

Conclusions HARP and SinMod methods show a high level of agreement for assessment of global mid-ventricular transmural circumferential strain, with good reproducibility for both individual methods. Agreement is much lower for segmental measurements; poor reproducibility for segmental measurements using both techniques probably reflect user variability in identification of right ventricular septal insertion points and contour tracing.

131 AETIOLOGICAL ROLE OF FOLATE DEFICIENCY IN CONGENITAL HEART DISEASE: EVIDENCE FROM MENDELIAN RANDOMISATION AND META-ANALYSIS

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Background The existence of a causal relationship between lower levels of plasma folate and congenital heart disease (CHD) remains contentious. Randomised trials of this question are not possible, in view of the known protective effect of folate against neural tube defects (NTDs). Folate fortification of flour is known to reduce the incidence of NTDs, but it is not known whether there is any effect on CHD. Clarity regarding the relationship between folate and CHD could potentially inform the practice of folate fortification. We present a genetic approach using “Mendelian randomisation” to determining the causality of folate in CHD risk.

Methods We compared genotype frequencies at the methylene tetrahydrofolate reductase (MTHFR) C677T single nucleotide polymorphism (SNP) in 1186 CHD cases and 4168 controls. The TT genotype at MTHFR C677T is known to be associated with lower activity of MTHFR and plasma folate, and higher levels of plasma homocysteine. The effect of TT genotype on plasma folate levels is greater in conditions of folate deficiency. Thus, if lower plasma folate had a causal effect on CHD risk, a higher frequency of TT genotype among CHD cases than among healthy controls would be anticipated, and this would be expected to be more marked in conditions of folate deficiency. We placed our results in the context of a meta-analysis of all previously published studies of this question (to September 2010), which together included 1883 cases and 3069 controls in 25 studies. Thus, the combined analyses included 3069 CHD cases and 7271 controls. We used random-effects models to combine the data. We conducted sensitivity analyses to examine folate fortification of flour as a potential source of heterogeneity.

Results The primary genotyping data in 1186 cases and 4168 controls revealed a trend towards increased risk with the TT genotype, but this did not reach statistical significance (OR 1.15 (95% CI 0.94 to 1.40)). Combination of our primary data with previous studies, however, revealed association in the larger dataset (OR 1.45 (95% CI 1.12 to 1.89); $p=0.005$). The population attributable fraction for the TT genotype was 3% of CHD. There was no evidence of publication bias among the contributing studies. We discovered folate fortification status to be a significant source of heterogeneity. Studies conducted in countries with mandatory folate fortification showed no effect of C677T genotype on CHD risk (OR 0.96 (95% CI 0.64 to 1.44)), whereas studies conducted in countries without mandatory fortification showed a significant effect of genotype (OR 1.63 (95% CI 1.19 to 2.25)). These ORs were significantly different from each other ($p=0.032$).

Conclusions We demonstrate genetic evidence in favour of a causal relationship between plasma folate and CHD. The absence of a genetic association in countries practicing folate fortification suggests that fortification largely abrogates the risk of CHD attributable to folate deficiency.

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NON-SYNONYMOUS SMAD6 MUTATIONS IMPAIRED INHIBITION OF BMP SIGNALLING IN PATIENTS WITH CONGENITAL CARDIOVASCULAR MALFORMATION

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Introduction Congenital cardiovascular malformation (CVM) exhibits familial predisposition but the specific genetic factors involved are unknown. Bone morphogenetic proteins (BMPs) regulate many processes during development, including cardiac development. Five genes of the BMP signalling were surveyed for novel variants predisposing to CVM risk. One of the genes, *SMAD6*, functions as an inhibitory SMAD which preferentially inhibits BMP signalling. The *SMAD6* knockout mouse is characterised by cardiac valve and outflow tract defects, including aortic ossification. We hypothesised that rare functional variation in *SMAD6* could predispose to congenital cardiovascular malformation (CVM).

Methods The coding regions of *BMP2*, *BMP4*, *BMP1A*, *BMPR2* and *SMAD6* were sequenced in 90 unrelated Caucasian cases of CVM. The MH2 domain of *SMAD6* were further sequenced in additional 346 CVM patients. Functional effects of the wild-type and variant *SMAD6* proteins were expressed in C2C12 cells and their capacity to inhibit ALK3 activated expression of a BMP-responsive reporter, or to inhibit osteogenic differentiation (using an alkaline phosphatase assay) was assessed.

Results We identified two novel non-synonymous variants, P415L and C484F, that were not present in 1000 ethnically-matched controls. P415L was identified in a patient with congenital aortic stenosis and C484F was identified in a patient with coarctation and calcification of the aorta. Both mutations are in evolutionarily conserved amino acid residues and are predicted to be damaging by in silico analysis. This was confirmed in functional assays as both *SMAD6* variants failed to inhibit BMP signalling compared with wild-type *SMAD6*. The P415L mutant appeared to be hypomorphic whereas C484F appeared to be a null allele in the luciferase assay. The C484F mutant had a significantly ($p<0.05$) lower capacity to inhibit alkaline phosphatase generation in response to BMP signalling.

Conclusions This is the first time that functional mutations in *SMAD6* have been described in patients with CVM, specifically those with calcific aortic malformations. Our data suggest that inadequate inhibition of BMP signalling pathway due to genetic variation in *SMAD6* may be an important factor in CVM.

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ACTIVITY AND PSYCHOSOCIAL HEALTH IN ADOLESCENTS WITH CONGENITAL HEART DISEASE (CHD)

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Many patients with CHD are now adolescents. Like other patients with chronic illnesses they may be at higher risk of psychological/emotional problems. Ability to exercise is an important quality of life measure and indicator of physical health. We aimed to ascertain if activity and psychosocial health were reduced in adolescents with major CHD compared to those with a minor diagnosis. Patients aged 12–20 years were identified using the Northern Ireland regional database (HeartSuite). Participants were categorised as having major or minor CHD and divided into four diagnostic

subgroups. Participants completed validated, age-appropriate questionnaires examining standard psychological parameters. Participants also underwent an evaluation of exercise, including formal exercise stress testing and measurement of free-living activity using an ActiGraph accelerometer. Results were analysed using parametric methods. 143 patients (mean age 15.6 years) consented to participate, 86 were male (60%) and 105 had major CHD (73%). Diagnostic subgroups included 39 acyanotic (27.3%), 61 acyanotic corrected (42.7%), 30 cyanotic corrected (21.0%) and 13 (9%) cyanotic palliated patients. Beck Youth Inventory demonstrated that individuals with major CHD, particularly cyanotic palliated patients, had higher anxiety scores (p value 0.01 ($-8.42, -1.13$)). There were no significant differences across study groups for self-esteem or other psychological parameters. 134 participants (93.7%) took part in regular exercise each week. There was no significant difference in activity score between study groups. On formal exercise testing, more complex patients performed worse at peak exercise. Exercise time for acyanotic group 11.73 mins (sd 3.74) compared to 8.26 mins (sd 4.08) in cyanotic palliated group, p value 0.002 (1.32, 5.61)). However, patients with major CHD had significantly higher activity counts. Correlation analysis showed that self-esteem and health locus of control were important predictor variables for activity. Self-esteem and mood seem well preserved in adolescents with CHD as a whole. The majority of young people with CHD, in this group, take part in regular exercise. Surprisingly, complex patients rate themselves to be as active as those with minor CHD. While accelerometer data indicate that the group may be more active day to day, they are limited in terms of peak exercise duration. The experience of growing up with a chronic condition may therefore have a positive effect on psychological health and interventions targeted around this area may influence activity.

134 MUTATIONS IN THE SARCOMERE PROTEIN GENE MYH7 IN EBSTEIN'S ANOMALY

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Background Ebstein's anomaly is a rare congenital heart malformation characterised by adherence of the septal and posterior leaflets of the tricuspid valve to the underlying myocardium. As there have been reports of abnormal left ventricular morphology and function in patients with Ebstein's anomaly we hypothesised that mutations in the β -myosin heavy chain (MYH7) may be associated with Ebstein's anomaly.

Methods MYH7 mutation analysis was undertaken in 141 unrelated affected individuals with Ebstein's anomaly using next-generation sequencing on the 454 platform. 64 probands had no associated cardiac anomalies. The most common associated cardiac malformation were atrial septal defect (48 probands) and left ventricular non-compaction (LVNC) (7 probands). Where mutations were discovered, family studies were undertaken and the segregation of the mutation with disease was investigated.

Results Heterozygous mutations were identified in eight of the probands including six of the seven with LVNC. Two patients had the same mutation; of the seven distinct mutations, five were novel (four missense changes and an in-frame deletion) and two have been previously reported in patients with hypertrophic cardiomyopathy. Family studies revealed additional members with LVNC for three of the probands, one of whom also had a relative with Ebstein's anomaly. In these three pedigrees the mutation segregated with disease.

Conclusions Mutations in MYH7 occur relatively frequently in Ebstein's anomaly accompanied by LVNC. This study is another

example of mutations in a sarcomere protein causing congenital heart malformation.

135 GENE SCREENING OF THE SECONDARY HEART FIELD NETWORK IN TETRALOGY OF FALLOT PATIENTS

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Background Tetralogy of Fallot (TOF) is the most common cyanotic heart defect, affecting 3–6 infants for every 10 000 births. TOF is phenotypically well defined; it consists of four heart abnormalities: a VSD, an over-riding aorta, a narrowed pulmonary valve and right ventricular hypertrophy. During heart development two heart fields can be distinguished. The first one gives origin to the left ventricle and contributes to the right and left atria. The secondary heart field gives origin to the right ventricle and the outflow tract. Each of these fields can be identified by the expression of specific markers. As TOF is a malformation of the outflow tract, we hypothesised genes involved in the regulatory network of the secondary heart field were particularly good candidates for TOF susceptibility.

Methods We examined by standard Sanger method the full exonic and intron boundary regions of 14 secondary heart field genes, namely *NKX2-5*, *GATA4*, *TBX20*, *MEF2C*, *BOP*, *HAND2*, *FOXC1*, *FOXC2*, *TBX1*, *FOXA2*, *FGF10*, *FGF8*, *ISL1* and *FOXH1*, in a panel of 93 TOF patients. All newly discovered rare variants were checked in a panel of 1000 control chromosomes by multiplex Sequenom assays. When available, parents of cases were screened to assess inheritance of the rare variant.

Results We re-sequenced a total of 80 exons and ~30 Kb. Among the 14 genes studied we found a total of 50 new variants, of which 23 were exclusive to the patient population, ie, were absent from 1000 normal chromosomes. Nine of these variants cause change in the amino acid sequence. We found a functional 19aa deletion of a highly conserved region of *TBX1*. In *FOXC1* we found a contraction of both alanine and glycine tracts. An alanine expansion, usually known to be deleterious, was found in *HAND2*. Four non-synonymous changes were found in *FOXA2*. Most patients presented just one variant, however 3 patients presented two, and one patient presented up to 3 variants. All patients were heterozygotes for the variants, and had inherited them from one of their phenotypically normal parents (when parental information was available). In addition, 75% of the variants were inherited from the mother.

Conclusions Although genes of the secondary heart field seemed good candidates for TOF susceptibility, thus far we have not found any strong indication of unique causal effect, as all variation found in probands was also present in their unaffected parents. However, the presence of multiple variants in the same proband may result in the disruption of gene-gene interactions in the secondary heart field pathway, which in turn may lead to outflow tract defects. Based on our results, it would seem more likely that susceptibility to TOF be determined by a larger number of small genetic contributions which are also modified by environmental factors. It is evident that larger scale analysis of significant numbers of whole genomes/exomes will be necessary to better understand the molecular aetiology of TOF.

136 SHOULD FAMILIAL SCREENING BE ROUTINELY OFFERED TO PATIENTS WITH BICUSPID AORTIC VALVE DISEASE?

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Background Bicuspid aortic valve (BAV) disease is one of the most common congenital cardiac abnormalities with prevalence in the