a variable phenotype characterized either by AF, Long QT syndrome (LQTS) or a combination of the two <sup>(1,2,3,4)</sup>. We present a large family with AF, LQTS and sudden cardiac death (SCD) who carry the KCNQ1 c.686G>A (p.G229D) mutation.

13 people have undertaken genetic testing. 3/13 people do not carry the mutation and do not have AF or a prolonged QT interval on ECG.

10/13 people were found to carry the mutation. 7/10 people are over 16 years of age. 3/10 people are less than 16 years of age.

Of the 7 adult mutation carriers: 6/7 developed AF but only 2 of these have prolonged QTc intervals. 1/7 mutation carrier had a normal QTc interval and currently no sign of AF.

Of the 3 mutation carriers under 16: none have AF but 2/3 had prolonged QTc intervals.

Three untested females died in their sleep (22, 23 and 51 years). Their relationship in the family proves they are obligate carriers.

Our data indicates KCNQ1 c.686G>A (p.G229D) is highly penetrant and can cause a variable phenotype of AF, long QT syndrome and sudden cardiac death. AF is more prevalent in this family and can present at an early age (the earliest diagnosis being 20 years of age).

KCNQ1 c.686G>A (p.G229D) is localised in the IV transmembrane segment of the KCNQ1 channel in close proximity to two other reported mutations linked with AF and LQTS <sup>(3,4)</sup>. It is possible this segment may represent a hot spot for mutations that cause both AF and LQTS.

### References

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## **RYR2 abnormalities in two paediatric patients with histologic evidence of arrhythmogenic right ventricular cardiomyopathy (ARVC)**

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Mutations in the cardiac ryanodine receptor (RYR2) have been implicated in both arrhythmogenic cardiomyopathy (ARVC2) and, more commonly, catecholaminergic polymorphic ventricular tachycardia (CPVT) in which there is an absence of structural heart disease. While the precise mechanism of RYR2 in the development of cardiomyopathy remains elusive, an understanding of the role of calcium release termination in cardiac pathophysiology is emerging. We report on two unrelated paediatric patients with histologic evidence of ARVC in whom RYR2 variants were detected. The first was resuscitated after sudden ventricular fibrillation (VF) arrest at the age of seven years. She underwent epicardial biopsy at the time of defibrillator implant and the results were consistent with ARVC. VF was not easily-inducible for defibrillator testing. She continued to have unusual arrhythmia which at times appeared to be both atrial and with some polymorphic component. Genetic analysis revealed a *de novo* mutation (Ala4091Val) in the "I" domain of RYR2 and no mutations detected in DSP, PKP2, DSG2 or DSC2. The second patient, a male, was evaluated for exercise-induced syncope at the age of six years. Polymorphic PVCs were revealed on exercise testing. Biopsy revealed abundant adipose tissue infiltrating the myocardium, compatible with ARVC. Genetic testing of PKP2, DSP, DSG2, DSC2, TMEM43 and RYR2 identified the previously-reported Glu1724Lys mutation in RYR2. These cases are an important addition to the increasing body of evidence of RYR2-related cardiomyopathy as well as the presentation of arrhythmogenic cardiomyopathy in children.

## It's a family affair – improving awareness and referrals of SAD cases

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### **Problem:**

Often the first presentation of an Inherited Cardiac Condition (ICC) is when a young person experiences a cardiac arrest or dies suddenly. If the post mortem does not identify any structural abnormality the cause of death is unascertained, however some may be caused by an undiagnosed arrhythmia. Coroners, pathologists and physicians involved in such cases are often unclear whether other family members are affected and who should investigate them. Furthermore, relatives have to deal with an unascertained cause of death and anxiety about their own risk of sudden cardiac death without any specialist support.

In South-West Wales clinical screening for first degree relatives is available at the Cardiac Centre, Morriston Hospital along with access to a well-established ICC multidisciplinary team (MDT) meeting with the All Wales Genetics service. However, there is limited awareness of these with many patients being seen in an adhoc manner in their local district general hospitals, by physicians or cardiologists with varying levels of expertise in the investigation and management of ICCs.

Therefore it was felt there was a need to increase awareness of ICC and the screening service amongst pathologists and coroners

### **ACTION:**

1. Targeted awareness drive for the Coroners and pathologists in South-West Wales
2. Service re-design to provide
  - central point of contact for coroners, pathologists, clinicians and relatives and development of referral algorithm
  - Prompt clinical assessment, risk stratification for 1st degree relatives with an option of investigations being undertaken locally or at Morriston,
  - Mechanism of review of investigation results and feedback by an EP consultant
  - When appropriate discussion at ICC MDT & genetic testing

**GOAL:** Improved referral rates to the ICC service from coroners and pathologists

### **RESULTS:**

- All coroners and teams of pathologists were offered a meeting to explain ICC and SAD. Only 1 coroner declined
- Agreed a standardised referral algorithm
- Drafted a relatives ICC information leaflet
- Improved referral rates from coroners/pathologists to ICC service and onward clinical screening (Table 1)

Table 1:

Date period	No: of direct referrals to EP team	No: of clinical screening for 1 <sup>st</sup> degree relatives
1997-2012	0	
May- Dec 2012	5	5 ( 2 declined)
Jan- September 2013	6	7 (1 undecided)

## Sequence analysis identifies variants in the *ELN* gene in patients with variable presentation aortic stenosis

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Pathogenic mutations in the gene encoding tropoelastin (*ELN*, MIM#130160) cause autosomal dominant Supravalvular Aortic Stenosis (SVAS), a congenital narrowing of the ascending aorta, and Cutis Laxa (CL), characterised by inelastic, loose-hanging skin. Variable phenotype and penetrance is noted in pedigrees.

In 2012 Bristol Genetics Laboratory submitted a UKGTN dossier for *ELN* screening, building on local clinical expertise to provide an accessible service for SVAS and CL cases. Genetic analysis confirms diagnosis to support patient management, allows cascade testing for at-risk relatives, and determines reproductive risks.

The 33-exon *ELN* gene (NM\_000501.2) is screened by Sanger sequencing in 27 amplicons (Beckman NX/ABI 3730). This will detect approximately 95% reported *ELN* mutations; dosage analysis may be added in future.

We present two families with multiple affected individuals, in whom *ELN* variants have been identified. In the first, the female proband has middle aortic syndrome and a heterozygous nonsense *ELN* variant c.1621C>T (p.Arg541\*), reported previously. Her sister, father, paternal aunt and cousin have joint hypermobility and myopia and exhibit variable cardiac phenotypes including SVAS, pulmonary artery stenosis (PAS), aortic regurgitation and VSD. All affected family members have the *ELN* mutation.

The second proband (also female) has SVAS and PAS with hypoplastic aorta. A novel heterozygous *ELN* variant of unknown significance was identified; c.1151-1G>A. Her father also has this variant and has an outflow tract anomaly (confirmation awaited). There are reported cases of aortic stenosis and bicuspid aortic valve in the wider paternal family.

These examples emphasise the importance of careful clinical examination and family assessment, which could implicate an *ELN* mutation as the cause of congenital heart disease in such cases.

## Retrospective analysis of Vertebral Tortuosity Index from patients seen in the CTD - Aortopathy clinic

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Arterial tortuosity of the head and neck vessels has been described as a frequent finding in Loeys-Dietz syndrome (LDS). Although not pathognomic, published reports suggest that tortuosity is present in 84% to 100% of patients with LDS. In contrast, arterial tortuosity has been described rarely in Marfan syndrome (MS).

It was also felt that there was no accepted definition or measure of proximal head and neck arterial tortuosity. A recent study by Morris et al has proposed the use of Vertebral Tortuosity Index (VTI) as an objective measure for vertebral tortuosity. Tortuosity was measured by Magnetic Resonance Angiography (MRA). The authors conclude that increased VTI is associated with increased aortic root size, increased frequency of surgery, and earlier age at surgery, dissection, and death. They propose that the use of this measurement tool may aid in evaluation, prognostication, and development of monitoring plans for children and young adults with various forms of CTD.

We retrospectively reviewed the MRA of our patients referred from the CTD – Aortopathy clinic over the last 6 months to assess the reproducibility of VTI measurements, as described by Morris et al. We identified MRA studies on 22 cases from Jan – Aug 2013. Table 1 demonstrates the VTI measurements for both vertebral arteries in this group. We hope to present the clinical features, presence of other arterial tortuosity and VTI of our cohort of patients. We also propose a grade for severity of VTI, which may aid in risk stratification.

**Table 1**

No	RIGHT VERTEBRAL ARTERY			LEFT VERTEBRAL ARTERY			Grade of VTI
	Actual length vertebral	straight length vertebral	VTI	Actual length vertebral a	straight length vertebral a	VTI	
1	14.4cm	13.6cm	5.9	15.9cm	14cm	13.6	Moderate
2	14.9cm	14cm	6.4	20.7cm	18.5cm	11.9	Moderate
3	21.6cm	19.6cm	10.2	22.7cm	19.7cm	15.2	Severe
4	13.9cm	10.8cm	28.7	12.6cm	10.5cm	20	Severe
5	13cm	12.7cm	2.4	13.5cm	13.1cm	3.1	Normal
6	18.3cm	16.7cm	9.6	19.3cm	17.8cm	8.4	Moderate
7	16.5cm	15.9cm	3.8	17.3cm	16.1cm	7.4	Moderate
8	15.3cm	14.7cm	4.1	17.6cm	16.8cm	4.8	Normal
9	18.2cm	18cm	1.1	15.7cm	15cm	4.7	Normal
10	16.5cm	16cm	3	19.7cm	18.4cm	7.1	Mild
11	19.2cm	18.2cm	5.5	19.7cm	18.8cm	4.8	Mild
12	14.2cm	13.8cm	2.9	15.6cm	14.7cm	6.1	Mild
13	17.9cm	16.9cm	5.9	19.5cm	19.2cm	1.6	Mild
14	26.4cm	22.7cm	16.3	22.1cm	19cm	16.3	Severe
15	15.7cm	14.9cm	5.4	17.4cm	16.1cm	8.1	Mild
16	16.5cm	15.6cm	5.8	15.9cm	13.4cm	18.7	Severe
17	18cm	17cm	5.9	18.6cm	17.1cm	8.8	Mild
18	11.4cm	11cm	3.6	13.9cm	13.5cm	3	Normal
19	23.2cm	22.3cm	4	18.2cm	17.3cm	5.2	Mild
20	14.6cm	14.1cm	3.5	15cm	13.8cm	8.7	Mild
21	16cm	14.5	10.3	18cm	16.6cm	8.4	Moderate
22	13.9cm	13cm	7	17cm	15.9cm	7	Mild

### Reference

*Increased Vertebral Artery Tortuosity Index Is Associated With Adverse Outcomes in Children and Young Adults With Connective Tissue Disorders. Morris et al Circulation. 2011; 124: 388-396*

## **Pre-symptomatic genetic testing for inherited cardiac conditions: a qualitative exploration of psychosocial and ethical implications**

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Inherited cardiac conditions (ICCs) can lead to sudden cardiac death at any age, yet are often asymptomatic and clinically undetected. Prophylactic interventions are available and cascade testing is recommended to identify family members at risk. When a disease-causing mutation has been identified in a family, pre-symptomatic genetic testing (PSGT) is available. This study explores perceptions of the cascade process, impact of PSGT and attitudes towards direct contact as an alternative to family-mediated dissemination for ICCs. In depth, interviews were conducted with 22 participants eligible for PSGT for Hypertrophic Cardiomyopathy or Long QT syndrome. Data were analysed using an inductive, thematic approach. Risk is perceived to be low pre-test in the absence of symptoms, and participants frequently test with the aim of ruling out risk to self and children. Testing of children is a complex decision; although older participants have concerns about possible adverse effects of genetic testing early in the life course, young participants are pragmatic about their result. The meaning of a positive genetic test result may be difficult to conceptualise in the absence of clinical evidence of disease, and this may deter further dissemination to at-risk family members. A majority of participants see advantages in direct contact from health professionals and support it in principle. Implications for practice include addressing risk perception pre-test, and presenting genetic test information as part of a risk stratification process rather than a binary outcome. Families may require more support or intervention in cascading genetic test information.

## **The role of Cardiac Genetics Nurses in developing ICC services**

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**Presenter:** Maggie Kirk

### **Presentation preference: POSTER**

Effective service provision is crucial for identifying and managing families affected by inherited cardiac conditions (ICC). Specialist cardiac nurses who have received additional training in genetics ('cardiac genetics nurses' - CGNs) can play a valuable role within the multidisciplinary ICC team in new and developing ICC services.

A three year initiative by the British Heart Foundation (2008-2011) to develop both new and existing ICC services, funded nine new CGN posts in England (n=8) and Wales (n=1). The nurses acted as a bridge between cardiology and genetics specialist services, served as the identified contact for the family, provided information and support, organised tests and contributed to clinical care. The initiative was independently evaluated by the authors using a case study approach. Service development was assessed using a Maturity Matrix. During the project, themed semi-structured interviews were held with all CGNs, Cardiology and Genetics Clinical Leads from each site, Genetic Counsellors and a patient representative (total 86 interviews). A patient focus group was also held. Qualitative data were analysed with NVivo 8.0 using an interpretative phenomenological approach.

The key findings were that the CGN: brings added value to ICC services and enhances the quality of the patient/family experience; plays an important role in prevention; enhances productivity, providing a value for money contribution to ICC services; adds capacity to innovate and enhance or develop aspects of (new) services and is a highly skilled nurse who plays a core role in facilitating seamless care between specialties in a patient-focussed integrated ICC care pathway.

For organisations considering the creation of an ICC service that would include CGN post(s) a 10 point checklist was compiled that addresses: induction; genetics education; cardiac training; mentoring; counselling supervision; administration support; management training; networks; lead times for clinical service set up and lead time for raising awareness of the service.

## **Familial hypercholesterolaemia; a comprehensive next generation sequencing diagnostic test to support national commissioning ?**

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Familial hypercholesterolaemia (MIM143890) is an autosomal dominant condition affecting 1/500 individuals leading to premature cardiovascular morbidity and mortality. NICE recommends comprehensive genetic testing in FH patients and cascade testing of at-risk relatives within CG71/DG2 and quality standard 41 (Aug13). Wales, Scotland and Ireland have comprehensive FH services, however England has not been universally commissioned with only 15% of 100,000 FH cases diagnosed.

Momentum is building for an England wide solution. The Cardiovascular Disease Outcome Strategy (Mar13) has designated improving ascertainment of FH cases to 50% as its specific action point 5. The British Heart Foundation are investing in nurse/counsellors to support cascade testing (Sept 13), and Heart UK/AstraZeneca are supporting the introduction of PASS software for family management. Laboratories need to address delivery of a comprehensive, high throughput, reduced cost FH genetic test to support national commissioning.

In 4 years we have tested 654 FH index cases (Wales/Midlands/SW) with a detection rate of 78% in dFH cases and 33% overall. 123 distinct variants (35 novel) were identified, the most frequent being *APOB* c.10580G>A (13%), *LDLR* c.1436T>C (5% SW severe mutation) and copy number variants (5%). 5 homozygous severe FH cases have been reported. Cascade testing is possible in 85% cases (pathogenic variant). Segregation analysis has clarified pathogenicity in 2 families with unclassified variants.

To facilitate increased uptake, we have developed a comprehensive FH NGS testing service processing batches of 16 patients at 25% less cost than current Sanger services. The capture assay (Agilent HaloPlex) targets coding regions of *LDLR*, *PCSK9*, *APOB* and *LDLRAP1* and *SLCO1B1* variants (*rs2306283* and *rs4149056*) associated with statin-induced myopathy with a bespoke bioinformatic pipeline based on Broad Institute GATK best practice for data analysis. The assay has been validated using 76 samples (251 variants) and a blind parallel trial of 16 patients showing 100% sensitivity.

*We will present the national picture of FH services, a 4 yr genetic laboratory audit highlighted by interesting case studies, and the changes in genetic technology that provide the cost improvements necessary to facilitate national commissioning and NICE/CDOS targets.*

## **Atrial fibrillation, long QT syndrome and sudden cardiac death found in an extended family with KCNQ1 c.686G>A (p.G229D) mutation.**

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Recent reports suggest some KCNQ1 mutations can cause a variable phenotype characterized either by AF, Long QT syndrome (LQTS) or a combination of the two <sup>(1,2,3,4)</sup>. We present a large family with AF, LQTS and sudden cardiac death (SCD) who carry the KCNQ1 c.686G>A (p.G229D) mutation.

13 people have undertaken genetic testing. 3/13 people do not carry the mutation and do not have AF or a prolonged QT interval on ECG.

10/13 people were found to carry the mutation. 7/10 people are over 16 years of age. 3/10 people are less than 16 years of age.

Of the 7 adult mutation carriers: 6/7 developed AF but only 2 of these have prolonged QTc intervals. 1/7 mutation carrier had a normal QTc interval and currently no sign of AF.

Of the 3 mutation carriers under 16: none have AF but 2/3 had prolonged QTc intervals.

Three untested females died in their sleep (22, 23 and 51 years). Their relationship in the family proves they are obligate carriers.

Our data indicates KCNQ1 c.686G>A (p.G229D) is highly penetrant and can cause a variable phenotype of AF, long QT syndrome and sudden cardiac death. AF is more prevalent in this family and can present at an early age (the earliest diagnosis being 20 years of age).

KCNQ1 c.686G>A (p.G229D) is localised in the IV transmembrane segment of the KCNQ1 channel in close proximity to two other reported mutations linked with AF and LQTS <sup>(3,4)</sup>. It is possible this segment may represent a hot spot for mutations that cause both AF and LQTS.

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## **A case of cerebrotendinous xanthomatosis presenting with marfanoid features.**

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Cerebrotendinous Xanthomatosis (CTX) is a rare autosomal recessive lipid storage disease. It is characterised by infantile onset diarrhoea, childhood onset cataracts, adolescent to young adult onset tendon xanthomas and adult onset progressive neurological dysfunction (including dementia and psychiatric disturbance, dystonia, peripheral neuropathy and seizures). Premature atherosclerosis has been described. Marfanoid features have been described before but are not typical of the condition. Diagnosis is confirmed by biochemical investigations or genetic testing of *CYP27A1*. Early diagnosis allows early treatment and may prevent or limit disease manifestations. Long term treatment with chenodeoxycholic acid (CDCA) normalises bile acid synthesis and improves neurophysiologic findings.

We present a 15 year old girl who was referred to our genetics service with a history of chronic diarrhoea, bilateral cataracts, learning difficulties, a high arched palate, arachnodactyly, scoliosis and hypermobility. She was born at 37/40 by normal vaginal delivery weighing 2.64Kg. She developed chronic diarrhoea and failed to thrive as a baby and was subsequently diagnosed with pancreatic insufficiency treated with enzyme supplementation. She developed bilateral cataracts which were removed at the ages of 9 and 12 years. She has mild-moderate learning difficulties and had a full statement of special needs. Normal investigations included array-CGH, homocystinuria, myotonic dystrophy and *Fibrillin-1* analysis.

She was reviewed 3 years after the original referral as a case of CTX with similar features was presented at a genetics conference. Analysis of her blood cholestanol showed raised levels consistent with the diagnosis of CTX. At the review appointment her behaviour had deteriorated and she had developed autistic rituals, poor balance, pins and needles in her hands and feet with a tremor. She also had raynauds phenomenon and osteoporosis. Treatment with CDCA has reduced her stool frequency significantly and she had gained a significant amount of weight.

## **Pre-symptomatic genetic testing for inherited cardiac conditions: a qualitative exploration of psychosocial and ethical implications**

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Inherited cardiac conditions (ICCs) can lead to sudden cardiac death at any age, yet are often asymptomatic and clinically undetected. Prophylactic interventions are available and cascade testing is recommended to identify family members at risk. When a disease-causing mutation has been identified in a family, pre-symptomatic genetic testing (PSGT) is available. This study explores perceptions of the cascade process, impact of PSGT and attitudes towards direct contact as an alternative to family-mediated dissemination for ICCs. In depth, interviews were conducted with 22 participants eligible for PSGT for Hypertrophic Cardiomyopathy or Long QT syndrome. Data were analysed using an inductive, thematic approach. Risk is perceived to be low pre-test in the absence of symptoms, and participants frequently test with the aim of ruling out risk to self and children. Testing of children is a complex decision; although older participants have concerns about possible adverse effects of genetic testing early in the life course, young participants are pragmatic about their result. The meaning of a positive genetic test result may be difficult to conceptualise in the absence of clinical evidence of disease, and this may deter further dissemination to at-risk family members. A majority of participants see advantages in direct contact from health professionals and support it in principle. Implications for practice include addressing risk perception pre-test, and presenting genetic test information as part of a risk stratification process rather than a binary outcome. Families may require more support or intervention in cascading genetic test information.

## **A review of families with SMAD4 mutations referred to the Yorkshire Regional Genetics Service. Should all affected relatives have aortopathy screening?**

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Juvenile Polyposis (JP) is an autosomal dominant disorder caused by mutations in SMAD4 or BMPR1A, characterised by multiple hamartomatous polyps of the GI tract and an increased risk of colorectal and gastric cancer. Hereditary Haemorrhagic Telangiectasia (HHT) is a vascular dysplasia syndrome, caused by mutations in ENG, ALK1 or SMAD4. Mutations in SMAD4 may cause a combined syndrome of JP and HHT. More recently, there have been reports of individuals with SMAD4 mutations presenting with an aortopathy. There are established guidelines for screening for both JP and HHT in patients with SMAD4 mutations. However, there are no guidelines for screening for aortopathies in these families. We performed an audit to determine whether individuals with SMAD4 mutations referred to the Yorkshire Regional Genetics Service in Leeds had been screened according to current guidelines and whether any of the patients were known to have an aortopathy. From 2007-2012, 29 individuals from 15 families (11 with JP, 4 with HHT) were tested for SMAD4 mutations. Three families (all with JP) were identified where at least 1 individual was found to have a pathogenic SMAD4 mutation. A total of 12 individuals had a pathogenic SMAD4 mutation. Ten patients (83%) had multiple GI tract polyps, 9 (75%) had epistaxis, 0 (0%) had telangiectasia, 12 (100%) had a family history of JP and 3 (25%) had pulmonary arteriovenous malformations (PAVMs). All 12 individuals had the appropriate screening recommended for JP and HHT. One of the 12 individuals we identified with a SMAD4 mutation also had an enlarged aortic root, raising the suggestion of additional echocardiographic screening in this group of patients. The natural history of the aortopathy associated with SMAD4 mutations is currently unknown leading to questions regarding benefits of screening, at what age to start and how frequently. Hence, long-term prospective studies are now required.

## Is Anderson-Fabry disease a common cause of isolated hypertrophic cardiomyopathy?

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Anderson-Fabry disease (AFD) is an X-linked disorder of glycosphingolipid metabolism caused by mutations in the *GLA* gene. It has an incidence of ~1 in 100,000 and usually presents in childhood with the following symptoms: pain crises, numbness or tingling of the extremities, decreased or absence of sweating, gastrointestinal symptoms, angiokeratoma, and corneal abnormalities. In later life, renal failure, heart manifestations (including left ventricular hypertrophy), and stroke can reduce life-expectancy. Tissue-specific AFD phenotypes have been proposed in multiple studies. Variants in the *GLA* gene have been detected in cohorts of patients with isolated stroke or isolated cardiac hypertrophy; however, there remains uncertainty with regards the clinical significance of these findings.

The Oxford Regional Molecular Genetics Laboratory provides analysis of *GLA* as part of a screen of 13 genes available to patients with hypertrophic cardiomyopathy (HCM). In 19 (1.5%) of ~1,250 probands referred for HCM or dilated cardiomyopathy (DCM), 11 *GLA* variants classed as highly likely to be pathogenic (5), likely to be pathogenic (2), or of unknown pathogenicity (4) were detected. No potentially pathogenic *GLA* variants were identified in patients with DCM. This suggests that *GLA* variants are responsible for disease in some individuals with idiopathic HCM. It is unclear whether or not these individuals will develop other manifestations of AFD in later life. One variant – p.Asn215Ser – was detected in five unrelated probands (~0.4% of cohort). In this presentation evidence supporting p.Asn215Ser as a disease-causing variant in individuals with isolated HCM will be described. The apparent effects of mosaicism and X-inactivation in individuals in whom a classic AFD presentation would be expected will also be considered.

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## **An Audit and Evaluation of Genetic Testing For Hypertrophic Cardiomyopathy in the Yorkshire Regional Genetics Service**

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The Yorkshire Genetic Cardiac Conditions Service is a tertiary multi-disciplinary service for inherited cardiovascular conditions supported by healthcare professionals from clinical genetics, adult & paediatric cardiology. Hypertrophic cardiomyopathy (HCM) is the most common familial cardiac disease and has vast genetic heterogeneity. The Yorkshire molecular laboratory has provided next-generation sequencing for HCM since August 2011 (basic panel: *MYBPC3*; *MYH7*; *TNNT2*; *TNNI3*). We present an audit of our service to assess the utility of genetic testing in HCM and to identify clinical indicators associated with identification of a pathogenic mutation.

43 patients underwent genetic testing for HCM between August 2011 and August 2012, 93% of referrals were from a secondary cardiac centre, whereas 7% of referrals were received from a General Practitioner or Paediatrician. Two patients underwent genetic testing following an equivocal cardiac assessment, whereas 41 had a clear diagnosis of HCM and cardiac investigations consistent with the diagnosis on assessment. 63% of patients were symptomatic or had suffered a recorded collapse or arrhythmia. The remainder were asymptomatic and were diagnosed after cardiac screening.

Of those families where inheritance was compatible with X-linked transmission, biochemical testing for Fabry's disease was carried out in all but one. 30% of patients were found to have a pathogenic or likely pathogenic mutation in one of the four HCM genes analysed. There was documented evidence of family screening for 84% of patients. 50% of patients with a first-degree relative affected with HCM had a pathogenic mutation on molecular analysis. However, only 4% of isolated cases without a family history were found to have a pathogenic mutation.

Our practice conforms to European Guidelines on genetic testing in HCM. In our patient cohort, families where two or more members have a clinical diagnosis of HCM are significantly more likely to have a pathogenic mutation than isolated cases.

## Clinical Characterization of 4 Swedish families With Arrhythmogenic Right Ventricular Cardiomyopathy and an identical PKP-2 mutation

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### Abstract

**Background:** Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a progressive disease of the desmosome characterised by increased risk of sudden death. The pathogenesis is desmosomal gene mutation and the most commonly affected gene is plakophilin-2. The trait is inherited in an autosomal dominant pattern with variable penetrance and a gene mutation does not always lead to signs and symptoms of disease. The aim of this study was to evaluate 4 families with an identical mutation in PKP2 c.2146-1G>C with the purpose to establish how many healthy mutation-carriers fulfilled the revised Task Force Criteria, and if any common clinical characteristics were present.

**Methods:** We evaluated the family members regarding the known mutation in PKP2 c.2146-1G>C. Mutation carriers were offered clinical follow-up. The evaluation was based on patient history and non-invasive testing including electrocardiography (ECG), signal-averaged ECG (SAECG), two-dimensional echocardiography, cardiac magnetic resonance imaging (MRI) and long term electrocardiogram recording (LTER).

**Results:** The familial mutation was analysed in 40 family members accepting testing, and the mutation was found in 24 individuals (including three index persons). Fourteen out of 21 family members were diagnosed with "possible ARVC", 3 with "borderline ARVC" and 4 had a definite diagnosis.

The relatives with borderline or definite ARVC demonstrated re-polarisation disturbances in most cases but arrhythmia was uncommon.

**Conclusion:** The vast majority of healthy family-members with an identical PKP2 mutation was found to have only "possible ARVC". Family-members with borderline or definite diagnoses frequently had ECG disturbances but rarely significant arrhythmia. Long term follow-up is mandatory in this relatively healthy patient group, however, with the ongoing aim to assess the risk for malignant arrhythmia. The possibility of modifying phenotypic influence must be regarded as well as the presence of additional genetic abnormality.

# Assessment of HaloPlex amplification for sequence capture and Next Generation Sequencing of Arrhythmogenic Right Ventricular Cardiomyopathy genes

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Introduction: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is mainly a genetically determined autosomal dominant form of cardiomyopathy with reduced penetrance. Sequence variations in at least ten genes *DES*, *DSC2*, *DSG2*, *DSP*, *JUP*, *PKP2*, *RYR2*, *TGTB3*, *TMEM43* and *TTN* involved in the function of desmosomes have been found to be important for ARVC development. Sequencing of gene panels by Next generation sequencing (NGS) can be a powerful tool for finding sequence variants involved in ARVC and other genetic disease.

Aims: In this study we designed and validated a NGS method for sequencing of a gene panel with exons and surrounding intronic sequences of ten genes.

Materials and methods: We used SureDesign to design a HaloPlex Custom kit (Agilent) for the genes involved in ARVC development. Twenty seven samples from ARVC- patients were sequenced using Haloplex and MiSeq-sequencer (2x150 bp, Illumina), multiplexing 6-8 samples/run. Bioinformatic analysis was performed using three different bioinformatic pipelines. For validation of the NGS method, all exons and splice-sites of five of the genes from the gene panel (*PKP2*, *DSP*, *DSG2*, *DSC2* and *JUP*) were Sanger-sequenced on a Genetic Analyzer using the same 27 samples.

Results and discussion: All samples were successfully sequenced using NGS with a mean coverage above 800x and 99% of bases were covered more than 20X. The sequence variations found by Sanger sequencing in the exons of the five genes were all found using NGS. Some additional genetic variants were found using the NGS technique and revising the Sanger data, these were found in low quality electropherograms regions or being due to placing primers over deletions or SNVs, leading to allele specific amplification, and thereby false negatives. All these variants could be confirmed by redesigning the Sanger sequencing. Validation of Haloplex and MiSeq sequencing showed equal or better sensitivity compared to Sanger sequencing.

## **Results of genetic testing and clinical screening in the first 500 Hypertrophic Cardiomyopathy (HCM) families attending the West of Scotland Inherited Cardiac Disease Clinic**

### Contributors

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Index cases from 500 HCM families underwent genetic testing, regardless of their age or family history (FH). The sarcomeric protein genes MYBPC3, MYH7, TNNT2, TNNI3, TPM1 and MYL2 were Sanger sequenced and alpha galactosidase measured on dried blood spot analysis. Three families with Fabry were identified.

Overall 231 (46%) cases were found to have significant genetic variants, including 20 (8.4%) variants of unknown significance (VUS). MYBPC3 accounted for 62% of the mutations and MYH7 for 26%. Detection rates in cases with or without a FH of HCM was 70% and 35% respectively. Twelve cases were found to have more than one variant, including one homozygote for TNNI3. VUS were more likely to be found amongst cases without a family history, perhaps suggesting lower penetrance alleles.

Mutation detection rates in single cases is still relatively high, reinforcing the benefit of testing individuals without a FH, which also identifies a FH negative and gene negative group, in whom relatives appear to be at lower risk of developing cardiomyopathy. We have audited the screening in first degree relatives of gene negative families and have compared this to the age related penetrance in gene positive relatives undergoing predictive testing from 137 families with a known mutation. The penetrance in relatives identified with a mutation by age 60 years is approximately 60%. Males with a pathogenic mutation are more likely to develop cardiomyopathy at an earlier age than females, with 40% of males being affected by 40 years compared to 30% of females. However screening in first degree relatives of gene negative, FH negative index cases has identified very few affected individuals, perhaps indicating that clinical screening in this group may need reviewing. Detailed results will be presented.

## Cases of Mitogenic Cardiomyopathy in the United Kingdom

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Dilated cardiomyopathy (DCM) is the most common type of cardiomyopathy in the paediatric population (Badertscher *et al* 2008). In a large number of cases, the aetiology and pathogenesis of DCM remains elusive but some pathologically distinct conditions are emerging. One of these is mitogenic cardiomyopathy which is thought to be a rare, recessive form of dilated cardiomyopathy and lethal in early infancy. This has been reported in five patients in Canada and characteristic histological features include myocyte hyperplasia and marked mitotic activity (Chang *et al* 2010).

We describe two unrelated cases of mitogenic cardiomyopathy from the UK wherein both previously healthy male infants were reported to be slightly unwell and had difficulty settling the night before their death. On post mortem, both patients had enlarged hearts for their age and histopathology revealed myocyte hypertrophy with frequent mitoses. The increased proliferation rate for both was confirmed by immunostaining with Ki 67 which showed approximately 10% of cells showing positive nuclear staining. Case 1 who died at four weeks had genetic investigations which did not reveal any chromosomal abnormalities or mutations in the TAZ gene which was tested due to an abnormal monolysocardiolipin/cardiolipin ratio. Cardiac screening for his parents revealed no abnormalities and there was no family history of sudden death. Case 2 who died at two weeks was also from non-consanguineous parents but no cardiac screening results are available, however, there is a strong maternal family history of death in infancy in her siblings and her maternal uncles and aunts which requires further clarification.

Recognition of this distinctive entity is important for genetic counselling and further genetic testing may reveal the underlying molecular pathogenesis.

### **References:**

Badertscher A, Bauersfeld U, Arbenz U, Baumgartner MR, Schinzel A, Balmer C. Cardiomyopathy in newborns and infants: a broad spectrum of aetiologies and poor prognosis. *Acta Paediatr* 2008;97:1523-8

## **Personalised External Aortic Root Support (PEARS) and the Big Aortic Root Study (BARS)**

Tom Treasure on behalf of the PEARS Project Group supported by the NIHR Cardiovascular Biomedical Research Unit of Royal Brompton and Harefield NHS Foundation Trust and Imperial College London

With technical refinements, and improving skill in practiced hands, root replacement to pre-empt dissection in people with Marfan Syndrome became a routine intervention. As perioperative mortality reduced there was growing confidence to intervene earlier to save those who dissect at smaller root size.

In 2000 Golesworthy proposed the use of computer assisted design, with input from the patient's digital images, to make a personalised external support. The first operation was in 2004 (*Lancet* 364:1582) and since then 40 PEARS procedures have been performed to the same engineered standard. The pliant macroporous mesh is inert and becomes incorporated in the aortic adventitia. Myocardial and cerebral hazards are obviated because there is no need to open the aorta. Cardiopulmonary bypass is infrequently needed. (*BMJopen* 2012) The aortic root and the valve architecture are stable over years. There has been prospective evaluation. The first 30 patients (median age 28 years; IQR 20-44) have been followed up for two to nine years. During a total of 150 patient years, among these patients with root and valve conserving surgery, there have been no cerebrovascular, aortic, or valve related events.

These results are better than reported for mechanical and valve sparing root replacement following which composite valve-related events are 1.3% and 1.9% per year respectively. Reoperation rates are 1.3% per year for valve sparing surgery. (*Heart* 97:955) The cumulative rates during a reasonable expectation of life are therefore considerable. We now propose a prospective comparative study (BARS) of the three operations available for patients with aortic root aneurysms considered to be at risk of dissection or regurgitation. Advice given to patients tends to lead to the surgery preferred by the team. BARS will ensure evidence based advice, uniform data acquisition, and the possibility of random allocation when equipoise exists.

**A cellular model to discern the functional impact of SCN5A mutation relating to a broad spectrum of arrhythmia disorders.**

*Names of all contributors:*

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A cellular model to discern the functional impact of SCN5A mutation relating to a broad spectrum of arrhythmia disorders.

The expertise from the Institute of Cancer & Genetics and the Wales Heart Research Institute are working together to identify and characterise the functional effects of variations identified in the SCN5A gene amongst arrhythmogenic patients. The SCN5A gene encodes the voltage-gated cardiac sodium channel (Na<sub>v</sub> 1.5), which is responsible for the initial upstroke of the action potential in an electrocardiogram (ECG). Opened or closed conformations in response to the voltage difference across the membrane results as the protein forms a sodium-selective channel through which Na(+) ions may pass in accordance with their electrochemical gradient.

Mutations confer intracellular trafficking and/or electrophysiological defects on SCN5A channels. The contribution of these abnormalities to the observed mutation-linked channel dysfunction needs to be properly determined. To address these issues an *in vitro* biophysical assay has been established. This model uses human embryonic kidney cells (HEK293) which are stably transfected with the wild-type SCN5A or a suspected mutant- SCN5A associated with an arrhythmia such as Brugada, Long QT, conduction disturbances, sick sinus syndrome, atrial standstill, atrial fibrillation, and dilated cardiomyopathy (DCM). Mutation-linked effects on cellular trafficking to the plasma membrane and an assessment of channel functionality by electrophysiology are combined to determine the mode of channel dysfunction.

## **Impact of predictive genetic testing for inherited cardiac conditions in young people (16-25 years)**

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Research on the psychosocial impact of presymptomatic testing for dominant cardiac conditions in young people has been so far been largely limited to children and their parents with Long QT syndrome. We present the results of a study investigating the experiences of young adults (16-25 years old) who had predictive testing for a range of inherited cardiomyopathies exhibiting variable penetrance (Dilated/Hypertrophic/Arrhythmogenic Right Ventricular). A qualitative methodology (Interpretative Phenomenological Analysis) was used to analyse data from semi-structured telephone interviews in ten young people. Of the ten participants five had tested positive for the familial presumed pathogenic gene change and five had tested negative.

Analysis identified a number of themes related to decision making and impact of predictive genetic testing in these young people. None of the participants expressed regret at being tested at a young age. Parents were a strong influence on the decision to be tested but participants were accepting of this and valued their support. Overall participants adapted well to their result, however one participant had marked anxiety about the future and required additional follow-up. Young people commonly only disclosed their predictive test to a small circle of family and friends. While the young people in this cohort demonstrated a good adjustment to the process with little emotional need to talk, they all appreciated the counselling they had received. In particular accessible information and feeling that they understood the process were identified as positive aspects of genetic counselling.

Implications commonly discussed in genetic counselling (insurance, employment, relationships etc) are often far in the future for young people and have little impact on their decision to test. Genetic counselling of young people should perhaps address this and explore the potential need for long-term follow up. This study provides some evidence that genetic testing is acceptable to this age group.

## **Assisting the Understanding of Molecular Genetic Reports**

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***Presenter : Peter Lunt***

In genetic cardiovascular disorders, molecular genetic testing is becoming increasingly important for making a definitive diagnosis, allowing optimum clinical management, and enabling anticipatory management for at-risk family members. The possibility of testing multiple genes simultaneously, rather than single ones sequentially, increases the importance of being able to recognise pathogenic gene alterations, and distinguish these from non-pathogenic or modifying variation. The process for this evaluation is a joint responsibility between the testing laboratory and the referring clinician, and may require additional expert advice.

In order to assist the referring clinician to have a level of understanding appropriate to assist this process, the NGGEC is developing an on-line educational resource on the reporting and interpretation of genetic test results. Based on written laboratory reports this resource illustrates and explains the terminology used, the different types of gene alteration which can be encountered, and their likely effect on the gene product.

The increasing use of next generation sequencing in panel tests or whole exome tests, thereby providing the DNA sequence of many genes in a person simultaneously, is often revealing multiple variants in different genes in a person's genome. Some variants may be reported as pathogenic or as definitely non-pathogenic, but for many the significance may at present be uncertain. Variants of uncertain significance (VUS) can present a major challenge for interpretation in a patient and in managing their wider family. The NGGEC resource will therefore explore in more detail: systems for classification of 'uncertain' variants, the language used for their description, and the methods which clinicians and scientists together use to try to minimise the number of variants whose significance remains truly 'unknown'.

## Evaluation of Diagnostic Genetic Testing in Hypertrophic Cardiomyopathy to Define Clinical Indicators that Predict the Likelihood of Identifying Pathogenic Mutations

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Hypertrophic cardiomyopathy (HCM) is the most common familial cardiac disease and has vast genetic heterogeneity. The Yorkshire Inherited Cardiac Conditions Service is a tertiary multi-disciplinary service supported by professionals from clinical genetics and adult & paediatric cardiology. The Leeds Clinical Molecular Genetics Laboratory has been providing next-generation sequencing for HCM since August 2011 (basic panel: *MYBPC3*; *MYH7*; *TNNT2*; *TNNI3* genes), underpinning the clinical service. We present an audit and evaluation of our service to assess the local utility of genetic testing in HCM and to define clinical indicators associated with pathological test outcomes.

A cohort of 43 individual patients, all from separate families, with a confirmed (41) or borderline (2) diagnosis of HCM, underwent diagnostic genetic testing for HCM between August 2011 and August 2012.

The majority of patients, 63%, were symptomatic or had suffered a recorded collapse or arrhythmia. The remainder were asymptomatic and were diagnosed after cardiac screening due to a family history of HCM or as part of an employment medical.

Pathogenic or likely pathogenic mutations were found in 30% of the patients tested. There was no correlation between the likelihood of identifying a pathogenic mutation and sex, age at diagnosis or symptomatic versus asymptomatic presentation. Those patients with an ICD or pacemaker inserted were more likely to have a pathogenic mutation than those managed medically. Considering the family history, 50% of patients with a first-degree relative affected with HCM had a pathogenic mutation identified, dropping to only 4% for isolated cases.

Family history was an important indicator of the likelihood of identifying a pathogenic mutation in our cohort. This observation has previously been described in other cohorts and should be considered when undertaking genetic counselling in this setting.

## **The Cardiff Study of Experiences of People with Copy Number Variation (ECHO Study)**

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The Cardiff Study of Experiences of People with Copy Number Variation (ECHO Study) (<http://medicine.cf.ac.uk/psychological-medicine-neuroscience/areas-research/copy-number-variant-research/research-projects/>) started in 2009. Our focus is on CNVs associated with high risk of schizophrenia, including deletions of 1q21.1, 3q29, 9q34.3 (Kleefstra Syndrome), 15q11.2, 15q13.3 and 22q11.2, as well as duplications of 16p11.2 and 16p13.11. By studying these high-risk CNVs we intend to gain insight into the developmental pathways into schizophrenia (following up children and adolescents over time) as well as the prevalence and nature of psychotic manifestations in adult patients.

We visit participants at their homes to characterise in detail the behavioural, neuro-cognitive and psychiatric manifestations associated with these CNVs. We also invite individuals to come to Cardiff to take part in structural and functional neuro-imaging. Furthermore, we collect blood/ spit samples for genotyping and gene expression analysis. We are supported by a number of charities including Unique as well as MaxAppeal.

Findings in our sample of youngsters with 22q11.2 Deletion Syndrome have indicated that more than half (54%) meet diagnostic criteria for one or more DSM-IV psychiatric disorders. Furthermore, the children had a wide range of neuro-cognitive impairments. However, we found no evidence that risk of psychiatric disorder was linked to level of intellectual function. That is, it is more likely that both neuro-cognitive impairment and psychiatric disorder occur as a result of shared genetic etiology, rather than that neuro-cognitive dysfunction increases risk of psychiatric disorder.

We have thus far recruited close to 200 individuals into our ECHO study; however, sample sizes for many of the individual CNVs are still small. We are keen to continue to recruit new patients through Medical Genetics clinics and would much appreciate your help.

# Oral Presentations

## Yield of 15 years of cardiogenetic testing: contribution of family history

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**Background:** During the consultation at the cardiogenetics outpatient clinic the chances of detecting a mutation are discussed with the proband. Although some studies suggest a higher yield of genetic testing in familial cases, a systematic approach is missing in literature. We show the results of 15 years of cardiogenetic counseling at the AMC specifically addressing the yield of molecular genetic testing in familial versus sporadic disease.

**Methods and results:** We collected data on 1438 index patients with a primary arrhythmia syndrome (LQTS, Brugada syndrome, CPVT) or cardiomyopathy (HCM, DCM) who visited our outpatient clinic from 1996 to 2010. Family history was considered positive if patients had  $\geq 1$  affected relative or had a relative (up to 3<sup>rd</sup> degree) who died suddenly  $\leq 40$  years (primary arrhythmia syndromes) or 45 years (cardiomyopathies). For all diseases the mutation detection rate was significantly higher in familial cases than in sporadic cases (LQTS: 82% vs. 29%; CPVT: 73% vs. 12%; Brugada syndrome: 44% vs. 21%; HCM: 65% vs. 40%, DCM: 39% vs. 10%). This was not due to more extensive genetic testing in familial cases. In both familial and sporadic cases mutation detection rates decreased over time.

**Conclusions:** Family history strongly determines the mutation detection rate in both primary arrhythmia syndromes and cardiomyopathies. These results may assist counselors in providing appropriate information on the expected yield of genetic testing based on the counselee's family history. We expect that family history will remain an important contributor to the yield of genetic testing when next generation sequencing is used.

## **Fifteen-year follow-up of mortality in a founder ARVC cohort after ICD therapy**

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**Background** We studied the impact of the implantable cardioverter-defibrillator (ICD) in arrhythmogenic right ventricular cardiomyopathy (ARVC) over 15 years in 24 Newfoundland families.

**Methods** Of 691 ascertained cardiomyopathy families, 24 had ARVC caused by an autosomal dominant founder p.S358L mutation in TMEM43. We compared mortality between ICD subjects, and controls derived from the deep pedigrees, matched for age, sex, and disease status. Subjects born at 50% *a-priori* risk (n=865) were classified as affected (n=398), unaffected (n=229), or unknown (n=238) based on clinical events, mutation analysis, and/or pedigree position. An ICD was provided to 150 affected subjects: 77 males, median age 28y (58 for primary prevention (PP), 19 for secondary prevention (SP) following ventricular tachycardia (VT)) and 73 females, median age 42y (71 for PP). Survival was compared to 150 affected historical controls who were alive at the same 'age to-the-day' at which the ICD subjects received their device. Time to first appropriate ICD intervention was compared to death in controls.

**Results** Median age to death in the untreated ARVC population was 40 years in males, 71 years in females: RR 5.1 (95% CI 3-8.5). The RR of death in control males compared with ICD subjects (PP) was 9.3 (95% CI 3.3-26), p<0.0001, and for ICD male subjects (SP) was 9.7 (95% CI 3.2-30), p<0.0001. The RR of death for female controls compared with ICD subjects (PP) was 3.6 (95% CI 1.3-9.5), p<0.011. Time to first appropriate device intervention in any ICD subject did not differ from death in controls.

**Conclusions** This genetic subtype of ARVC leads to early death. ICD therapy for PP in both sexes and SP in males significantly improves survival. Appropriately aborted VT is analogous to a death event when compared to controls. The ICD should be the prophylactic treatment of choice in ARVC caused by TMEM43 p.S358L.

## Unusual presentations of thoracic aortic aneurysm in children

**Names of all contributors:** Marlies Kempers (MD, PhD), Simone Salemink (MSc), Liesbeth Spruijt (MD, PhD), Carlo Marcelis (MD), Professor Bart Loeys (MD, PhD)

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Thoracic aortic aneurysm in children is a rare but important disease entity. Marfan and Loeys-Dietz syndrome are amongst the most frequent syndromic causes. We present three children with an unusual presentation of known syndromes associated with aortic pathology.

**Case 1:** This boy had failure to thrive, prominent forehead, malar hypoplasia, pectus carinatum, arachnodactyly, hypermobility, delayed motor development, frequent pulmonary infections and a striking progeroid appearance. The aortic root diameter was normal (z-score +0.8). He had a de novo *FBN1* mutation (c.8226+5G>A).

**Case 2:** This girl was known with intestinal malrotation, PDA, mydriasis, recurrent respiratory infections, constipation and urinary incontinence. She had pectus carinatum, thin skin, 2-3 syndactyly of the feet, and hypermobility. Cardiological examination showed dilatation of the ascending aorta. A de novo *ACTA2* mutation (c.535C>T, p.Arg179Cys) was found.

**Case 3:** This girl had neonatal hypotonia, neonatal convulsions, frequent respiratory infections and delayed motor development. Cerebral MRI showed periventricular heterotopia. Physical examination showed pes plani, mild scoliosis, hypermobility and hyperelastic skin with normal scars. Cardiac ultrasound shows a dilated aortic root. A de novo *FLNA* mutation (c.5184C>T) was shown.

The first two cases illustrate that specific mutations in well-known genes may give a specific, recognizable phenotype: mutations near the 3'end of the *FBN1* gene are associated with a distinct Marfanoid phenotype with progeroid features and lipodystrophy; the Arg179Cys mutation in *ACTA2*, usually associated with (non-syndromic) TAAD, gives a syndromic presentation with disseminated smooth muscle cell dysfunction. The third case illustrates that *FLNA* mutations, associated with Ehlers Danlos syndrome with periventricular nodular heterotopy, can be easily missed, especially when cerebral imaging is not performed.

The recognition of these syndromes is of utmost importance because all diagnosed syndromes are associated with increased risk of aortic pathology. Adequate and timely (molecular) diagnosis enables optimized screening and follow-up.

## Elastin in inherited cardiovascular disorders – stretching the associated phenotypes

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The *ELN* gene at 7q11.23 spans 45kb genomic DNA and encodes tropoelastin, the 72kD polypeptide subunit of elastin. Elastin is the major component of elastic fibres, which provide strength and flexibility to connective tissue, for example in the skin and arterial wall. Elastin has been implicated in a number of ICC including deletions at 7q11.23 (including *ELN*) in Williams Beuren Syndrome, in which supravalvular aortic stenosis is a common feature; sporadic or autosomal dominant non-syndromic SVAS or pulmonary artery stenosis (PAS) resulting from elastin haploinsufficiency and autosomal dominant cutis laxa caused by frameshift variants at the 3' end of the *ELN* gene. *ELN* gene mutation analysis has now been developed as a UKGTN service in Bristol.

We have investigated a three generation family identified through the proband who presented with middle aortic syndrome. Other members had SVAS, PS and ASD. Non-cardiac features included recurrent hernias, varicose veins, joint hyper extensibility and cutis laxa were subtle and variable. Mutation analysis identified a heterozygous nonsense *ELN* variant c.1621C>T (p.Arg541\*), reported previously. This enabled us to carry out a family study to clarify affected status in borderline cases. In addition wider screening of the vascular system identified intra-cranial aneurysms confirming that this is a systemic vascular phenotype which is important to diagnose in order to offer correct management and genetic counselling to family members.

We present this and other cases analysed and discuss the widening of the spectrum of vascular disorders associated with abnormality of Elastin.

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## **Sudden Cardiac Death: Diagnostic effectiveness of Molecular Autopsy in a Multidisciplinary Team Setting**

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Sudden cardiac death occurring between age 1 and 40 has a frequency of around 1-5 per 100000 patient years. In one series, 16% had cardiomyopathy at autopsy, while in 31%, no cause was found and death was presumed to be arrhythmic. In Aberdeen, we have developed a multidisciplinary team approach to the investigation of sudden cardiac death involving forensic pathology, genetics and cardiology. 82 cases have been referred for molecular autopsy over 11 years. 39 cases aged between 1 and 40 years had normal hearts and a presumed arrhythmic death. 31 cases have completed genetic investigation - all had Long QT testing (5 genes) and 9 were also tested for RYR2. 6 pathogenic mutations were detected (2 KCNH2, 2 SCN5A, 1 KCNQ1 and 1 RYR2). Most cases with mutations were aged 10 or less but 2 cases were aged between 36 and 40. 43 cases had evidence of cardiomyopathy – 23 Arrhythmogenic Right Ventricular Cardiomyopathy, 10 Dilated Cardiomyopathy and 10 Hypertrophic Cardiomyopathy. There was no age limit for genotyping in cases with suspected inherited cardiomyopathy. Six pathogenic mutations were detected. Three ARVC cases had PKP2 DSC2 or DSP. 1 DCM case had a mutation in MYH7, another in LMNA. Two HCM cases had MYBPC3 mutations. The peak age group in which mutations were detected was 40 – 49. Despite a conservative approach to defining pathogenicity of sequence variants a genetic diagnosis was made in 19% of sudden arrhythmic deaths and 16% of cardiomyopathy deaths. This illustrates the utility of careful use of new technology in diagnosing sudden cardiac death. There may be a role for molecular autopsy in people dying after age 40 with presumed arrhythmia or suspected inherited cardiomyopathy.

## **Paralogue Annotation: a new approach to accurately identify pathogenic genetic variants in patients with Inherited Cardiac Conditions**

James Ware

### **Background**

Distinguishing genetic variants that cause disease from variants that are rare but benign is arguably the principal challenge in contemporary genetics. We present a novel computational approach, called Paralogue Annotation, which accurately identifies disease-causing missense variants by transferring published functional annotations across families of related proteins (paralogues).

### **Methods and results**

We first tested the approach using long QT syndrome (LQT) genes. Paralogue Annotation accurately classified 185 known LQT-causing variants with a positive predictive value (PPV) of 98.4%, outperforming established tools ( $p < 0.05$ ). We also identified 1078 new putative disease loci in these genes.

Next, we applied the approach to variants found in 2,266 patients with catecholaminergic polymorphic ventricular tachycardia (CPVT) or Brugada syndrome (BrS), to evaluate applicability to a clinical diagnostic setting. More than one-third of novel non-synonymous variants found, that would otherwise be reported as “variants of unknown significance”, were re-annotated as likely disease-causing (55/153).

Considering variants of known consequence in BrS and CPVT genes, the PPV was 98.7%, in line with our previous estimate. 500 new putative disease loci for BrS and CPVT were identified.

This method is widely transferable to other human diseases. More than 1800 human disease genes belong to protein families to which this method can be applied, with ~150,000 potentially informative annotations.

### **Conclusion**

Paralogue Annotation accurately discriminates disease-causing genetic variation, and can be applied to interpret variants found through diagnostic genetic testing. Although not all variants can be annotated by this method, we found informative annotations for more than one-third of novel unclassified variants here, with a high PPV exceeding existing methods.

Our web resource ([http://cardiodb.org/Paralogue\\_Annotation/](http://cardiodb.org/Paralogue_Annotation/)) provides a user-friendly implementation of our approach, together with a referenced compendium of known variants in inherited arrhythmia syndrome genes, helping clinicians and researchers to interpret novel variants that might otherwise remain unclassified.

## A review of new evidence for variant pathogenicity - the impact of variant re-classification

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Genetic variants, other than those known to be benign polymorphisms, are identified in over 50% of our cardiomyopathy and cardiac arrhythmia patient cohorts. These variants are classified either as 'highly likely / likely to be pathogenic', 'unknown clinical significance' or 'unlikely to be pathogenic', based on information available at the time of classification. Classification is important for ensuring that patients (proband and their families) receive appropriate genetic counselling and follow-up. For at risk relatives, this may be pre-symptomatic genetic testing followed by clinical evaluation of those in whom the variant is present, or genetic testing combined with clinical screening to inform pathogenicity.

Over the past 10 years, there has been an increase in the information available for the genes offered by our diagnostic service, including: genotype data from population-based cohorts<sup>[2,3]</sup>, larger cohorts of normal controls, more case studies including details of segregation, and a better understanding of protein structure and function.

We report several cases where recent review of variants has led to the re-classification from 'highly likely or likely to be pathogenic' to 'unknown clinical significance' and even 'unlikely to be pathogenic'.

The impact of re-classification can be significant for the patient and their family. New information may mean that clinical follow up for additional individuals or further molecular analysis becomes appropriate, both of which may increase patient anxiety.

Our cases highlight the importance of regularly reviewing newly emerging data, considering variant classification in light of this new data, and where necessary, communicating new findings to clinical and laboratory colleagues.

### References

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## The Phenotypic Spectrum of Titin Truncations in Health and Disease

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### Background

TTN truncating variants (TTNtv) cause severe dilated cardiomyopathy (DCM), but sometimes occur in healthy individuals. The molecular consequences of TTNtv that impact clinical outcomes are poorly understood.

### Methods

We sequenced *TTN* in 4,440 subjects phenotyped by quantitative cardiac MRI (CMR) and/or echocardiography including 308 healthy volunteers, 3,603 Framingham Heart Study (FHS) and Jackson Heart Study (JHS) participants, 374 prospective, unselected and 155 end-stage DCM patients. RNA and protein studies were performed using 84 left ventricular (LV) specimens.

### Results

TTNtv were identified in 2.9% of healthy volunteers, 1.3% of FHS and JHS participants, and in 14% of unselected and 19% of end-stage DCM patients ( $P=7.6 \times 10^{-40}$ , DCM vs controls). TTNtv in DCM patients were enriched in highly expressed exons and isoforms ( $P=9 \times 10^{-5}$ ), unlike TTNtv in controls with normal cardiac physiology. TTNtv-positive FHS subjects had increased risk for DCM (RR=16,  $p=0.008$ ). TTNtv-positive DCM patients had more depressed LV ejection fraction (LVEF:  $P=0.02$ ), thinner LV walls ( $P<0.02$ ), and a higher incidence of sustained ventricular tachycardia ( $P=0.001$ ). C-terminus TTNtv were especially associated with lower LVEF ( $\beta=-18 \pm 7.4$ ,  $p=0.01$  versus N-terminus) and were more common in end-stage disease. LV function recovered in five of six TTNtv-positive end-stage DCM patients following mechanical unloading. No change was detected in total LV TTN mRNA or protein levels in TTNtv-positive hearts.

### Conclusions

Most individuals with TTNtv do not develop DCM, but TTNtv in highly expressed, particularly C-terminus, exons commonly cause DCM, likely through dominant-negative mechanisms. TTNtv-positive DCM exhibits increased arrhythmic risk worse indices of cardiac function and and recoverable LV impairment. Incorporation of exon-specific expression and variant position improves interpretation of TTNtv, enabling precision medicine.

## The Molecular Dissection of Dilated Cardiomyopathy.

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### Abstract:

Familial dilated cardiomyopathy (DCM) exhibits considerable clinical heterogeneity both within and between families; penetrance is incomplete and age dependant. Combined clinical screening and molecular analysis is therefore essential to identify affected individuals. The genetic basis of DCM is complex. Currently, there are in excess of 30 genes reported to cause nonsyndromic familial DCM, few are associated with any differentiating phenotype and the majority of genes account for less than 1% of cases. The Oxford Molecular Genetic Laboratory has developed and validated a diagnostic NGS workflow for the analysis of 28 DCM genes using Haloplex enrichment technology (Agilent Technologies) followed by sequencing on the MiSeq (Illumina). Here we report on the genetic heterogeneity observed in the first 50 familial DCM patients screened using this new test. On average, 97.5% of target regions were consistently covered to x30 read depth and clinically relevant variants were detected in 37% of patients. Pathogenic variants were found within genes responsible for the sarcomere and desmosome structure, and the sodium ion channel. Our test sensitivity of 37% is higher than previously reported studies (17-35%) <sup>[1,2]</sup> and demonstrates the utility of an extensive DCM gene panel in a diagnostic setting . Frequency, distribution and classification of clinically relevant variants will be discussed.

[1] Hershberger & Siegfried, *Am Coll Cardiol* 2011; **57**:1641–9.

[2] Lakdawala *et al.*, *J Card Fail.* 2012; **18**(4):296-303.

## **Informed Consent? The Minefield Of Complex Results**

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In 2013, nineteen UK Clinical Genetics Centres participated in a National Cardiac-Genetics Audit of diagnostic genetic testing for hypertrophic cardiomyopathy (HCM) and Long QT (LQT) syndrome. A total of 379 HCM and 197 LQT referrals between April 2011-12 were audited.

The audit found that of the 487 patients offered a genetic test, 36% of gene tests identified a single pathogenic mutation, whilst in 43% no abnormality was detected. One in five (20%) HCM and LQT diagnostic gene tests produced a 'complex result' i.e an unclassified variant (UV) or a 'dual mutation' - a result with more than one possible mutation. The remaining 1% of gene test results were pending at data collection.

Furthermore, in over half of the patients' notes the audit identified there was no documented evidence that a pre-test discussion had taken place regarding the possibility of finding a complex result. Either discussion occurred, but documentation was inadequate, or more worryingly, no pre-test discussion of a complex result was undertaken. Either way, this audit highlights a training need for clinicians taking consent for diagnostic gene testing for inherited cardiac conditions.

Without appropriate discussion of all possible outcomes of a genetic test, including complex results, the consent taken can not be considered informed and is therefore invalid. For a patient who is expecting a definite answer, receiving a complex and uncertain result may lead to post decision regret, resulting in a detrimental psychological outcome for the patient. It also demonstrates suboptimal counselling with regard to the promotion of empowerment, autonomy and effective decision making and it represents a poor standard of care delivered by the clinician.

The presentation will cover:

- possible outcomes for diagnostic gene testing for inherited cardiac conditions
- the implications of complex results for the patient and their wider family;
- the critical importance of informed patient consent