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Use of low-density lipoprotein cholesterol gene score to distinguish patients with polygenic and monogenic familial hypercholesterolaemia: a case-control study

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Summary

Background Familial hypercholesterolaemia is a common autosomal-dominant disorder caused by mutations in three known genes. DNA-based cascade testing is recommended by UK guidelines to identify affected relatives; however, about 60% of patients are mutation-negative. We assessed the hypothesis that familial hypercholesterolaemia can also be caused by an accumulation of common small-effect LDL-C-raising alleles.

Methods In November, 2011, we assembled a sample of patients with familial hypercholesterolaemia from three UK-based sources and compared them with a healthy control sample from the UK Whitehall II (WHII) study. We also studied patients from a Belgian lipid clinic (Hôpital de Jolimont, Haine St-Paul, Belgium) for validation analyses. We genotyped participants for 12 common LDL-C-raising alleles identified by the Global Lipid Genetics Consortium and constructed a weighted LDL-C-raising gene score. We compared the gene score distribution among patients with familial hypercholesterolaemia with no confirmed mutation, those with an identified mutation, and controls from WHII.

Findings We recruited 321 mutation-negative UK patients (451 Belgian), 319 mutation-positive UK patients (273 Belgian), and 3020 controls from WHII. The mean weighted LDL-C gene score of the WHII participants (0·90 [SD 0·23]) was strongly associated with LDL-C concentration ($p=1{\cdot}4\times10^{-77}$; $R^2=0{\cdot}11$). Mutation-negative UK patients had a significantly higher mean weighted LDL-C score (1·0 [SD 0·21]) than did WHII controls ($p=4{\cdot}5\times10^{-16}$), as did the mutation-negative Belgian patients (0·99 [0·19]; $p=5{\cdot}2\times10^{-20}$). The score was also higher in UK (0·95 [0·20]; $p=1{\cdot}6\times10^{-5}$) and Belgian (0·92 [0·20]; p=0·04) mutation-positive patients than in WHII controls. 167 (52%) of 321 mutation-negative UK patients had a score within the top three deciles of the WHII weighted LDL-C gene score distribution, and only 35 (11%) fell within the lowest three deciles.

Interpretation In a substantial proportion of patients with familial hypercholesterolaemia without a known mutation, their raised LDL-C concentrations might have a polygenic cause, which could compromise the efficiency of cascade testing. In patients with a detected mutation, a substantial polygenic contribution might add to the variable penetrance of the disease.

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Introduction

Familial hypercholesterolaemia (Online Mendelian Inheritance in Man number 143890) is a common autosomal-dominant disorder that is thought to be monogenic, and is characterised by substantially raised plasma concentrations of low-density lipoprotein cholesterol (LDL-C) and a five to eight times higher than average risk of early coronary heart disease.[1] The National Institute for Clinical Health and Excellence (NICE)-endorsed Simon Broome Register criteria for a clinical diagnosis of familial hypercholesterolaemia include a high LDL-C concentration (>4·9 mmol/L in adults) plus a family history of raised cholesterol, or early coronary heart disease in a first-degree relative.[1,2]

Patients who also have tendon xanthomas—a hallmark of long-term high cholesterol—are designated as having definite familial hypercholesterolaemia, whereas patients without tendon xanthomas are given the clinical diagnosis of possible familial hypercholesterolaemia. The prevalence of familial hypercholesterolaemia is about 1 per 500 people in most countries in Europe, although this might be an underestimate because a population-based study reported a frequency of one per 137 in Denmark.[3] 120 000 people in the UK could therefore have heterozygous familial hypercholesterolaemia with an increased coronary heart disease risk, but only around 15 000 are being treated in lipid clinics.[4]

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LDL-C concentrations in patients with familial hypercholesterolaemia can be effectively lowered by statins,[5,6] which can significantly improve life expectancy.[6–8] The charity HEART UK has written a report on the health, social, and economic advantages of treating familial hypercholesterolaemia,[9] and estimated that high-intensity lipid-lowering statin therapy would lead to 101 fewer cardiovascular deaths per 1000 patients with familial hypercholesterolaemia given treatment (aged 30–85 years) than if no treatment was given, and that the UK could save £378·7 million from cardiovascular events avoided if all relatives of index cases were identified and treated optimally over 55 years, equating to £6·9 million per year.

The 2008 UK NICE guidelines for the identification and management of patients with familial hypercholesterolaemia[10] recommend that all patients with clinical and biochemical features of familial hypercholesterolaemia should be offered a DNA test to confirm their diagnosis.[2] Mutation testing would then enable unambiguous identification of affected relatives. This cascade testing strategy[11] has been successfully applied in the Netherlands in families of familial hypercholesterolaemia probands with a detected mutation, thus allowing statin treatment of family members at risk of early coronary heart disease.[12] A DNA-based cascade testing strategy for patients with this disorder has been implemented in Wales, Scotland, and Northern Ireland,[4] but has not been widely commissioned in England.

Mutations in one of three genes are known to cause familial hypercholesterolaemia. In the UK, about 93% of identified mutations are in the gene encoding the receptor for LDL-C removal (*LDLR*), a further 5% are in *APOB,* which codes for apolipoprotein B (the major apoprotein component of LDL-C that acts as a ligand for the LDL receptor), and about another 2% are in *PCSK9*, which codes for a protein involved in the degradation of the LDL receptor.[13,14] With standard molecular diagnostic techniques, a familial hypercholesterolaemia-causing mutation can be detected in 20–30% of patients with possible familial hypercholesterolaemia and 60–80% of patients with definite familial hypercholesterolaemia.[14–16] Roughly two-thirds of patients have possible familial hypercholesterolaemia;[5,14] thus, overall no mutations are detected in about 60% of tested patients with this disorder.[14] This finding has led to a search for additional familial hypercholesterolaemia-causing genes, using genome-wide linkage approaches[17] and next-generation sequencing of all coding exons.[18] However, a proportion of all clinically diagnosed cases of familial hypercholesterolaemia could possibly be polygenic, due to the inheritance of a greater than average number of common LDL-C-raising alleles (each causing a slight effect) leading to an increase in LDL-C above the diagnostic cutoff. The inclusion of probands with a polygenic rather than monogenic cause of hypercholesterolaemia would reduce the efficiency of any cascade screening programme, since much less than the expected 50% of first-degree relatives would be affected. Therefore, identification and exclusion of individuals with polygenic hyperlipidaemia would enhance and enrich any cascade testing programme.

See **Online** for appendix

The Global Lipid Genetic Consortium (GLGC) meta-analysis of genome-wide association studies identified several loci where common variants affect LDL-C concentration,[19] and results of another study[20] showed that a proportion of individuals carrying several LDL-C-raising single nucleotide polymorphisms (SNPs) have LDL-C concentrations that exceed the diagnostic LDL-C threshold of 4·9 mmol/L (used to diagnose familial hypercholesterolaemia).

We aimed to calculate gene scores, derived from 12 common LDL-C-raising SNPs in 11 genes (two in *APOE*), in a sample of UK patients with European ancestry who had a clinical diagnosis of familial hypercholesterolaemia with or without an identified familial hypercholesterolaemia-causing mutation and compare them with those of healthy men and women of European ancestry from the UK Whitehall II (WHII) cohort study to test the hypothesis that familial hypercholesterolaemia can be caused by an accumulation of common small-effect LDL-C-raising alleles. We also aimed to validate our results by repeating the analysis in a sample of patients with familial hypercholesterolaemia from Belgium.

Methods

Patients

In November, 2011, we assembled our study sample, which consisted of all British patients with familial hypercholesterolaemia from three sources: the Simon Broome British Heart Foundation study,[21] the Oxford familial hypercholesterolaemia study,[22] and the Department of Health familial hypercholesterolaemia audit project.[14] For validation analyses, we also recruited Belgian patients with familial hypercholesterolaemia from one lipid clinic (Hôpital de Jolimont, Haine St-Paul, Belgium).[23] See appendix for further details on inclusion and diagnostic criteria used in each study. We used a healthy comparison group of white men and women from the UK Whitehall II study (appendix).[24]

The Whitehall II study was approved by the University College London Research Ethics Committee and participants gave informed consent to each aspect of the study. All other studies from which we recruited our study samples received ethical approval from their respective ethics committees.

Procedures

We isolated genomic DNA from whole blood samples using standard methods.[25] Details of mutation detection in *LDLR, APOB*, and *PCSK9* genes are presented in the appendix. We then used standard methods (appendix) to genotype the selected SNPs.

For the gene score calculation—the primary endpoint of the study—we selected only the lead SNP from each locus, and if a SNP was associated with more than one lipid

fraction, we only included it if LDL-C was the lead trait (most strongly associated) for the SNP. For each individual, we calculated LDL-C-specific gene scores using the weighted sum of the risk allele (ie, the LDL-C-raising allele). The weights used were the corresponding per-allele (risk) beta coefficients reported by the GLGC (appendix).

Statistical analysis

We did linear regression of observed baseline LDL-C concentrations with the weighted LDL-C gene scores. We also calculated the risk ratio of having a measured LDL-C concentration of higher than 4·9 mmol/L by deciles of the GLGC weighted score in WHII controls. We used the statistical program R-2.14.2 to analyse the data.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full

	UK patients with familial hypercholesterolaemia			WHII controls (n=3020)
	FH with no known mutation (n=321)	FH with known mutation (n=319)	p value*	
Men	73/156 (46·8%)	126/237 (53·2%)	0·47	2308/3020 (76·4%)
Age (years)	54·9 (13·9) (n=320)	49·5 (14·0) (n=316)	0·02	49·0 (6·0)
Pretreatment total cholesterol (mmol/L)	9·16 (4·92) (n=263)	10·28 (1·90) (n=246)	0·03	6·4 (1·1)
Pretreatment LDL-C (mmol/L)	5·87 (1·57) (n=71)	7·03 (1·49) (n=27)	0·002	4·4 (1·0)
Post-treatment LDL-C (mmol/L)	4·22 (1·58) (n=136)	5·49 (1·34) (n=237)	0·05	4·4 (1·0)
Post-treatment HDL-C (mmol/L)	1·42 (0·34) (n=67)	1·37 (0·36) (n=237)	0·35	1·4 (0·4)
Post-treatment triglycerides (mmol/L)	1·82 (0·86) (n=67)	1·33 (0·67) (n=237)	$1{\cdot}54{\times}10^{-6}$	1·4 (1·1)

Data are number (%) or mean (SD), unless otherwise stated. The denominators vary because these measurements were only available in patients not receiving lipid lowering therapy. WHII=Whitehall II. FH=familial hypercholesterolaemia. LDL-C=low-density lipoprotein cholesterol. HDL-C=high-density lipoprotein cholesterol. *We adjusted p values according to the study source that the patient belonged to using a two-way ANOVA; age (p=0·02), post-treatment LDL (p<0·0001), and post-treatment HDL (p<0·0001) significantly differed between each familial hypercholesterolaemia cohort after adjustment for presence or absence of mutation; sex (p=0·39), pretreatment total cholesterol (p=0·30) and pretreatment LDL (p=0·62) did not differ significantly after adjustment.

Table 1: **Basic characteristics of UK participants**

	Chromosome number	Gene	Minor allele	Common allele	GLGC weight for score calculation	Minor allele frequency		
						FH with known mutation (n=319)	FH without known mutation (n=321)	WHII controls (n=3020)
rs2479409	1	*PCSK9*	G*	A	0·052	0·33	0·39	0·35
rs629301	1	*CELSR2*	G	T*	0·15	0·19	0·12	0·21
rs1367117	2	*APOB*	A*	G	0·10	0·35	0·37	0·33
rs4299376	2	*ABCG8*	G*	T	0·071	0·37	0·37	0·32
rs1564348	6	*SLC22A1*	C	T*	0·014	0·19	0·17	0·17
rs1800562	6	*HFE*	A	G*	0·057	0·06	0·08	0·07
rs3757354	6	*MYLIP*	T	C*	0·037	0·21	0·17	0·21
rs11220462	11	*ST3GAL4*	A*	G	0·050	0·14	0·13	0·13
rs8017377	14	*NYNRIN*	A*	G	0·029	0·48	0·47	0·48
rs6511720	19	*LDLR*	T	G*	0·18	0·10	0·08	0·13
rs429358	19	*APOE*†	C	T	··	0·19	0·21	0·15
rs7412	19	*APOE*†	T	C	··	0·04	0·03	0·08
ε2ε2	19	*APOE*	··	··	–0·9	··	··	··
ε2ε3	19	*APOE*	··	··	–0·4	··	··	··
ε2ε4	19	*APOE*	··	··	0·2	··	··	··
ε3ε3	19	*APOE*	··	··	0	··	··	··
ε3ε4	19	*APOE*	··	··	0·1	··	··	··
ε4ε4	19	*APOE*	··	··	0·2	··	··	··

LDL-C=low-density lipoprotein cholesterol. FH=familial hypercholesterolaemia. WHII=Whitehall II. G=guanine. A=adenine. T=thymidine. C=cytosine. *Risk alleles (LDL-C-raising). †*APOE* weights were based on haplotypic effects taken from Bennet and colleagues' study,[26] as described in the Methods section of our study.

Table 2**: Global Lipid Genetic Consortium 12-SNP LDL-C gene score calculation**

access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We had complete genotype data for 640 UK patients with familial hypercholesterolaemia—308 from the Simon Broome British Heart Foundation study, 242 from the Oxford familial hypercholesterolaemia study, and 90 from the Department of Health familial hypercholesterolaemia audit project (appendix). Complete genotype data was available for 3020 healthy controls from the WHII study. 319 (50%) UK patients with familial hypercholesterolaemia had a known mutation, and of these, 297 (93·1%) had mutations in *LDLR*, 16 (5·0%) in *APOB*, and 6 (1·9%) in *PCSK9*. The characteristics of the mutation-negative and mutation-positive patients with familial hypercholesterolaemia and healthy controls are shown in table 1, and the characteristics of patients in each study sample are presented in the appendix. Total cholesterol, LDL-C, HDL-C, and triglyceride concentrations were available for all healthy controls and for some patients with familial hypercholesterolaemia receiving lipid-lowering treatment (table 1). Compared with mutation-positive patients, mutation-negative patients were older, but had lower pretreatment total cholesterol and LDL-C concentrations (table 1). We recorded no difference in LDL-C concentrations between groups after statin treatment. In both familial hypercholesterolaemia groups, triglyceride concentrations were not raised in either group compared with the other, and none of the patients was likely to have a combined hyperlipidaemia phenotype.

The 12 SNPs used to define the LDL-C gene score are listed in table 2. The median number of risk alleles present in the WHII controls was 13 (range 6–20), with a mean of 12·7 (SD 2·0) risk alleles and a per-allele raising effect of 0·12 mmol/L (95% CI 0·10–0·14; appendix). The mean weighted LDL-C gene score of the WHII participants was 0·90 (SD 0·23), and WHII individuals in the bottom decile (decile 1) of the LDL-C gene score distribution had a mean LDL-C concentration of 3·76 mmol/L (0·95), whereas those in the top decile (decile 10) had a mean LDL-C concentration of 4·90 mmol/L (0·99), the diagnostic cutoff point for familial hypercholesterolaemia (table 3). A 1 SD increment in the weighted LDL-C score was associated with 0·33 mmol/L (95% CI 0·30–0·37) higher LDL-C concentration ($p=1\cdot4\times10^{-77}$), explaining 11% of the variance in this trait ($R^2=0\cdot11$). This association was robust to adjustment for sex, age, lipid-lowering drug use, body-mass index, diabetes status, smoking status, and blood pressure (Beta 0·34, 95% CI 0·31–0·38). Participants from WHII in decile 10 of the LDL-C score distribution had a much higher likelihood than those in decile 1 of having observed LDL-C concentrations above the UK NICE recommended diagnostic threshold of 4·9 mmol/L (table 3, figure 1), with 146 (50%) of 292 individuals in decile 10 having observed LDL-C concentrations of greater than 4·9 mmol/L compared with 35 (12%) of 295 individuals in decile 1 (table 3). Based on the combined GLGC risk allele effects, and using the WHII population mean LDL-C concentration of 4·4 mmol/L, 50 (17%) of 295 WHII individuals in decile 1 of the LDL-C weighted score distribution had predicted LDL-C concentrations of greater than 4·9 mmol/L, whereas 126 (43%) of 292 WHII individuals in decile 10

	LDL-C weighted score in WHII controls		Measured LDL-C (mmol/L) in WHII controls, mean (SD)	WHII controls with LDL-C >4·9 mmol/L		Risk ratio (95% CI) of LDL-C >4·9 mmol/L*
	Mean (SD)	Range		Measured	Predicted	
Decile 1	0·43 (0·14)	−0·5 to 0·58	3·76 (0·95)	36/299 (12%)	51/302 (17%)	NA
Decile 2	0·66 (0·04)	0·58 to 0·73	3·99 (0·88)	43/296 (15%)	69/302 (23%)	1·21 (0·80–1·82)
Decile 3	0·77 (0·03)	0·73 to 0·81	4·21 (0·96)	71/300 (24%)	82/302 (27%)	1·97 (1·36–2·84)
Decile 4	0·85 (0·02)	0·81 to 0·88	4·34 (0·95)	85/298 (29%)	88/303 (29%)	2·37 (1·66–3·38)
Decile 5	0·91 (0·02)	0·88 to 0·93	4·36 (0·94)	80/300 (27%)	94/302 (31%)	2·21 (1·55–3·17)
Decile 6	0·96 (0·01)	0·94 to 0·98	4·48 (0·91)	96/298 (32%)	100/302 (33%)	2·68 (1·89–3·79)
Decile 7	1·00 (0·01)	0·98 to 1·02	4·50 (1·00)	102/295 (35%)	106/302 (35%)	2·87 (2·04–4·05)
Decile 8	1·05 (0·02)	1·02 to 1·08	4·56 (0·93)	96/292 (33%)	108/301 (36%)	2·73 (1·93–3·87)
Decile 9	1·12 (0·02)	1·08 to 1·16	4·68 (1·05)	120/294 (41%)	118/302 (39%)	3·39 (2·42–4·74)
Decile 10	1·23 (0·06)	1·16 to 1·46	4·90 (0·99)	148/295 (50%)	130/302 (43%)	4·17 (3·01–5·78)

Please see appendix for details of how we predicted the LDL-C values. LDL-C=low-density lipoprotein cholesterol. WHII=Whitehall II. NA=not applicable. *Decile 1 used as reference.

Table 3: **Outcome data in Whitehall II controls according to weighted LDL-C gene score deciles**

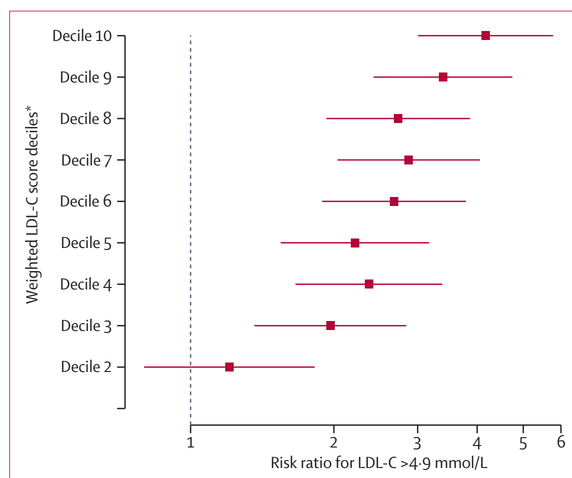

Figure 1: **Risk ratio of participants in the WHII population having LDL-C >4·9 mmol/L according to gene score decile**
LDL-C=low-density lipoprotein cholesterol. WHII=Whitehall II. *Decile 1 used as reference.

were predicted to be above this threshold, which is similar to the proportions based on actual measured LDL-C in the WHII population of 12% versus 50% (table 3). This supports the validity of the 12-SNP weighted GLGC score in UK individuals.

The familial hypercholesterolaemia group without a known mutation had a significantly higher mean weighted LDL-C gene score of 1·0 (SD 0·21; $p=4·5\times10^{-16}$) than did WHII participants (figure 2A). This suggests that a substantial proportion of the mutation-negative familial hypercholesterolaemia group's raised LDL-C concentrations can be explained by co-inheritance of common LDL-C-raising SNPs. 64 (20%) of 321 patients with familial hypercholesterolaemia without a known mutation had a score that fell within decile 10 of the WHII LDL-C score distribution. 167 (52%) had a score within deciles 7–10, whereas only 35 (11%) had a score within deciles 1–3.

When we calculated the weighted LDL-C gene score in the familial hypercholesterolaemia group with a known mutation, the mean weighted score (0·95 [SD 0·20]) was significantly higher than the score in the WHII group (0·90 [0·23]; $p=1·6\times10^{-5}$; figure 2B, 2C), but was significantly lower than that of the mutation-negative group (1·00 [0·21]; p=0·0014). This result suggests that even in patients with familial hypercholesterolaemia who have a detected causative mutation, their raised LDL-C concentrations have an additional polygenic component.

DNA was available from 451 patients with familial hypercholesterolaemia without a known mutation from one lipid clinic in Belgium, and 273 patients with familial hypercholesterolaemia with a known mutation from the same clinic. All patients had a cholesterol concentration above the 95th percentile for age and sex (appendix) and a family history of early cardiovascular disease. The mean weighted score of the mutation-negative Belgian patients (0·99 [SD 0·19]) was significantly higher than the score of the WHII participants ($p=5·2\times10^{-20}$), with a smaller difference in score between the mutation-positive patients (0·92 [0·20]) and the WHII group (p=0·04; appendix). The weighted score in the mutation-negative group was also significantly higher than in the mutation-positive group ($p=4·0\times10^{-6}$). Overall, 73 (16%) had a LDL-C gene score that fell within decile 10 of the WHII LDL-C gene score distribution, and 211 (46%) fell within deciles 7–10.

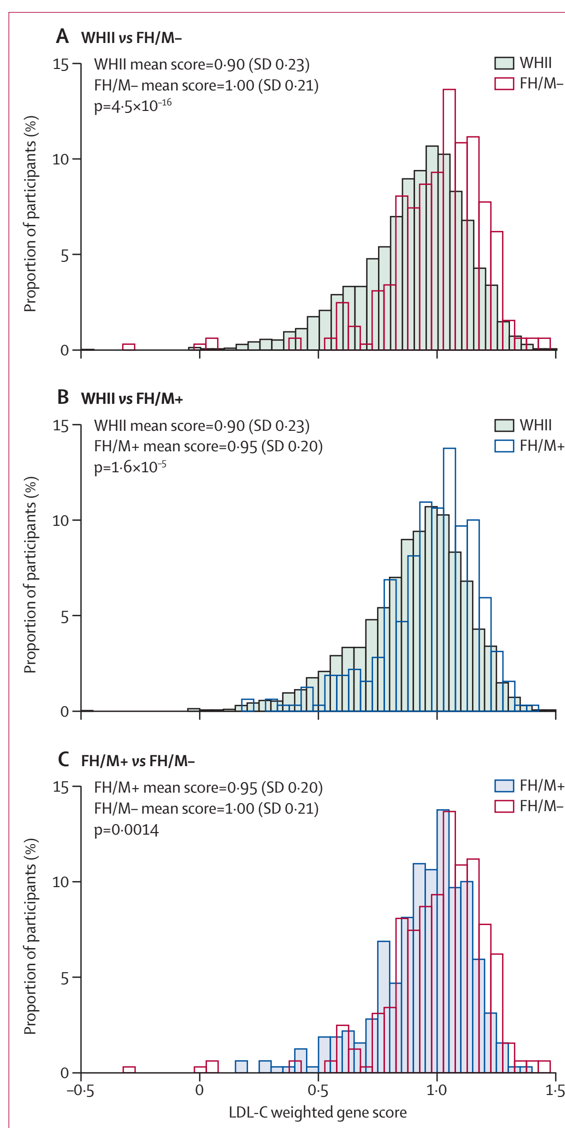

Figure 2: **Distribution of weighted LDL-C gene scores**
(A) Whitehall II controls (WHII) versus patients with familial hypercholesterolaemia without a known mutation (FH/M–). (B) WHII controls versus patients with familial hypercholesterolaemia with a known mutation (FH/M+). (C) FH/M– versus FH/M+. In both patients with definite familial hypercholesterolaemia (DFH) and those with possible familial hypercholesterolaemia (PFH), patients without a detected mutation had a significantly higher mean weighted LDL-C gene score than did those with a detected mutation (DFH/M+ 0·95 [SD 0·012] *vs* DFH/M– 1·03 [0·018], p=0·001; PFH/M+ 0·91 [0·043] *vs* PFH/M– 1·00 [0·015], p=0·03; appendix). LDL-C=low-density lipoprotein cholesterol.

Discussion

The major finding of our study is that a substantial proportion of the raised LDL-C concentrations measured in patients with a clinical diagnosis of familial hypercholesterolaemia with no detected causative mutation might have a polygenic rather than a monogenic cause. This observation was replicated in an independent sample of patients with familial hypercholesterolaemia from Belgium. In patients with polygenic familial hypercholesterolaemia, cascade testing of their relatives will be compromised, because less than the 50% of relatives expected for a proband with monogenic disease will be affected. If cascade testing was restricted to the 40% (roughly) of patients with a clinical diagnosis of familial hypercholesterolaemia with an identified causative mutation, this would eliminate staff and screening costs associated with the remaining 60% of patients who would not benefit.

The second finding is that, even in patients with familial hypercholesterolaemia with a detected causative mutation, an additional polygenic contribution might explain their highly increased LDL-C concentrations, a finding confirmed in the Belgian patient samples. This result partly explains the reduced penetrance reported in families with familial hypercholesterolaemia, whereby the relatives have a milder form of the disease with a lower mean LDL-C concentration than the proband.[27] A polygenic component is also likely to contribute to the large overlap in LDL-C concentrations reported in mutation-carrier and non-carrier relatives,[28] since the proband has more than the average number of LDL-C-raising SNPs, but the inheritance of these SNPs will be independent of each other and the causative major mutation.

The LDL-C gene score we used is based on GLGC data from more than 100 000 participants,[19] and is therefore an unbiased and robust genetic instrument for LDL-C-raising alleles. The weighted score was strongly associated with LDL-C concentrations in the healthy men and women from the WHII study, with a 1·1 mmol/L (24%) difference in LDL-C concentrations between those in the bottom and top score deciles, which is similar to the effect achieved by taking a 40 mg daily dose of simvastatin.[29] Although 27 (75%) of the 36 GLCG consortium LDL-C SNPs were present on the genotyping array, to generate a LDL-C-specific score we initially only included SNPs for which LDL-C was the lead trait associated with the SNP. However, when we repeated the analysis using the full range of GLGC LDL-C-raising SNPs (plus the *APOE* haplotype; appendix), it explained a slightly higher proportion of the variation in LDL-C concentration, but did not materially improve discrimination (appendix), confirming that the addition of further SNPs with very small effects to a gene score is unlikely to be useful.

In patients with the strongest clinical suspicion of familial hypercholesterolaemia—ie, tendon xanthomas, providing a clinical diagnosis of definite familial hypercholesterolaemia—the mutation detection rate is between 60–80%,[14–16] and this rate could be improved if additional genes with rare mutations causing monogenic autosomal familial hypercholesterolaemia are identified. Together with the reduced price of whole genome sequencing, in the future additional rare familial hypercholesterolaemia-causing mutations could be identified. A better definition of familial hypercholesterolaemia might expedite this process. A diagnosis of familial hypercholesterolaemia in the UK is based on the Simon Broome criteria—namely, LDL-C concentration of higher than 4·9 mmol/L, a family history of raised cholesterol or early coronary heart disease in a first-degree relative, or the presence of tendon xanthomas. Clearly, polygenic hypercholesterolaemia would not be excluded. Groups in the Netherlands[12] and the USA[30] have proposed other diagnostic scores, but these scores are also likely to include polygenic hypercholesterolaemia. Additionally, no major differences were reported in the specificity and sensitivity of these three diagnostic criteria used to correctly predict whether a causative mutation would be detected in patients with familial hypercholesterolaemia from Denmark.[31]

There are limitations to our study. The three genes in which rare mutations cause familial hypercholesterolaemia (*LDLR*, *APOB*, and *PCSK9*) also have GLGC LDL-C-raising common alleles, and the inclusion of these SNPs could have biased the results in the unlikely event that they show linkage disequilibrium with undetected rare familial hypercholesterolaemia-causing mutations. However, we can rule this out because when we repeated the analysis excluding the three SNPs in these genes in the score, the findings were not substantially affected (appendix).

We have not carried out a complete gene screen of *PCSK9,* nor for all the introns of *LDLR*, and we examined only the region of the *APOB* gene where familial hypercholesterolaemia-causing mutations have been reported. Therefore, some of the mutation-negative group could have an undetected mutation in one of these three genes, and this might explain why a proportion of the patients had a low LDL-C SNP score. The use of more LDL-C-raising SNPs or of other functional variants such as copy number variants might improve the discriminatory power of the score, but increasing the number of GLGC-reported LDL-C SNPs in the score had only a marginal, non-significant improvement. Because the accepted frequency of familial hypercholesterolaemia in the general population is 1 in 500,[1] six or seven of the 3020 WHII participants were likely to have had undiagnosed familial hypercholesterolaemia, and if they had been identified and excluded from the study, we would have had a cleaner control sample, which might have marginally improved discrimination between the WHII participants and the familial hypercholesterolaemia groups. The mean total cholesterol concentration of 6·4 mmol/L in the WHII participants is typical of the general population in the late 1980s when these participants were recruited, and concentrations were only marginally lower more than a decade later, with the Health Survey of England 2003[32] reporting mean total cholesterol concentrations of men and women aged 45–54 years to be 5·9 mmol/L and 5·8 mmol/L, respectively (with the 90th percentile cutoffs being 7·3 mmol/L and 7·2 mmol/L, respectively). Since the WHII control sample cholesterol concentration falls within this range, it is probably representative of the current population. The replication of the effect in an independent sample of familial hypercholesterolaemia patients, selected with different criteria, validates the findings, and although we did not have a sample of healthy Belgian participants for comparison, SNP frequencies are not likely to differ greatly between the two countries, since the GLGC SNP frequencies are representative of populations of European origin, and the comparison between the Belgian mutation-negative and mutation-positive groups was also statistically significant.

Our findings have relevance for the management of patients with familial hypercholesterolaemia (panel). All individuals with raised LDL-C concentrations qualify for statin treatment, whether or not their high cholesterol concentration has a mainly monogenic or polygenic cause. Results of modelling suggest that because patients with familial hypercholesterolaemia have a life-long burden of LDL-C accumulation, these patients need intensive lipid-lowering therapy, and although this treatment is more expensive than non-intensive therapy, it is cost effective.[33] However, patients with no detected mutation have lower mean LDL-C concentrations and coronary heart disease risk,[15,34,35] and less atherosclerosis in their carotid arteries,[36] than do patients with clinically diagnosed familial hypercholesterolaemia and a detected mutation. Although further research is needed, NICE-recommended intensive lipid-lowering therapy might only be cost effective in patients with familial hypercholesterolaemia with a known mutation.

The other NICE recommendation that might need to be examined in view of these data is that cascade testing in mutation-negative patients with familial hypercholesterolaemia is also cost effective and should be done using LDL-C measures with so-called affected status (to identify individuals for secondary cascading) identified by age-specific and gender-specific thresholds.[28] When a proband's raised LDL-C is polygenic, the proportion of their relatives who are likely to also have raised LDL-C is substantially less than the 50% predicted for monogenic familial hypercholesterolaemia. In one study,[11] the proportion of relatives with LDL-C above the NICE recommended cutoff value was only 30%, and further cascade testing from these individuals (eg, their children) would be even less effective. Family studies will need to be done to identify the score threshold above which cascade testing from mutation-negative patients with familial hypercholesterolaemia (more than 60% of all those with a clinical diagnosis of familial hypercholesterolaemia in UK lipid clinics) will no longer be cost effective. Based on the probability distribution shown in figure 1, we propose that cascade testing from these patients should be restricted to those in the bottom 20% of the distribution (ie, with a score lower than 0·73) who are highly unlikely to have an LDL-C concentration of greater than 4·9 mmol/L that can be explained by the inheritance of LDL-C-raising SNPs. The successful cascade testing programme running in the Netherlands is based entirely on families in which a familial hypercholesterolaemia-causing mutation has been detected.[12] We therefore propose the diagnostic workflow shown in figure 3. Patients with familial hypercholesterolaemia who have no detected *LDLR/APOB/PCSK9* mutation and an LDL-C weighted gene score below 0·73 are highly likely to have an unidentified monogenic cause, which would warrant family studies and further research. If the next-generation sequencing library capture of the known familial hypercholesterolaemia-causing genes were expanded to include fragments containing the 12 SNPs, this information could be obtained at essentially no additional cost. The actual usefulness of these cutoffs for triaging

***Panel:* Research in context**

Systematic review

In 2008, the National Institute for Clinical Health and Excellence published the results of their systematic review of the identification and management of familial hypercholesterolaemia. This report recommended that all patients with clinical and biochemical features of this disorder should be offered a DNA test to confirm their diagnosis and enable unambiguous identification of affected relatives through a systematic cascade testing strategy. However, in roughly half of patients with a clinical diagnosis of familial hypercholesterolaemia, a causative mutation cannot be found. In 2011, we used the meta-analysis data of genome-wide association studies including more than 100 000 individuals published by the Global Lipid Genetic Consortium (GLGC) to identify possible loci where common variants influence LDL-C concentration. The combined information from these single nucleotide polymorphisms (SNPs) was then used as a genetic instrument, since we predicted that in individuals carrying several LDL-C-raising SNPs, LDL-C concentrations would exceed the diagnostic LDL-C threshold of 4·9 mmol/L used in the diagnosis of familial hypercholesterolaemia.

Interpretation

In a substantial proportion of patients with familial hypercholesterolaemia in whom no mutation can be identified, their raised LDL-C probably has a polygenic cause. This compromises the efficacy of cascade testing in this group, and such testing should be restricted to mutation-positive patients, as is done in some countries already. We propose that the clinical diagnosis of familial hypercholesterolaemia should be restricted to those in whom a mutation can be identified, whereas those with no detected mutation should be given the clinical diagnosis of polygenic hypercholesterolaemia. Even in patients with familial hypercholesterolaemia with a detected causative mutation, a substantial polygenic contribution occurs, which might explain the variable penetrance of the disease, and suggests that polygenes might contribute to diseases formerly considered to be solely monogenic.

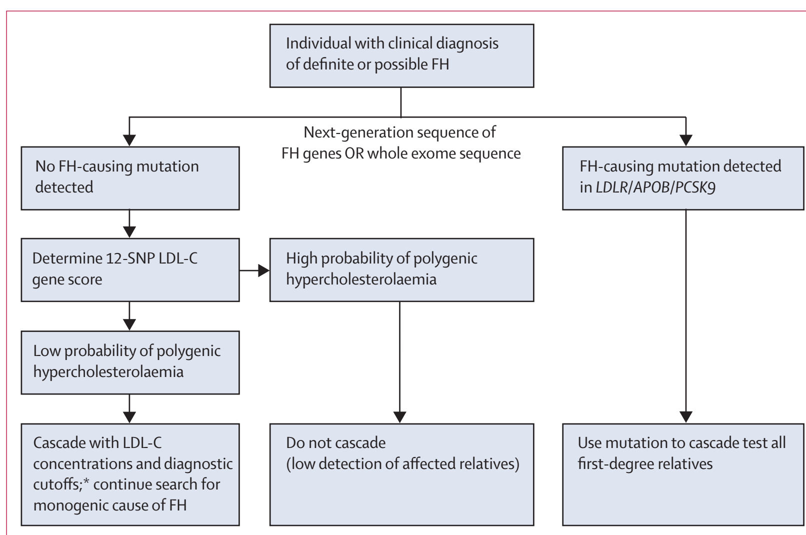

***Figure 3:* Diagnostic workflow for cascade testing in patients with familial hypercholesterolaemia**
FH=familial hypercholesterolaemia. SNP=single nucleotide polymorphism. LDL-C=low-density lipoprotein cholesterol. *As recommended by NICE.[27]

probands needs to be researched further to establish the proportion of affected relatives in the two groups.

Would it, therefore, be clinically useful to restrict the clinical diagnosis of familial hypercholesterolaemia to those patients in whom a causative major gene mutation can be identified? We propose that, from this perspective, patients with a familial hypercholesterolaemia phenotype and no such mutations could be given the clinical diagnosis of polygenic hypercholesterolaemia, and not familial hypercholesterolaemia. This should not, however, affect the treatment of these patients,[37] but would influence the decision to undertake cascade testing in their relatives.

Contributors

PJT and SEH devised the study design. SS and JAC did the data analysis. RW, MF, PH, KL, and CL contributed to the genotyping. FK, HAWN, OSD, NL, MKi, ADH, and MKu provided study samples for the analysis. PJT, SS, SCH, FD, JW and SEH interpreted the data interpretation and wrote the manuscript. OSD and his colleagues in the Centre de Recherche de Jolimont performed the genetic testing for the low-density lipoprotein receptor and *APOB* mutations in all Belgian samples. All authors read the manuscript and contributed to the final version.

Conflicts of interest

HAWN and OSD have served as consultants to pharmaceutical companies marketing lipid-lowering drugs, and have received travel expenses, payment for speaking at meetings and funding for research from some of these companies. SEH is the medical director and minority shareholder of the University College London start-up coronary heart disease risk genetic testing company StoreGene, and has received honoraria for speaking at educational meetings with a pharmaceutical sponsor, but has donated all personal payments to various medical charities. JW is employed by, and holds shares in, GlaxoSmithKline. All the other authors declare that they have no conflicts of interest.

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