GENETICS OF CARDIOVASCULAR DISEASE IN 2016

Title COMMON AND RARE GENETIC VARIANTS AND CARDIOVASCULAR DISEASE RISK

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Much of the progress in cardiovascular genetics in 2016 has been driven by next-generation sequencing studies, and the clinical utility of knowing an individual's genotype for predicting their risk of cardiovascular disease is gaining credibility, both for monogenic and polygenic disorders. Additionally, phenotype data are increasingly abundant, although databases linking genotype with clinically relevant phenotypes require optimization.

Next-generation sequencing (NGS) has driven major advances in our understanding of the monogenic cause of elevated LDL-C and premature coronary heart disease (CHD) in familial hypercholesterolemia (FH), focussing on the three proven FH-causing genes (*LDLR, APOB*, and *PCSK9*). NGS has a detection rate approaching 100% and a false-positive rate of almost 0%, but NGS is currently too expensive for population-scale screening. Although the prevalence of FH has historically been estimated at 1/500 in outbred populations, the likely true prevalence of FH-causing mutation carriage now seems to be between 1/250 and 1/300. In Denmark, 98,098 participants from the Copenhagen General Population Study were genotyped for three common *LDLR* mutations and the most common *APOB* mutation (p.R3527Q)[1]. The prevalence of the four FH mutations was 1/565, accounting for approximately 39% of all pathogenic mutations in the country, and equating to an estimated total prevalence of FH-mutation carriers of 1/217 in the general population [1].

Wald and colleagues[2] reported a UK study aimed to identify adults with FH by measuring cholesterol in 10,094 children at the time of routine immunizations (median age 12.7 months). Using diagnostic criteria of either total cholesterol >5.31 mmol/l (205 mg/dl; 95th percentile) plus any FH-mutation, or two cholesterol values of ≥5.90 mmol/l (228 mg/dl; 99th percentile), the investigators identified 45 children with FH, 37 children had a detected mutation and eight had repeated LDL-cholesterol (LDL-C) over the prespecified threshold with no identified mutation. The prevalence of mutation carriers was thus 1/273 (37/10,094). We have previously demonstrated that such individuals (without a monogenic cause of FH) probably have a polygenic aetiology for their FH phenotype, with co-inheritance of a greater than average number of common LDL-C-raising gene variants of modest effect, and have proposed that *only* those patients with a detected mutation should be designated as monogenic FH[3]. When testing the parents of the children with confirmed FH, Wald and colleagues identified 40 parents who also met the criteria for FH diagnoses[2], demonstrating the feasibility of the approach in primary care at routine child immunization visits.

In the UK, in patients with FH where a monogenic cause is found, approximately 93% have a mutation in *LDLR*, around 5% in *APOB*, and approximately 2% in *PCSK9*, and although mutations in other novel genes have been proposed, none have been independently confirmed yet. By contrast, in the Wald study 15/37 (40%) of the detected FH-mutations were the *APOB* (p.R3527Q) mutation — a well-documented cause of generally less severe FH than most *LDLR* mutations. Universal screening, therefore, had detected many individuals with a genetic predisposition to milder hypercholesterolaemia, but who might be at less elevated risk of CHD than carriers of *LDLR* Thus,

although this screening approach has value in identifying new families with FH, universal screening will also identify individuals with low risk of cardiovascular disease. In these patients, the genetic data might contribute to decision-making for intensive statin treatment, where the risk–benefit balance would otherwise not be as clear as for relatives of index patients with clinically diagnosed FH, whose personal or family history of early CHD is an essential diagnostic component.

This elevated risk for CHD in patients with FH with a detected mutation has been confirmed convincingly by Khera *et al*.[4] in a population-based analysis. Khera and colleagues used NGS for the known FH genes in 20,485 individuals without CHD, of whom 1,386 (6.7%) had LDL-C >4.9 mmol/l (190 mg/dl), and of these 24 (1.7%) carried a known FH mutation. Those individuals with LDL-C >4.9 mmol/l and no FH mutation had a six-fold higher risk of CHD than individuals with LDL-C <3.7 mmol/l (130 mg/dl) and no mutation, but individuals with both LDL-C >4.9 mmol/l and an FH mutation had a 22-fold higher risk. This increased risk is explained by the substantially higher accumulated 'LDL-C burden', because these patients have had genetically-determined, lifelong, high LDL-C levels.

Dilated cardiomyopathy has a prevalence of up to 1/250 in the general population and is the most common reason for heart transplantation. Dilated cardiomyopathy is frequently caused by truncating mutations in the titin gene (*TTN*). *TTN*-truncating variants occur in approximately 1% of the general population, but not all carriers of such variants develop dilated cardiomyopathy. To explore the mechanisms of this 'variable penetrance', Shafer *et al*.[5] used rat models and human cardiac imaging and genomic data. The rat studies showed that the truncation variants led to nonsense-mediated degradation of the mutant protein and altered cardiac metabolism. However, cardiac physiology in the mutant rats was normal in young animals, but became impaired during stress. To define the effects of *TTN*-truncating variants on the heart in the general population, Shafer *et al.* sequenced the *TTN* gene in 1,409 healthy volunteers, with cardiac morphology and function examined by standard 2D cardiac magnetic resonance (CMR) imaging and with high-resolution, high-fidelity 3D CMR imaging. Carriage of a *TTN*-truncating variant was associated with eccentric cardiac remodelling, with a phenotypic spectrum from healthy to severe disease[5]. Similar to familial hypercholesterolemia, rare, highly penetrant mutations lead to severe disease, but carriage of less deleterious variants in the same gene cause a spectrum of cardiac dysfunction in the general population.

Using a genetic risk score (GRS) combining 50 variants identified in genome-wide association studies (GWAS) to be associated with risk of CHD, Khera *et al*.[6] compared genetically-determined and lifestyle-related risk of CHD in three prospective, USA and Swedish cohorts (total participants ≈ 56,000; 5,103 CHD events). A healthy lifestyle was defined as a composite of no current smoking, no obesity, regular physical activity, and a healthy diet. Results were consistent across the studies, and the relative risk of incident CHD was 91% higher among those individuals in the highest quintile of the GRS than in those in the lowest quintile[6]. The association between GRS and a family history of premature CHD was modest but significant (15.1% of individuals in the low GSR group had a family history of premature CHD, 16.9% in the intermediate GSR group, and 18.7% in the high GSR group; P >0.0001). Unsurprisingly, an unhealthy lifestyle (absence of at least three of the four factors above) resulted in a substantially higher (≈80%) risk of CHD, and the genetic and lifestyle risk scores had essentially cumulative effects. Individuals at high genetic risk but who followed a favourable lifestyle had lower overall risk than those with high genetic risk and an unhealthy lifestyle. In a substudy including 4,260 individuals, both genetic and lifestyle factors were associated with coronary artery

calcification. One of the studies (ARIC) included African American participants (2,269 individuals; 350 coronary events and showed consistency in the magnitude of effects across both ancestral groups[6].

The debate over the relationship between blood lipids and risks of CHD and type 2 diabetes mellitus (T2DM) continues to intensify as the results of outcome trials of novel proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor agents are awaited. As shown in FIG. 1, there is little doubt that reducing LDL-C leads to lower risk of CHD, and doing so using statins increases the risk of T2DM[7]. White *et al*.[8] used Mendelian randomization to explore the causal relationships between the three major lipid fractions — HDL cholesterol (HDL-C), LDL-C, and triglycerides — and risk of CHD and T2DM. Importantly, these analyses were executed with novel methods and exclusively with publicly available GWAS data. As expected, both LDL-C and triglycerides demonstrated positive causal relationships with the risk of CHD, but higher HDL-C was not associated with lower risk of CHD once pleiotropy of the genetic instruments had been accounted for[8]. Higher concentrations of LDL-C and HDL-C were associated with lower risk of T2DM, suggesting that altered lipid fraction concentrations *per se*, rather than specific therapeutic mechanisms, can alter glucose homeostasis.

The risk of T2DM associated with individual, therapeutic, lipid-modulatory mechanisms remains an area of interest[9]. Given the now confirmed increased risk of T2DM with statin treatment, much attention rests on whether a similar risk increase will be observed in phase III trials on PCSK9 inhibitor agents. Identification of functional gene variants in proposed cardiovascular disease aetiological pathways has allowed exploration of causal contributions of specific cardiovascular disease-associated lipid traits in coronary heart disease by use of Mendelian randomization. By the use of common variants in the gene encoding PCSK9 as proxies for pharmacological inhibition of the enzyme, Schmidt and colleagues[10] demonstrated genetic associations of lower LDL-C, higher blood glucose levels, and bodyweight, and higher risk of T2DM (OR 1.29, 95% CI 1.11–1.50 per 1mmol/l LDL-C reduction). These findings have been confirmed by other genetic analyses, and have emphasized the importance of diabetes outcomes in the forthcoming PCSK9 inhibitor phase III trials.

The year 2016 has brought substantial advances in our understanding of the genetic architecture of cardiomyopathy, coronary disease, and dyslipidaemia. However, much uncharted territory remains in cardiovascular genetics and, perhaps most strikingly, in the case of common, non-monogenic heart failure. The HERMES consortium to investigate heart failure genetics is set to address this issue in 2017, and the findings of this consortium, along with those of many other ongoing efforts, are hotly awaited.

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Competing interests statement

D.I.S. has been a consultant to Pfizer. S.E.H. declares no competing interests.

Further information
HERMES consortium: www.hermesconsortium.org

Key advances

- The prevalence of familial hypercholesterolaemia has now been established convincingly by next generation sequencing and by genotyping of European, population-based studies to be roughly 1/250, twice as common as previously thought[1]

- Common truncating mutations in the gene encoding titin increase the risk of dilated cardiomyopathy in humans, but only under stress conditions[5]

  A genetic risk score combining common single-nucleotide polymorphisms identified in genome-wide association studies showed clinical utility in identifying individuals at high risk of coronary heart disease (CHD), which can be offset with healthy lifestyle choices[6]
- Mendelian randomization studies have shown the directionally consistent effect of genetically-lowered or pharmacologically-lowered LDL-cholesterol in increasing risk of type 2 diabetes mellitus, while reducing risk of CHD, demonstrating a causal role of low LDL-cholesterol[7,8,9,10]

- Trials of LDL-cholesterol lowering with agents such as proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors should monitor for development of type 2 diabetes

Figure 1 | **genetically lower or pharmacologically Lipid-lowering therapies, lipid fractions, and risk of coronary heart disease and type 2 diabetes.** Evidence from randomized clinical trials (RCT) and Mendelian randomization (MR) studies on the relationship between lipid-modifying drug targets or commonly measured lipid fractions — LDL-cholesterol (LDL-C), triglycerides (TG), and HDL-cholesterol (HDL-C) — and the risk of coronary heart disease and type 2 diabetes mellitus. Common targets of lipid-modifying drugs are 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), which is the essential enzyme in the cholesterol synthesis cascade and is inhibited by statins[7]; Niemann-Pick C1-like protein 1 (NPC1L1), which is inhibited by the LDL-C-lowering agent ezetimibe[9]; and proprotein convertase subtilisin/kexin type 9 (PCSK9), the main protein involved in the degradation of LDL receptors during receptor recycling[10]. Risk reduction is shown in green and risk increase is shown in red.