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Abstract: Mutations in any of three genes (LDLR, APOB and PCSK9) are known to cause autosomal dominant FH, but a mutation can be found in only ~40% of patients with a clinical diagnosis of FH. In the remainder a polygenic aetiology may be the cause of the phenotype, due to the co-inheritance of common LDL-C raising variants. In 2013 we reported the development of a 12-SNP LDL-C "SNP-Score" based on common variants identified as LDL-C raising from genome wide association consortium studies, and have confirmed the validity of this score in samples of no-mutation FH adults and children from more than six countries with European-Caucasian populations. In more than 80% of those with a clinical diagnosis of FH but with no detectable mutation in LDLR/APOB/PCSK9, the polygenic explanation is the most likely for their hypercholesterolaemia. Those with a low score (in the bottom two deciles), may have a mutation in a novel gene, and further research including whole exome or whole genome sequencing is warranted. Only in families where the index case has a monogenic cause should cascade testing be carried out, using DNA tests for an unambiguous identification of affected relatives. The clinical utility of the polygenic explanation is that it supports a more conservative (less aggressive) treatment care pathway for those with no mutation. The ability to distinguish those with a clinical diagnosis of FH who have a monogenic or a polygenic cause of their hypercholesterolaemia is a paradigm example of the use of genomic information to inform Precision Medicine using lipid lowering agents with different efficacy and costs.

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### Highlights

A mutation in *LDLR/APOB/PCSK9* is found in only ~40% of patients with clinical FH.

At least 80% of these no-mutation patients have a high LDL-C “SNP-Score”

Atherosclerosis and CHD risk is lower in Polygenic patients than in monogenic FH

Monogenic FH requires more aggressive lipid lowering than Polygenic Hypercholesterolaemia

Only from monogenic FH index cases should cascade testing of relatives be performed.

This is a paradigm example of the use of genomic data to inform Precision Medicine

## Clinical utility of the Polygenic LDL-C SNP score in Familial Hypercholesterolemia

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**Keywords:** Familial Hypercholesterolemia, LDL-C SNP-Score, Polygenic hypercholesterolemia, Variants of Unknown Significance (VUS)

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## Abstract

1  
2 Mutations in any of three genes (*LDLR*, *APOB* and *PCSK9*) are known to cause autosomal  
3 dominant FH, but a mutation can be found in only ~40% of patients with a clinical diagnosis  
4 of FH. In the remainder a polygenic aetiology may be the cause of the phenotype, due to the  
5 co-inheritance of common LDL-C raising variants. In 2013 we reported the development of a  
6 12-SNP LDL-C “SNP-Score” based on common variants identified as LDL-C raising from  
7 genome wide association consortium studies, and have confirmed the validity of this score  
8 in samples of no-mutation FH adults and children from more than six countries with  
9 European-Caucasian populations. In more than 80% of those with a clinical diagnosis of FH  
10 but with no detectable mutation in *LDLR/APOB/PCSK9*, the polygenic explanation is the  
11 most likely for their hypercholesterolaemia. Those with a low score (in the bottom two  
12 deciles), may have a mutation in a novel gene, and further research including whole exome  
13 or whole genome sequencing is warranted. Only in families where the index case has a  
14 monogenic cause should cascade testing be carried out, using DNA tests for an  
15 unambiguous identification of affected relatives. The clinical utility of the polygenic  
16 explanation is that it supports a more conservative (less aggressive) treatment care pathway  
17 for those with no mutation. The ability to distinguish those with a clinical diagnosis of FH  
18 who have a monogenic or a polygenic cause of their hypercholesterolaemia is a paradigm  
19 example of the use of genomic information to inform Precision Medicine using lipid  
20 lowering agents with different efficacy and costs.  
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## Introduction

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36 A clinical diagnosis of Familial Hypercholesterolemia (FH) can be made using any one of  
37 several different published criteria. Across Europe, the most widely used is the Dutch Lipid  
38 Clinic Network (DLCN) criteria (1), which uses a point system based on patient’s cholesterol  
39 levels, personal and family history of premature coronary heart disease (CHD), physical  
40 examination and the presence of a detected mutation. Those with a score over 8 are given  
41 the diagnosis of Definite FH (DFH), those with a score between 6-8 the diagnosis of Probable  
42 FH, between 3-5 the diagnosis of Possible FH, while a score of below 3 is not FH. The criteria  
43 have been modified by clinicians in Wales to take into account that an elevated triglyceride  
44 level in a suspected FH patient makes it less likely that the patient has monogenic FH (2),  
45 which is supported by findings in an lipid clinic in England (3). In the UK, the National  
46 Institute for Health and Care Excellence (NICE) guideline recommends use of the Simon  
47 Broome criteria (4). These criteria include raised cholesterol levels, physical stigmata e.g.  
48 tendon xanthomata or an evidence of these signs in first- or second-degree relatives, and  
49 having a family history of premature coronary artery disease. A definite diagnosis of FH is  
50 made if a patient has elevated cholesterol levels and tendon xanthomata or a disease  
51 causing mutation is found. A possible diagnosis of FH is made if the patient has only high  
52 levels of cholesterol levels and a family history of hypercholesterolemia or premature CHD.  
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1 Finally, the MedPed criteria are used for diagnosis of probable FH in the US and are mainly  
2 based on the total cholesterol and LDL-C cut offs stratified by age and family history. The cut  
3 offs are different in individuals with first, second and third degree relatives with FH (5).  
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5 In the majority of cases, a single mutation in one of three genes (*LDLR*, *APOB* or *PCSK9*)  
6 causes autosomal dominant FH, with a single deletion variant in *APOE* also reported to  
7 cause the FH phenotype in a few families (6). In diagnostic laboratories, a mutation in one of  
8 the three genes can be found in 60-80% of patients with a clinical diagnosis of definite FH  
9 but only in about 25-30% of patients with possible FH (PFH) (7). In those mutation positive  
10 families cascade testing (CT) using the family mutation for unambiguous identification of FH  
11 relatives is recommended by all recent published guidelines (eg (1, 8, 9)), with identified  
12 subjects treated with high intensity lipid lowering therapies to reduce their very  
13 considerable risk of early CHD. CT has been shown to be a feasible and highly cost-effective  
14 strategy in many countries (eg (10, 11)). However, this leaves a diagnostic problem in  
15 patients with no causative mutation, who have a strong possibility of a polygenic cause for  
16 their FH (12)  
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### 25 **Development of the LDL-C SNP-Score**

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27 In 2010 the Global Lipids Genetics Consortium (GLGC) meta-analysis identified over 100 loci  
28 where common variants influence LDL-C levels (13). Thus we hypothesised that in patients  
29 where no mutation can be found, the LDL-C and total cholesterol level is raised above the  
30 FH diagnostic cut-off by having inherited a greater-than-average number of these common  
31 cholesterol-raising variants with modest effect. To test this we selected 12 key single  
32 nucleotide polymorphisms (SNPs) with common variants that raise LDL-C by the largest  
33 amount. Using these we generated an LDL-C “SNP-Score” by summing the number of raising  
34 alleles an individual carries, and improved the precision of the score by “weighting” the  
35 carriage of each SNP by the size of its effect (Table 1). In a sample of no-mutation FH  
36 patients from the UK (FH/M-) we showed that the mean weighted score was significantly  
37 higher than in a group of ~3000 healthy UK men and women, and confirmed this effect in a  
38 sample of FH/M- patients from Belgium (12). The decile SNP-Score cut-offs are shown in  
39 Table 2. In the FH/M- group 52% had a score that fell within the range observed in the top  
40 three deciles of the WHII weighted LDL-C gene score distribution, and only 11% fell within  
41 the range observed in the lowest three deciles. We estimated that in more than 80% of  
42 those with a clinical diagnosis of autosomal dominant FH but with no detectable mutation in  
43 *LDLR/APOB/PCSK9*, the polygenic explanation is most likely cause of their  
44 hypercholesterolaemia. In the remainder, a mutation in a novel gene may be present (14,  
45 15). We proposed that only those with a monogenic cause for their phenotype be given the  
46 diagnosis of FH, and the remainder be termed “Polygenic Hypercholesterolaemia”. In this  
47 group cascade testing will be less cost effective, since only ~30% of relatives will have  
48 elevated LDL-C compared to the 50% that is seen in monogenic families.  
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1 It is interesting to note in figure 1 that the mean weighted SNP-Score in the FH/M+ group is  
2 intermediate between the healthy subjects and the FH/M- group. This suggests that, even in  
3 those with an identified FH-causing mutation, a polygenic contribution to their phenotypes  
4 is occurring. The additional polygenic contribution, (with also contribution from  
5 environmental factors such as diet) could potentially explain some of the variation in the  
6 LDL-C concentrations seen among the family members of an FH patient. In different  
7 relatives, the LDL-C levels seen will be determined by the combined contribution of the  
8 single mutation of large effect *plus* the contribution of the number of LDL-C-raising alleles  
9 inherited, which will of course differ between family members, since the genes are on  
10 different chromosomes and will segregate independently.  
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### 17 **Can the LDL-C SNP score be improved?**

19 It is unlikely that there are additional common SNPs in the genome that will outrank the 12  
20 chosen for the SNP-Score, since any common SNPs with larger effects would have been  
21 detected by the size of the datasets currently available. However it may be possible to  
22 improve the performance of the SNP-Score by including additional SNPs previously  
23 identified by the GLGC meta-analysis as influencing LDL-C (See Supplementary Table 3 in  
24 (16)). In the 2013 analysis, to maintain a high specificity for LDL-C, SNPs were selected with a  
25 major effect only on LDL-C and not on another lipid trait. To see if the SNP-Score could be  
26 improved, other SNPs were included, and also lipid traits other than LDL-C (eg *CETP*).  
27 However, addition of 21 LDL-C-raising SNPs did not significantly improve the ability of the  
28 SNP-Score to discriminate between FH/M- and healthy subjects (16). Following this, the  
29 sequential removal of SNPs of smaller effects and/or lower minor allele frequencies showed  
30 that a weighted score of six SNPs performed as well as the 12 SNP-Score. Thus, to improve  
31 cost-efficiency, SNP-Score calculations in a number of replication cohorts were based on  
32 genotypes of only the six SNPs with greatest effects on LDL-C shown in bold in Table 1.  
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41 Replication of the SNP-Score effect was carried out in samples from six different countries  
42 (16) as shown in Figure 2. In all samples the mean score in the FH/M- subjects was higher  
43 than in the UK healthy subjects, and overall the effect was highly statistically significant. The  
44 highest LDL-C SNP-Score was observed in the two M- hypercholesterolaemic children  
45 cohorts (one from the Netherlands and one from Greece), showing that the SNP-Score  
46 discriminates well in children as well as adults. Similar data have been obtained in a sample  
47 of children with FH from Portugal (17). 92 FH/M- children were genotyped for the 6 LDL-C  
48 genetic risk score SNPs, and the weighted score compared with 1563 healthy Portuguese  
49 subjects. Polygenic hypercholesterolaemia was conservatively defined as a score above the  
50 top quartile. The FH/M- group had a significantly higher mean (SD) score than the healthy  
51 group ( $0.73 \pm 0.17$  vs  $0.62 \pm 0.22$ , ( $p < 0.001$ ), with over 42% being in the top quartile of the  
52 score and only 13% in the bottom quartile. Interestingly, there was no significant difference  
53 in mean score between the UK and Portuguese healthy subjects (17). These data have  
54 validated the score in the Portuguese population, and suggests that about half of the FH/M-  
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1 patients could have polygenic hypercholesterolaemia. Overall, these data suggest that in a  
2 child, once a single gene cause for highly elevated LDL-C is ruled out, a polygenic cause is  
3 highly likely. A simple spreadsheet is available to calculate the weighted SNP-Score for an  
4 individual based on their 12-SNP genotype (Supplementary data).  
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6 One of the limitations in the SNP-Score as presented is that all samples are from Caucasian  
7 patients and we currently have no data to allow us to extrapolate the utility of this score to  
8 patients from other ethnic backgrounds, where the minor allele frequency will differ  
9 considerably, and where the raising effect of the SNPs on LDL-C, although in general are  
10 directionally consistent, may not be of similar size (18). Thus further data on this is required  
11 for the extension of the SNP-Score to different ethnic groups.  
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### 18 **Estimation of the proportion of FH/M- subjects likely to be polygenic by SNP-Score**

19 In the clinical use of the SNP-Score we need to estimate the probability that the elevated  
20 LDL-C seen in an FH/M- individual can be explained by their weighted SNP-Score. The first  
21 estimate needed for this calculation is the underlying rate of undetected monogenic  
22 mutations in FH/M- subjects. Accumulating evidence from genotyping and Next Generation  
23 Sequencing (NGS) data suggests that, at least in most European populations, around 1/250  
24 people carry an FH-causing mutation (19-22). Therefore the baseline frequency of FH-  
25 causing mutations is 0.004. Assuming that we have found 90% of all mutations, the  
26 frequency of remaining undetected mutations will be 0.0004. Based on the lack of novel FH  
27 causing genes reported to date (eg (23)), this is a reasonable estimate. If we have identified  
28 only 75% of all mutations, the frequency of remaining undetected mutations would be  
29 0.0005, and this seems likely to be the upper limit of undetected mutations. We modelled  
30 the likelihood of an individual having an LDL-C >4.9mmol/l (the FH diagnostic cut-off used by  
31 the UK diagnostic criteria), at an undetected mutation frequency of 0.0005. Our analysis  
32 (16), shown in Figure 4, suggests that the probability of a polygenic cause for  
33 LDL>4.9mmol/L in all the assessed FH/M- individuals with a score above the first decile is  
34 >95%, which reduces when the frequency of an undetected monogenic cause increases. This  
35 suggests that, except for those with a SNP score in decile 1, all other FH/M- subjects have a  
36 probability of >90% of a polygenic case explaining their elevated LDL-C (>4.9mmol/l). In  
37 individuals with a clinical diagnosis of FH with a SNP-Score in the lowest decile, it is very  
38 unlikely that there is a polygenic cause, and, research to identify whether the individuals  
39 have a mutation in a yet to be identified gene would be valuable.  
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### 54 **Variants of Unknown Significance (VUS)**

55 DNA diagnostic labs have now developed methods whereby all the protein coding and  
56 splicing regions of the genome for the known FH genes can be captured and sequenced  
57 together as a gene “panel” with high accuracy (24-27), and with the addition of small  
58 ‘barcoding’ sequences attached to the primers used to PCR-amplify the regions of interest,  
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1 it is now possible to mix the samples from up to 95 individuals (keeping one slot for a no-  
2 template control) and analyse them in one run with high accuracy (25). This economy of  
3 scale is helping to drive down prices so that now a full FH diagnostic scan can be completed  
4 for around £250, and the data can also be used to review if an individual has a large  
5 duplication or deletion of the gene, which occurs in about 5-10% of patients. Other FH  
6 phenocopy genes (*APOE*, *LDLRAP1*, *LIPA*, *ABCG5/8*) and the polygenic SNP score can also be  
7 added to the panel without increasing substantially the cost. These methods produce a large  
8 amount of sequence data, requiring statistical and bioinformatics analytical approaches, and  
9 this has increased the number of occasions whereby a Variant of Uncertain Significance  
10 (VUS) is identified (27). This creates a diagnostic conundrum which clearly cannot be  
11 reported as FH-causing to the clinician or patient, but which requires either *in vitro*  
12 molecular assays to examine impact on transcription (28) or splicing (29), or by family  
13 studies to see if the variant tracks with high LDL-C levels in the family, while the relatives  
14 without the inherited variant have normal levels of LDL-C. However a number of relatives in  
15 a single family, or many families with the same variant, are needed to use co-segregation as  
16 a functional proof of disease-causing, and such families are not always available.

17 It is clearly of great importance to be able to reliably assess the pathogenicity of variants  
18 identified in clinical settings, or as incidental findings in genomics projects, in order to allow  
19 the appropriate and consistent cascade testing of their relatives. This is not always  
20 straightforward in *LDLR/APOB/PCSK9* especially for synonymous and missense variants. For  
21 *APOB*, in addition to the two common pathogenic variants (p.(Arg3527Gln) (30) and  
22 p.(Arg3527Trp) (31)) the ApoB protein is highly polymorphic, with many common and rare  
23 variants that do not cause FH (eg (32)]. Several novel variants in the *APOB* gene have been  
24 shown to be FH-causing using *in vitro* assays (33, 34). For *PCSK9* the situation is complicated  
25 in that *in silico* prediction algorithms may predict that a missense change is likely to affect  
26 function, but cannot distinguish between a gain-of-function, LDL-C raising (possibly FH-  
27 causing) variant, and a loss-of-function, LDL-C lowering variant. Additionally, *in silico* analysis  
28 is not recommended for complex proteins like *APOB* and *PCSK9*.

29 In 2015, the Association for Clinical Genetic Science (ACGS) published guidelines for the  
30 classification of variants (35), with categories ranging from 1 and 2 (clearly not or unlikely to  
31 be pathogenic), to 3 (variants of unknown significance), to 4 and 5 (likely to be or clearly  
32 pathogenic). The recently updated *LDLR* variant database with variants classified according  
33 to these guidelines may be accessed via: <http://databases.lovd.nl/shared/genes/LDLR>. (36).  
34 Although 93% of *LDLR* variants in the current upgrade of the database have been assigned  
35 as pathogenic or likely pathogenic, 7% (115) remain as variants of unknown significance.  
36 The American College of Medical Genetics and Genomics has developed an algorithm for  
37 variant classification and following this algorithm about half (~1000) of all FH associated  
38 variants are considered VUS (37) mainly because of a lack of available data that fits into the  
39 specified scoring system.

40 However, for a VUS in any of the three FH-genes, knowing the LDL SNP-Score may help,  
41 since finding a VUS in an individual with a particularly high score would suggest that the

1 variant is less likely to be pathogenic (since the high LDL-C can be explained by the polygenic  
2 component), while a low score in an individual with a novel VUS would mean that the  
3 variant is more likely to be pathogenic. Such families could then be recruited into co-  
4 segregation studies.  
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### 7 8 **Identifying new FH genes** 9

10 The identification of each FH-causing gene has stimulated the development of novel lipid-  
11 lowering drugs (*LDLR* and statins, *APOB* and Mipomersen and *PCSK9* and monoclonal  
12 antibodies), and the identification of additional FH-causing genes may also reveal novel  
13 pathways for which therapeutic agents could be sought. In the UK the 100,000 genomes  
14 project (<https://www.genomicsengland.co.uk/the-100000-genomes-project/>) has been  
15 established to undertake whole genome sequencing in ~70,000 patients (and relatives) with  
16 a rare disease, or patients with cancer (and their tumour material). The aim of the project is  
17 primarily to create a new genomic medicine service for the NHS, by transforming care  
18 pathways for patients. It is hoped that many patients will be offered a diagnosis where there  
19 wasn't one before, and the work will help develop best practice for the use of genomics in  
20 healthcare, and how best to interpret genomic data to improve patient care. Within 100,000  
21 genomes, patients with a clinical diagnosis of definite FH but with no detectable mutation in  
22 *LDLR/APOB/PCSK9* are being recruited. Importantly, only those with a low LDL SNP-Score are  
23 being included (for example the five subjects shown as having a score below the 95  
24 percentile bars of the box-whisker plot in Figure 2), to enhance the chances of finding a new  
25 monogenic cause.  
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### 37 **Clinical utility of a diagnosis of Monogenic FH vs Polygenic Hypercholesterolaemia** 38

39 There are several lines of evidence to suggest that the extent of atherosclerosis is higher in  
40 monogenic compared to polygenic hypercholesterolaemia patients. Many papers report  
41 that the prevalence of CHD is higher in groups of mutation positive FH patients compared to  
42 those with a clinical diagnosis of FH but where no mutation can be found (30, 39, 39).  
43 Support for this also comes from the UK Simon Broome register (40), which showed that  
44 patients with a clinical diagnosis of definite FH had a higher Standardized Mortality Ratio  
45 (SMR) for CHD than those with a clinical diagnosis of possible FH (2.94 vs 2.05). Since a  
46 mutation is found in ~80% of DFH patients, the majority of this group will have a monogenic  
47 cause. By contrast, a mutation is detected in 25-30% of possible FH patients (7), meaning  
48 the majority of this group will have a polygenic cause of their elevated LDL-C. The lower CHD  
49 mortality rate in the possible FH patients suggests that the extent of atherosclerosis in  
50 polygenic FH is likely to be less.  
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57 This elevated risk for CHD in FH patients with a detected mutation has been convincingly  
58 confirmed in a population-based analysis (39). Using NGS for the known FH genes among  
59 20,485 CHD-free individuals, 1,386 (6.7%) had LDL-C >4.9mmol/l, and of these, 24 (1.7%)  
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1 carried a known FH mutation. Compared with individuals with LDL-C<3.7mmol/l and no  
2 mutation, those with LDL-C >4.9mmol/l and no FH mutation had a 6-fold higher risk for CHD,  
3 but those with both LDL-C >4.9mmol/l and an FH mutation had a 22-fold higher risk. This  
4 risk is likely explained by the substantially higher accumulated 'LDL-C burden' in monogenic  
5 FH subjects, since these individuals will have had genetically-determined lifelong high LDL-C.  
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8 Finally, we have recently demonstrated (41) that the degree of thickening in the carotid  
9 artery, as measured by ultrasound, is considerably greater in a group of monogenic FH  
10 patients compared to a group of patients with a polygenic aetiology, even though total and  
11 LDL-C levels were similar. In addition, coronary calcium score was significantly higher in  
12 monogenic vs polygenic patients. While all patients with a clinical diagnosis of FH need  
13 cholesterol and CHD risk management, the demonstration of higher levels of atherosclerotic  
14 burden in the monogenic patients supports recommendations that they warrant intensive  
15 LDL-C lowering under the management of a lipid specialist. In some patients this may  
16 include treatment with PCSK9 inhibitors in order to achieve LDL-C target values. By contrast,  
17 in those who do not have a monogenic cause for their lipid phenotype, estimation of their  
18 CHD risk using risk algorithms is appropriate, and they may be able to be managed in  
19 general practice. This use of genetic information to risk stratify patients with a clinical  
20 diagnosis of FH is a paradigm example of the utility of genetic in Precision Medicine. As NGS  
21 becomes cheaper, and the bioinformatics analysis has developed further, this may expand  
22 to whole genome sequencing to give an individual a more complete picture of their future  
23 risk of disease.  
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### 32 33 34 **Acknowledgments**

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### 45 46 **Conflict of Interest**

47  
48 MF reports speakers' fees from Sanofi. SH is the Medical Director of a UCL spin-out company  
49 StoreGene that offers to clinicians genetic testing for patients with familial  
50 hypercholesterolaemia. SH Directs the Children's FH Register which is supported by a grant  
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## References

1. Nordestgaard BG, Chapman MJ, Humphries SE, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur Heart J*. 2013;34(45):3478-90a.
2. Marks D, Thorogood M, Neil HA, Humphries SE. A review on the diagnosis, natural history, and treatment of familial hypercholesterolaemia. *Atherosclerosis*. 2003;168(1):1-14.
3. Futema M, Whittall RA, Kiley A, et al. Analysis of the frequency and spectrum of mutations recognised to cause familial hypercholesterolaemia in routine clinical practice in a UK specialist hospital lipid clinic. *Atherosclerosis*. 2013;229(1):161-8.
4. Haralambos K, Whatley SD, Edwards R, et al. Clinical experience of scoring criteria for Familial Hypercholesterolaemia (FH) genetic testing in Wales. *Atherosclerosis*. 2015;240(1):190-6.
5. Williams RR, Hunt SC, Schumacher MC, et al. Diagnosing heterozygous familial hypercholesterolemia using new practical criteria validated by molecular genetics. *Am J Cardiol*. 1993;72(2):171-6.
6. Marduel M, Ouguerram K, Serre V, et al. Description of a large family with autosomal dominant hypercholesterolemia associated with the APOE p.Leu167del mutation. *Hum Mutat*. 2013;34(1):83-7.
7. Taylor A, Wang D, Patel K, et al. Mutation detection rate and spectrum in familial hypercholesterolaemia patients in the UK pilot cascade project. *Clin Genet*. 2010;77(6):572-80.
8. Goldberg AC, Hopkins PN, Toth PP, et al. Familial hypercholesterolemia: screening, diagnosis and management of pediatric and adult patients: clinical guidance from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. *J Clin Lipidol*. 2011;5(3 Suppl):S1-8.
9. Watts GF, Sullivan DR, Poplawski N, et al. Familial hypercholesterolaemia: a model of care for Australasia. *Atheroscler Suppl*. 2011;12(2):221-63.
10. Kerr M, Pears R, Miedzybrodzka Z, et al. Cost effectiveness of cascade testing for familial hypercholesterolaemia, based on data from familial hypercholesterolaemia services in the UK. *Eur Heart J*. 2017;38(23):1832-9.
11. Lazaro P, Perez de Isla L, Watts GF, et al. Cost-effectiveness of a cascade screening program for the early detection of familial hypercholesterolemia. *J Clin Lipidol*. 2017;11(1):260-71.
12. Talmud PJ, Shah S, Whittall R, et al. Use of low-density lipoprotein cholesterol gene score to distinguish patients with polygenic and monogenic familial hypercholesterolaemia: a case-control study. *Lancet*. 2013;381(9874):1293-301.
13. Teslovich TM, Musunuru K, Smith AV, et al. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature*. 2010;466(7307):707-13.
14. Fouchier SW, Dallinga-Thie GM, Meijers JC, et al. Mutations in STAP1 are associated with autosomal dominant hypercholesterolemia. *Circulation research*. 2014;115(6):552-5.
15. Lange LA, Hu Y, Zhang H, et al. Whole-exome sequencing identifies rare and low-frequency coding variants associated with LDL cholesterol. *Am J Hum Genet*. 2014;94(2):233-45.
16. Futema M, Shah S, Cooper JA, et al. Refinement of variant selection for the LDL cholesterol genetic risk score in the diagnosis of the polygenic form of clinical familial hypercholesterolemia and replication in samples from 6 countries. *Clin Chem*. 2015;61(1):231-8.
17. Mariano C. FM, Humphries S.E., Bourbon M. . Applicability of the low-density lipoprotein cholesterol gene score in a South European population. *Atherosclerosis*. 2017;263:e99-e100.

18. Coram MA, Duan Q, Hoffmann TJ, et al. Genome-wide characterization of shared and distinct genetic components that influence blood lipid levels in ethnically diverse human populations. *Am J Hum Genet.* 2013;92(6):904-16.
19. Benn M, Watts GF, Tybjaerg-Hansen A, Nordestgaard BG. Mutations causative of familial hypercholesterolaemia: screening of 98 098 individuals from the Copenhagen General Population Study estimated a prevalence of 1 in 217. *Eur Heart J.* 2016;37(17):1384-94.
20. Consortium UK, Walter K, Min JL, et al. The UK10K project identifies rare variants in health and disease. *Nature.* 2015;526(7571):82-90.
21. Do R, Stitzel NO, Won HH, et al. Exome sequencing identifies rare LDLR and APOA5 alleles conferring risk for myocardial infarction. *Nature.* 2015;518(7537):102-6.
22. Wald DS, Bestwick JP, Morris JK, Whyte K, Jenkins L, Wald NJ. Child-Parent Familial Hypercholesterolemia Screening in Primary Care. *The New England journal of medicine.* 2016;375(17):1628-37.
23. Futema M, Plagnol V, Li K, et al. Whole exome sequencing of familial hypercholesterolaemia patients negative for LDLR/APOB/PCSK9 mutations. *J Med Genet.* 2014;51(8):537-44.
24. Hinchcliffe M, Le H, Fimmel A, et al. Diagnostic validation of a familial hypercholesterolaemia cohort provides a model for using targeted next generation DNA sequencing in the clinical setting. *Pathology.* 2014;46(1):60-8.
25. Yarram-Smith L, , Dean P, et al. The impact of routine next generation sequencing testing for familial hypercholesterolaemia - 5 months service experience. *Atherosclerosis.* 2014;236(2):3304.
26. Norsworthy PJ, Vandrovцова J, Thomas ER, et al. Targeted genetic testing for familial hypercholesterolaemia using next generation sequencing: a population-based study. *BMC Med Genet.* 2014;15:70.
27. Maglio C, Mancina RM, Motta BM, et al. Genetic diagnosis of familial hypercholesterolaemia by targeted next-generation sequencing. *J Intern Med.* 2014;276(4):396-403.
28. Khamis A, Palmen J, Lench N, et al. Functional analysis of four LDLR 5'UTR and promoter variants in patients with familial hypercholesterolaemia. *European journal of human genetics : EJHG.* 2015;23(6):790-5.
29. Webb JC, Patel DD, Shoulders CC, Knight BL, Soutar AK. Genetic variation at a splicing branch point in intron 9 of the low density lipoprotein (LDL)-receptor gene: a rare mutation that disrupts mRNA splicing in a patient with familial hypercholesterolaemia and a common polymorphism. *Hum Mol Genet.* 1996;5(9):1325-31.
30. Humphries SE, Whittall RA, Hubbart CS, et al. Genetic causes of familial hypercholesterolaemia in patients in the UK: relation to plasma lipid levels and coronary heart disease risk. *J Med Genet.* 2006;43(12):943-9.
31. Gaffney D, Reid JM, Cameron IM, et al. Independent mutations at codon 3500 of the apolipoprotein B gene are associated with hyperlipidemia. *Arterioscler Thromb Vasc Biol.* 1995;15(8):1025-9.
32. Rabes JP, Varret M, Devillers M, et al. R3531C mutation in the apolipoprotein B gene is not sufficient to cause hypercholesterolemia. *Arterioscler Thromb Vasc Biol.* 2000;20(10):E76-82.
33. Thomas ER, Atanur SS, Norsworthy PJ, et al. Identification and biochemical analysis of a novel APOB mutation that causes autosomal dominant hypercholesterolemia. *Molecular genetics & genomic medicine.* 2013;1(3):155-61.

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34. Alves AC, Etxebarria A, Soutar AK, Martin C, Bourbon M. Novel functional APOB mutations outside LDL-binding region causing familial hypercholesterolaemia. *Hum Mol Genet.* 2014;23(7):1817-28.
35. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in medicine : official journal of the American College of Medical Genetics.* 2015;17(5):405-24.36. Leigh S, Futema M, Whittall R, et al. The UCL low-density lipoprotein receptor gene variant database: pathogenicity update. *J Med Genet.* 2017;54(4):217-23.
37. Chora J, Medeiros A, Alves A, et al. Analysis of publicly available LDLR, APOB and PCSK9 variants associated with Familial Hypercholesterolemia: applications of ACMG guidelines and implications for Familial Hypercholesterolemia diagnosis. *Genet Med; gim2017151.* Epub ahead of print 2017. DOI: 10.1038/gim.2017.151.
38. Austin MA, Hutter CM, Zimmern RL, Humphries SE. Genetic causes of monogenic heterozygous familial hypercholesterolemia: a HuGE prevalence review. *Am J Epidemiol.* 2004;160(5):407-20.
39. Khera AV, Won HH, Peloso GM, et al. Diagnostic Yield of Sequencing Familial Hypercholesterolemia Genes in Patients with Severe Hypercholesterolemia. *Journal of the American College of Cardiology.* 2016.
40. Neil HA, Huxley RR, Hawkins MM, et al. Comparison of the risk of fatal coronary heart disease in treated xanthomatous and non-xanthomatous heterozygous familial hypercholesterolaemia: a prospective registry study. *Atherosclerosis.* 2003;170(1):73-8.
41. Sharifi M, Higginson E, Bos S, et al. Greater preclinical atherosclerosis in treated monogenic familial hypercholesterolemia vs. polygenic hypercholesterolemia. *Atherosclerosis.* 2017;263:405-11.
42. Bennet, AM, Di Angelantonio, E, Ye, Z et al. Association of apolipoprotein E genotypes with lipid levels and coronary risk. *JAMA.* 2007; 298: 1300–1311

## Figure Legends

**Figure 1.** Probability of having a polygenic cause of hypercholesterolaemia (LDL-C > 4.9mmol/l) by deciles of SNP-Score, depending on the frequency of undetected mutations (monogenic cause)

A. when the frequency of unknown mutation is 0.001,

B. when the frequency of unknown mutation is 0.005,

C. when the frequency of unknown mutation is 0.01.

**Figure 2.** Boxplot of the LDL-C weighted SNP score in WHII control cohort and patients groups.

Footnote: Data from (11). [11]. In the WHII participants, the mean weighted LDL-C gene score was  $0.90 \pm 0.23$ ). Compared to WHII participants, a significantly higher mean weighted LDL-C gene score was seen in 321 UK FH/M- patients ( $1.0 \pm 0.21$ ;  $p = 4.5 \times 10^{-16}$ ). The score was also higher in 329 UK FH/M+ patients ( $0.95 \pm 0.20$ ;  $p = 1.6 \times 10^{-5}$ ).

**Figure 3.** Meta-analysis of the LDL-C SNP score in nine independent FH mutation negative cohorts in comparison to the WHII population.

Footnote: data from (15). Highlighted in red box are two cohorts studied in the original report (11). The overall SMD was 0.381.

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**Statement of Originality**

I confirm that the material contained in this manuscript is original and has not been submitted elsewhere for publication. The final version has been approved by all authors

17<sup>th</sup> April 2018

Dr Raul Santos  
Associate Editor  
*Atherosclerosis*

Dear Raul,

I am writing to submit our invited Review titled: '**Clinical utility of the Polygenic LDL-C SNP score in Familial Hypercholesterolemia.**' to *Atherosclerosis*. The final version has been approved by all authors.

The manuscript contains the following Conflict of Interest statement "MF reports speakers' fees from Sanofi. SH is the Medical Director of a UCL spin-out company StoreGene that offers to clinicians genetic testing for patients with familial hypercholesterolaemia. SH Directs the Children's FH Register which is supported by a grant from Pfizer given by the International Atherosclerosis Society. MB has received project grants from Alexion and Regeneron."

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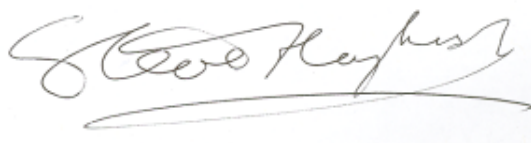
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Yours sincerely,



Professor Steve Humphries

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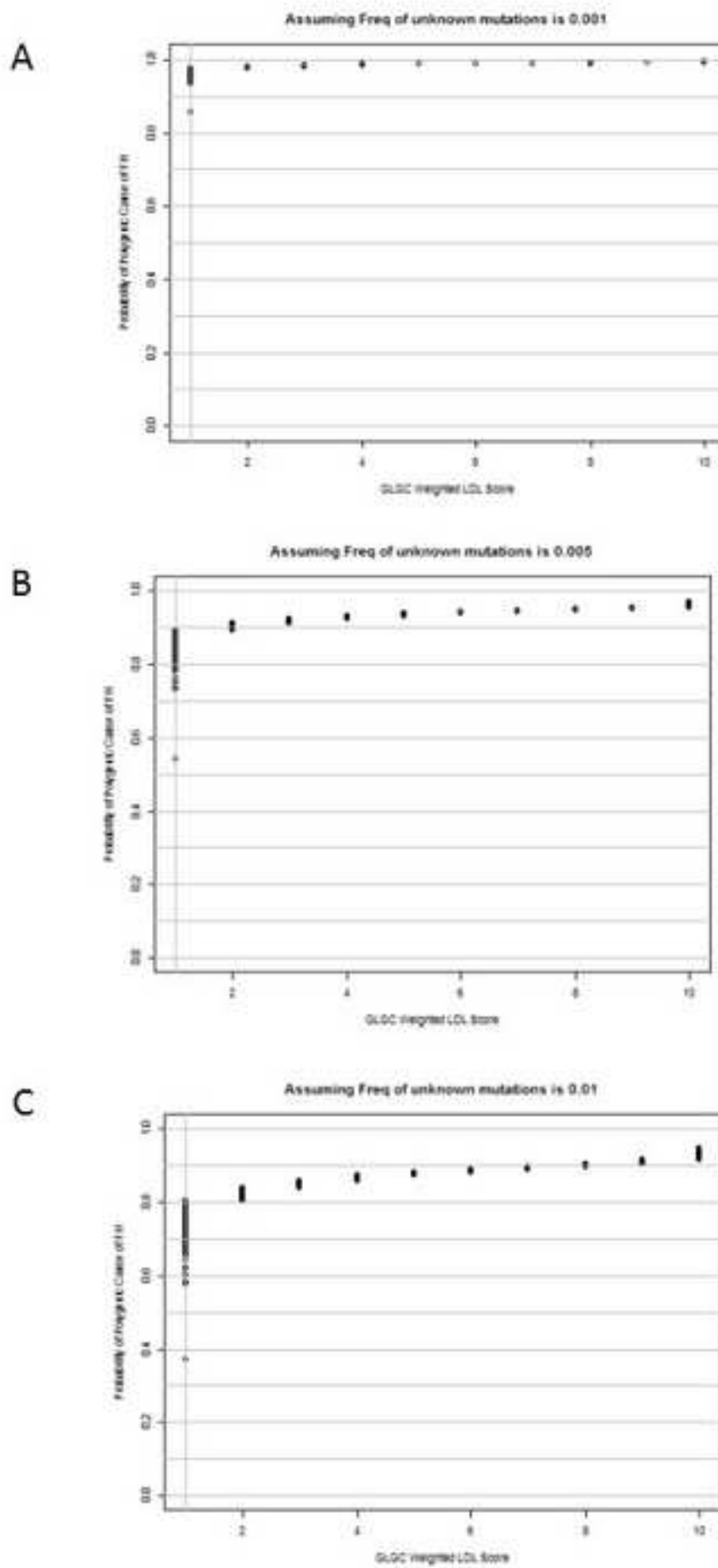


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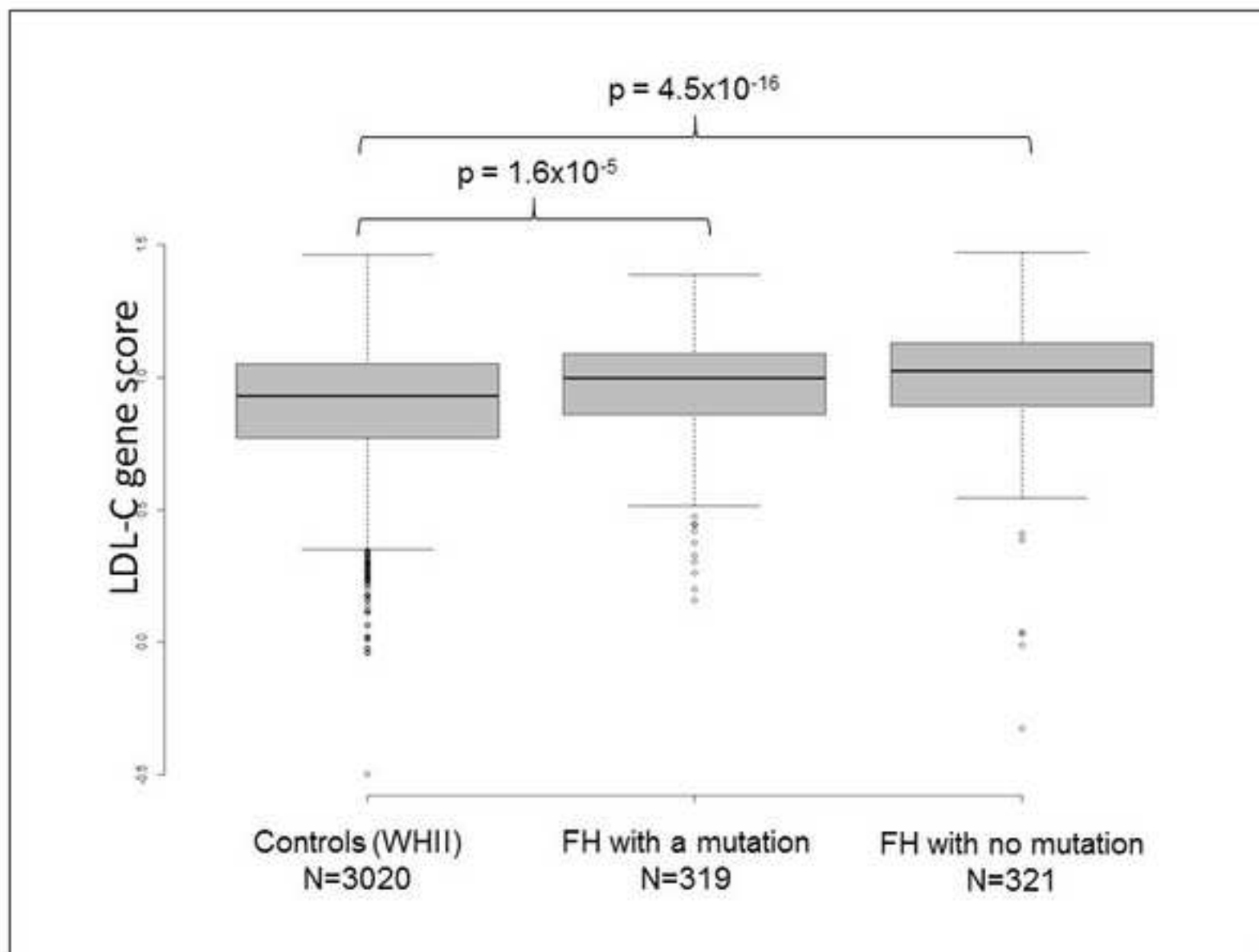
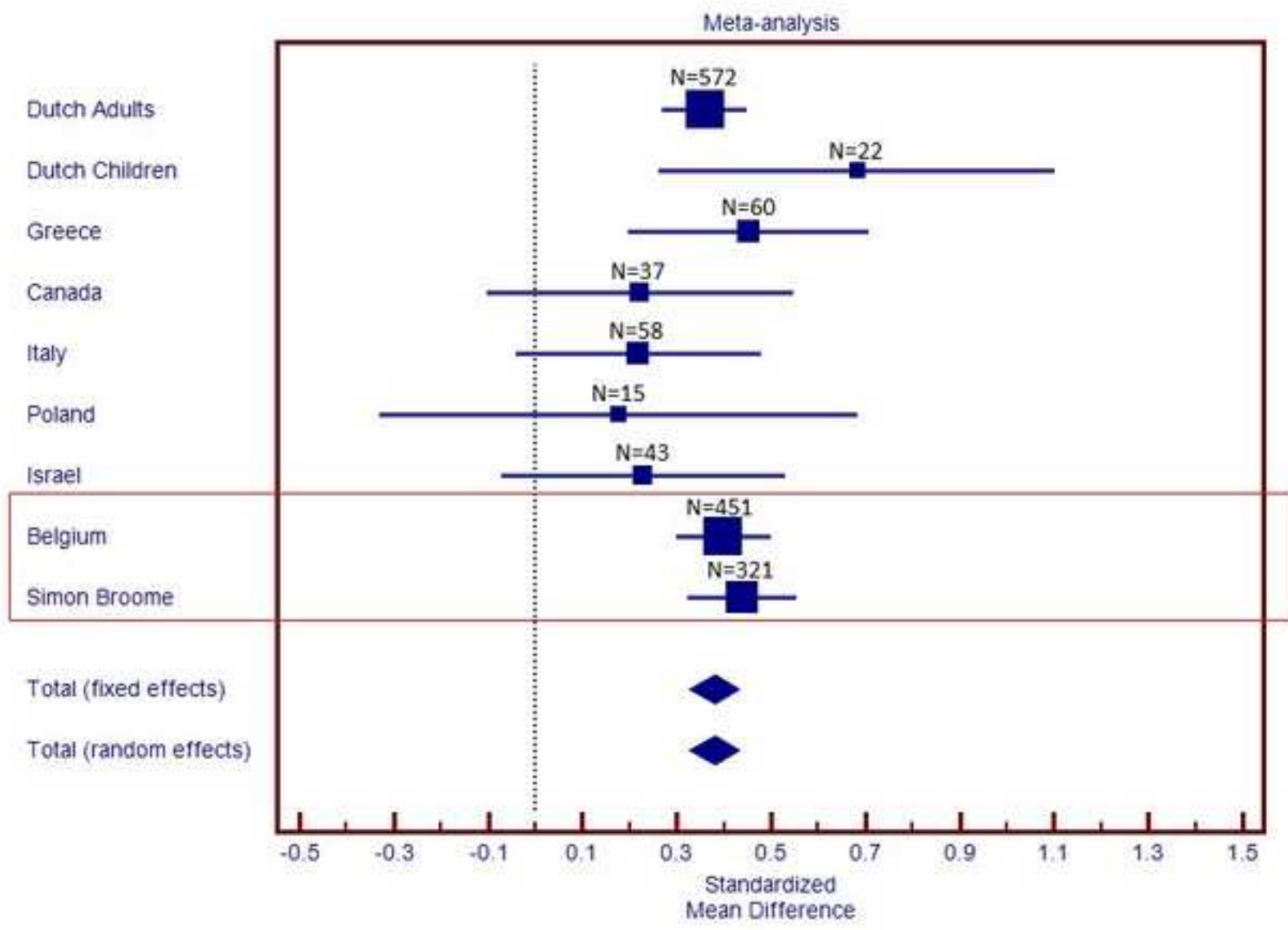


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**Table 1:** Global Lipid Genetic Consortium [10] 12 SNP LDL-C Gene Score, showing the LDL-C-raising allele and the published raising effect (in mmol/l).

CHR	SNP	Gene	Minor*	Common*	GLGC Weight
1	rs2479409	<i>PCSK9</i>	<b>G</b>	A	0.052
1	<b>rs629301</b>	<b><i>CELSR2</i></b>	G	<b>T</b>	0.15
2	<b>rs1367117</b>	<b><i>APOB</i></b>	<b>A</b>	G	0.10
2	<b>rs4299376</b>	<b><i>ABCG8</i></b>	<b>G</b>	T	0.071
6	rs1564348	<i>SLC22A1</i>	C	<b>T</b>	0.014
6	rs1800562	<i>HFE</i>	A	<b>G</b>	0.057
6	rs3757354	<i>MYLIP</i>	T	<b>C</b>	0.037
11	rs11220462	<i>ST3GAL4</i>	<b>A</b>	G	0.050
14	rs8017377	<i>KIAA1305</i>	<b>A</b>	G	0.029
19	<b>rs6511720</b>	<b><i>LDL-R</i></b>	T	<b>G</b>	0.18
19	<b>rs429358</b>	<b><i>APOE</i></b>	C	T	
19	<b>rs7412</b>	<b><i>APOE</i></b>	T	C	
19	ε2ε2	<i>APOE</i>			-0.9
19	ε2ε3	<i>APOE</i>			-0.4
19	ε2ε4	<i>APOE</i>			-0.2
19	ε3ε3	<i>APOE</i>			0
19	ε3ε4	<i>APOE</i>			0.1
19	ε4ε4	<i>APOE</i>			0.2

\*Risk alleles (LDL-C-raising) are indicated in bold. rs numbers and gene symbols in bold indicate the 6-SNPs used in [15].

GLGC weights from [10] and *APOE* weights were based on haplotypic effects taken from [42]



**Table 2.** Mean (SD) of weighted LDL-C gene score deciles range as calculated in the WHII study of 3000 healthy UK men and women.

<b>Score Deciles</b>	<b>Mean (SD)</b>	<b>Min and max</b>
1	0.43 (0.14)	-0.5 -0.58
2	0.66 (0.04)	0.58 -0.73
3	0.77 (0.03)	0.73 -0.81
4	0.85 (0.02)	0.81 -0.88
5	0.91 (0.02)	0.88 -0.93
6	0.96 (0.01)	0.94 -0.98
7	1.00 (0.01)	0.98 -1.02
8	1.05 (0.02)	1.02 -1.08
9	1.12 (0.02)	1.08 -1.16
10	1.23 (0.06)	1.16 -1.46