



# Improving the cost-effectiveness equation of cascade testing for familial hypercholesterolaemia

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## Purpose of review

Many international recommendations for the management of familial hypercholesterolaemia propose the use of cascade testing using the family mutation to unambiguously identify affected relatives. In the current economic climate DNA information is often regarded as too expensive. Here, we review the literature and suggest strategies to improve cost-effectiveness of cascade testing.

## Recent findings

Advances in next-generation sequencing have both speeded up the time taken for a genetic diagnosis and reduced costs. Also, it is now clear that, in the majority of patients with a clinical diagnosis of familial hypercholesterolaemia in whom no mutation can be found, the most likely cause of their elevated LDL-cholesterol (LDL-C) is because they have inherited a greater number than average of common LDL-C raising variants in many different genes. The major cost driver for cascade testing is not DNA testing but treatment over the remaining lifetime of the identified relative. With potent statins now off-patent, the overall cost has reduced considerably, and combining these three factors, a familial hypercholesterolaemia service based around DNA-cascade testing is now less than 25% of that estimated by NICE in 2008.

## Summary

Although all patients with a clinical diagnosis of familial hypercholesterolaemia need to have their LDL-C lowered, cascade testing should be focused on those with the monogenic form and not the polygenic form.

## Keywords

next-generation sequencing, polygenic familial hypercholesterolaemia, statin price

## INTRODUCTION

Familial hypercholesterolaemia is an autosomal dominant disorder, characterized clinically by elevated LDL-cholesterol (LDL-C) levels, and as a consequence premature mortality and morbidity from coronary heart disease (CHD). There is a 50% risk of CHD in a man with familial hypercholesterolaemia by the age of 50 years and in a woman with familial hypercholesterolaemia by the age of 60 years, which has been reported in several studies, as previously summarized [1]. Once identified, individuals with heterozygous familial hypercholesterolaemia can be successfully treated by lipid-lowering agents particularly statins which reduce their CHD mortality to that of the general population. Familial hypercholesterolaemia affects about one of 500 [2] to one of 200 [3,4<sup>\*\*\*</sup>] individuals of the Caucasian population, with therefore between 120 000 and 240 000 people in the UK with heterozygous familial hypercholesterolaemia, and an estimated 1.8–4.5 million people affected in Europe, of whom at least 75% are undiagnosed [2]. Cascade testing, using the family mutation to identify carrier relatives unambiguously,

is a cost-effective method of finding additional familial hypercholesterolaemia patients, and has been used extensively in some countries in Europe, most notably in Holland, with great success [5<sup>\*</sup>]. In 2008, the UK agency NICE published a familial hypercholesterolaemia guideline for implementing genetic testing of index case and cascade testing of their relatives on the basis of compelling evidence of

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## KEY POINTS

- Recent developments in NGS methods have improved the cost and time efficiency of genetic diagnosis for familial hypercholesterolaemia.
- Cascade testing to identify relatives affected by familial hypercholesterolaemia is most cost-effective in families diagnosed with the monogenic form of familial hypercholesterolaemia.
- The cost of cascade testing in familial hypercholesterolaemia families has significantly fallen mainly due to the reduction in costs of lipid-lowering therapy.
- A 'dual care' approach for the familial hypercholesterolaemia patients' management, and avoiding cascade testing in relatives of polygenic hypercholesterolaemia cases, can provide further savings on cascade testing.

clinical efficacy and cost-effectiveness [6], and several other country specific and international consensus guidelines have made similar recommendations [7]. Using the NICE model, it has been estimated that DNA-based cascade testing for identifying and managing affected relatives had an incremental cost-effectiveness ratio (ICER) of ~£3666 (~€4600) per quality adjusted life year (QALY) gained [8], which is much more cost-effective than many current ongoing screening programmes. However, in the UK and many countries in Europe, DNA-based cascade testing is still not being undertaken, in large part because of the perception that the provision of the service is too expensive in difficult economic times. Here, we review the literature regarding the cost-effectiveness of DNA-based cascade testing for familial hypercholesterolaemia and examine how recent advances in genetic knowledge and methods, and the cost reduction achieved by changing the model of care and using off-patent statins can influence overall costs.

## GENETIC TESTING FOR FAMILIAL HYPERCHOLESTEROLAEMIA

Roughly, 93% of patients with identified genetic cause have a mutation in the receptor for LDLs (*LDLR*) and the University College London worldwide database currently holds more than 1200 published and confirmed genetic causes of familial hypercholesterolaemia [9]. The second most common cause of familial hypercholesterolaemia worldwide is a single mutation in the gene for apolipoprotein B (*APOB*) which prevents efficient binding of the LDL-C particle to the receptor and

therefore reduces clearance of LDL-C from the blood by the liver (~5% of familial hypercholesterolaemia patients in the UK [10]). The other molecular cause of familial hypercholesterolaemia is that of mutations in the gene for proprotein convertase subtilisin/kexin type 9 (*PCSK9*), which encodes the PCSK9 protein which is involved in the degradation of the LDL-receptor during its recycling and cellular trafficking. Mutations causing familial hypercholesterolaemia are gain-of-function, and in the UK a common mutation in this gene explains about 2% of patients with familial hypercholesterolaemia [10].

Since the announcement of the entire human genome sequence in 2007 there has been huge technological advances, such that it is now possible to use 'next-generation sequencing' (NGS) methods to sequence the whole of the genome of an individual, or more usefully, all the parts of the genome that code for proteins, known as 'exome sequencing' [11,12,13<sup>11</sup>,14]. DNA diagnostic labs have now developed methods whereby all the protein coding regions of the genome for the known familial hypercholesterolaemia genes can be captured and sequenced in one run with high accuracy [15<sup>12</sup>,16<sup>13</sup>,17<sup>14</sup>], and with the addition of small 'barcoding' sequence runs into the primers used to PCR amplify the regions of interest, it is now possible to mix the samples from up to 96 individuals and analyse them in one run with high accuracy [17<sup>14</sup>]. This economy of scale is helping to drive down prices so that now a full familial hypercholesterolaemia diagnostic scan can be completed for roughly £250 and the data can also be used to look to see if an individual has a large insertion or deletion of the gene, which occurs in about 5% of patients [15<sup>12</sup>]. Using these methods produces a large amount of sequence data, which must be analysed using statistical and bioinformatics approaches and this has increased the number of occasions whereby a variant of uncertain significance is identified [18<sup>15</sup>]. This creates a diagnostic conundrum which clearly cannot be reported as familial hypercholesterolaemia-causing to the clinician or patient but which requires either in-vitro molecular assays to examine impact on transcription or correct splicing, or by family studies to see if other relatives who have inherited this variant have also shown high LDL-C levels. Several novel variants in the *APOB* gene have also been identified and shown to be familial hypercholesterolaemia-causing using in-vitro assays [19,20<sup>16</sup>].

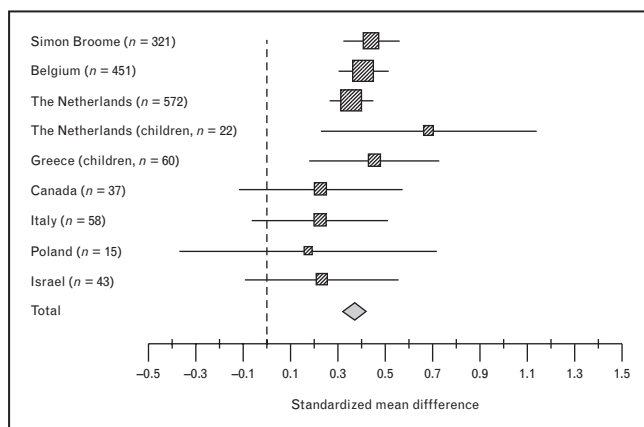
## POLYGENIC FAMILIAL HYPERCHOLESTEROLAEMIA

Overall, ~60% of patients with a clinical diagnosis of possible or definite familial hypercholesterolaemia

(i.e. having a Dutch Lipid Clinic Score of over 5) are mutation negative by conventional testing, and in this group it is now clear that the familial hypercholesterolaemia phenotype can be reproduced by an accumulation of common small-effect LDL-C raising alleles. This was first reported in 2013 [21<sup>■</sup>], and now (Fig. 1) confirmed in samples from an additional six different countries and in both adults and children [22<sup>■</sup>]. The data indicate that in clinical familial hypercholesterolaemia but mutation-negative patients, at least 80% are likely to have a polygenic cause of the elevation in LDL-C rather than an as yet unidentified single gene mutation. Cascade testing would be less effective in such cases, since the number of them inheriting this high number of LDL-raising single nucleotide polymorphisms (SNPs) would be considerably lower than the 50% affected relatives expected for monogenic inheritance. We proposed [21<sup>■</sup>] that patients with a familial hypercholesterolaemia phenotype, in whom no familial hypercholesterolaemia-causing mutation can be found, should be given the clinical diagnosis of polygenic hypercholesterolaemia, and not familial hypercholesterolaemia. This should not, however, affect the treatment of these patients, but would influence the decision to conduct cascade testing in their relatives [21<sup>■</sup>].

### CLINICAL UTILITY OF DNA TESTING

There are several reasons why, based on published evidence, the NICE guidelines for familial hypercholesterolaemia published in 2008, and the NICE quality standards, published in 2013 emphasized the clinical utility of DNA testing. The first is because there is a significant overlap in the



**FIGURE 1.** The overall difference between the combined mutation-negative familial hypercholesterolaemia groups and the approximately 3000 healthy individuals from the Whitehall II study was 0.38 ( $\pm 0.027$ ),  $P < 10^{-15}$ . Data from [22<sup>■</sup>].

distribution of LDL-C levels in mutation carriers and their first-degree relatives who do not carry a mutation [23,24], and this overlap reduces the efficiency of cascade testing. The cascade testing programmes that have been carried out so successfully in Holland [5<sup>■</sup>,24] and that are now being actively pursued in both Wales [25] and Scotland [26], therefore use DNA testing to identify those with a mutation and only cascade test in mutation-positive families. Although false-positive results based on LDL-C levels identify individuals whose LDL-C is high and may need to be treated, if they do not carry the family mutation, testing their children will clearly result in no (or very low) frequency of new familial hypercholesterolaemia patients. The opposite of this is that in the false-negative individuals who carry a mutation but whose LDL-C level is below the diagnostic threshold, their low LDL-C may be because they have luckily inherited LDL-C lowering genetic variants or are adopting an LDL-C lowering environment, but clearly 50% of their children are at risk of inheriting the mutation and may not be lucky enough to take on the lifestyle or have inherited the LDL-C-lowering variants.

### COST-EFFECTIVENESS OF DIFFERENT STRATEGIES FOR CASCADE TESTING IN FAMILIAL HYPERCHOLESTEROLAEMIA FAMILIES

The cost-effectiveness literature for familial hypercholesterolaemia has been recently reviewed [27]. Six studies reported on the cost-effectiveness of familial hypercholesterolaemia screening and subsequent treatment of the identified relatives. All such papers use published estimates of the high risk of CHD in men and women with familial hypercholesterolaemia (as summarized in [1]), and the efficacy of the early-in-life initiated treatment, as has been demonstrated in the UK Simon Broome Study and in Holland [28,29]. These CHD estimates were examined in detail by NICE CG71 and accepted as robust and used in their published analysis [8]. Compared with no screening the ICER of screening ranged from €3177 to €29 554 per life year gained. The results were sensitive to the underlying prevalence of familial hypercholesterolaemia among the population being examined, the validity of the screening test and the price and efficacy of lipid-lowering therapy. The authors concluded that overall, cascade testing for new cases of familial hypercholesterolaemia was cost-effective, but there were uncertainties in the modelling methods, especially with regard to the use of different approaches to assess the outcomes of treatment. It was recognized

that cost-effectiveness would improve further once atorvastatin and rosuvastatin lost exclusivity.

These issues were examined in more detail in a follow-up paper [30<sup>11</sup>] using data from an Australian healthcare perspective. Using a Markov model and a 10-year time horizon to simulate the onset of first-ever CHD and death in close relatives of monogenic familial hypercholesterolaemia index cases the decision-analysis compared the clinical consequences and costs of cascade testing versus no testing. The annual risk of CHD and benefits of treatment were estimated from a cohort study, and the underlying prevalence of familial hypercholesterolaemia, sensitivity, specificity, cost of screening, treatment and clinic follow-up visits were derived from a cascade screening service for familial hypercholesterolaemia in Western Australia. The model was cost-effective. It estimated that testing would reduce the 10-year incidence of CHD from 50.0 to 25.0% among people with familial hypercholesterolaemia. Of every 100 people tested, there was an overall gain of approximately 25 life-years and 29 QALY, with an ICER in Australian dollars, \$4155 (€5850) per years of life saved and \$3565 (€5093) per QALYs gained.

A recent paper by Pears *et al.* [31<sup>12</sup>] examined the cost-effectiveness of three alternative models of care for familial hypercholesterolaemia. The first model considered was a specialist-led model. This model was similar to the NICE model with the exception that a lower proportion of patients would have annual reviews in secondary care. The second model involved primary care taking responsibility for the entire adult familial hypercholesterolaemia care pathway. In this model, no patients would be referred to a familial hypercholesterolaemia specialist (locally this means a lipidologist), and all patients would be reviewed annually by their GP. The final model was a 'dual care' model in which primary care would manage the majority of the familial hypercholesterolaemia cascade testing pathway. In this final model, GPs would be able to refer patients to lipidologists if they needed further advice about management, or to genetic services, when advice on cascade testing was needed because a genetic mutation had not been identified.

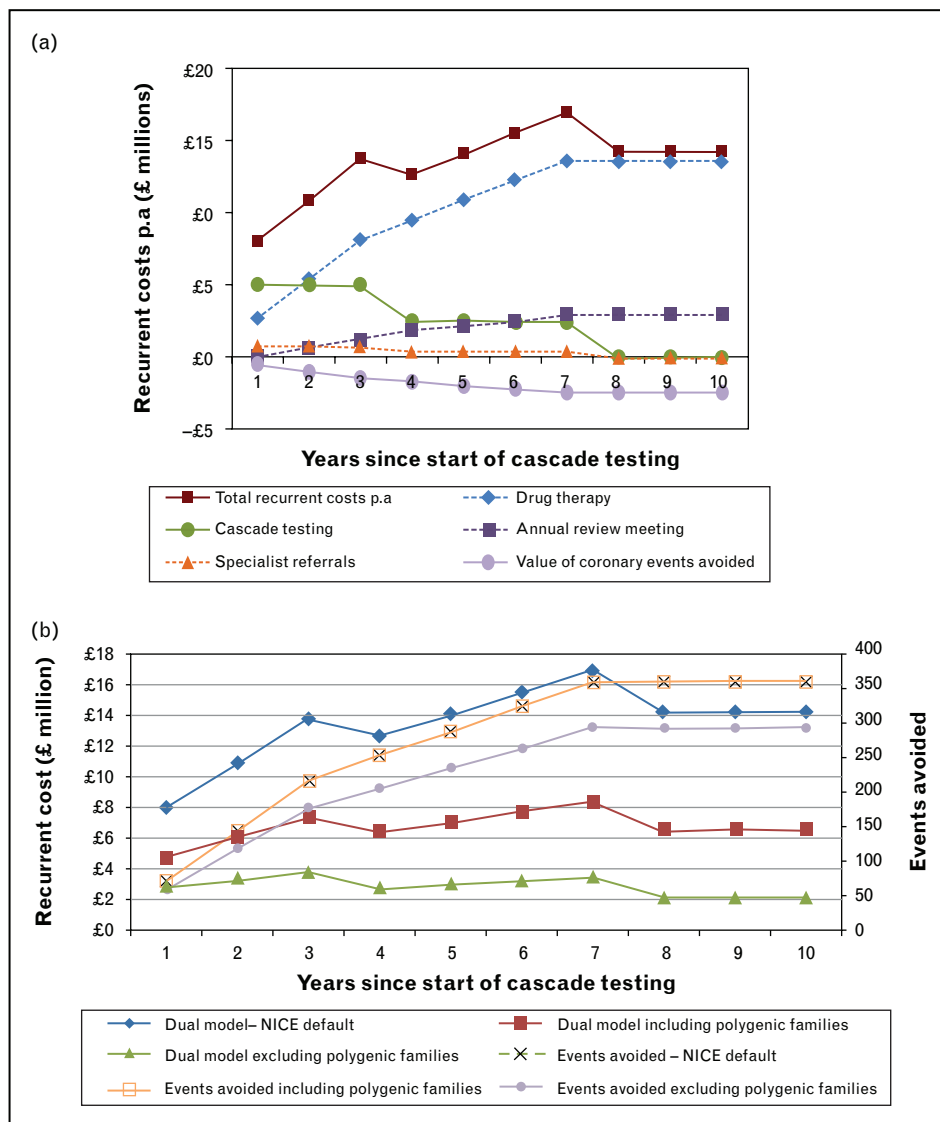
The authors conclude that costs for all three models were now less than 50% of the cost of the original estimates undertaken by NICE. By using the latest statin costs and reduced DNA testing costs, by reducing the proportion of patients prescribed more expensive proprietary owned rosuvastatin and by managing more patients with familial hypercholesterolaemia in primary care, providing an familial hypercholesterolaemia service is now much more affordable than predicted by NICE in 2008. The

reduction in costs with generic atorvastatin now available (~£368–25 pa for 40 mg or £45 pa for 80 mg) this reduces the cost of a 10-year service by 42.5% (~£4.8–2.8 million). Compared with the generic atorvastatin costing estimates, the specialist-led model reduced 10-year costs by a further 27.2%, the dual care model by a further 32.5% and the GP-led model by a further 35.8%. The three alternative models of care were now less than 50% of the cost of the original estimates undertaken by NICE. If 50% of patients with familial hypercholesterolaemia are diagnosed early in life and then treated optimally over a 55-year period, £94.7 million (£1.97 million per 1000 cases) can be saved through reduced CV events, or £1.7 million (€1.2 million) per year.

Pears *et al.* estimated their models in a population of 1.95 million, estimating the dual care model to cost £1.89 million (~€2.6 million) over 10 years. If we extrapolate the figure to that of the entire population of England (53.9 million in 2013), the original NICE model will cost approximately £134.5 million (€95.49 million) over 10 years and the dual care model will cost approximately £67.3 million (€47.8 million). Such a programme would include the cost of medicines, management in primary care, referral for specialist attention and genetic testing and assessment of family members. However, as we now understand that the majority of those with no detectable mutation do not have 'monogenic' familial hypercholesterolaemia, costs can be reduced further if we also only cascade test from familial hypercholesterolaemia patients with a monogenic cause of familial hypercholesterolaemia. Based on (31) this would be only approximately 45% of those with a clinical diagnosis of familial hypercholesterolaemia, and would reduce costs still further to approximately £28.5 million (~€40 million) as shown in Fig. 2a (see [28] and supplementary material for detailed assumptions of this model). The dual model of care, excluding polygenic hypercholesterolaemia cascade testing, is now less than 25% of the cost of the original 2008 estimates undertaken by NICE. Restricting cascade testing to monogenic families reduces the number of cardiac events avoided over 10 years from 2736 to 2229 (18.5% reduction) as shown in Fig. 2b. However, relative cost-effectiveness jumps. For instance, in year eight onwards the cost per cardiac event avoided is £39 489 (€28 037) in the original NICE model, £18 088 (€12 842) in the dual model and £7 155 (€5080) in the monogenic cascade testing model.

## CONCLUSION

Although almost all recent guidelines for the identification and management of familial hypercholesterolaemia recognize the utility of DNA-based



**FIGURE 2.** Estimate over 10 years of the costs in a monogenic cascade testing familial hypercholesterolaemia service. (a) A comparison of costs and clinical benefits (i.e. a population of 53.9 million), assume costs based on atorvastatin off-patent, only mutation-positive patients included as index cases for cascade testing and a cost of £250 per index test and £70 per relative test, and based on the dual care pathway in [28]. € costs estimated assuming 1 € = £0.71. (b) A comparison of costs per cardiac events avoided. Adapted with permission from [28].

cascade testing for the identification of at-risk relatives, few countries to date have implemented this as a national strategy. The advent of NGS methods and the recognition that the vast majority of patients with a clinical diagnosis of familial hypercholesterolaemia but with no identified mutation have a polygenic cause of their elevated LDL-C suggests sensible targeting strategies for cascade testing. It is likely that individuals with polygenic familial hypercholesterolaemia will not have so severe atherosclerosis as monogenic familial hypercholesterolaemia, or at least will not manifest their cardiovascular disease at such an early age, although further research is urgently needed to

explore this. Such individuals could be easily managed in general practice, whereas monogenic familial hypercholesterolaemia may well require management in expert centres because they require multiple drug therapies in order to reduce their LDL-C levels to acceptable target levels, or the use of novel lipid lowering agents such as monoclonal antibodies to PCSK9. Use of this dual care approach also reduces overall costs of the cascade testing and management programme, but the major factor in the huge reduction in costs is the availability of potent off-patent statins. As such, the cost of providing a familial hypercholesterolaemia service has reduced by three-quarters in the past 5 years.

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## Conflicts of interest

There are no conflicts of interest.

## REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Marks D, Thorogood M, Neil HA, Humphries SE. A review on the diagnosis, natural history, and treatment of familial hypercholesterolaemia. *Atherosclerosis* 2003; 168:1–14.
2. 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; 34:3478–3490a.
3. Benn M, Watts GF, Tybjaerg-Hansen A, Nordestgaard BG. Familial hypercholesterolemia in the danish general population: prevalence, coronary artery disease, and cholesterol-lowering medication. *J Clin Endocrinol Metab* 2012; 97:3956–3964.
4. Do R, Stitzel NO, Won HH, *et al.* Exome sequencing identifies rare LDLR and APOA5 alleles conferring risk for myocardial infarction. *Nature* 2015; 518:102–106.

Exome sequencing of 9793 early myocardial infarction (MI) patients ( $\leq 50$  years in males and  $\leq 60$  years in females) along with MI-free controls. *LDLR* mutation carriers were at 4.2-fold increased risk for MI; carriers of null alleles at *LDLR* were at even higher risk (13-fold difference). Approximately 2% of early MI cases harbour a rare, damaging mutation in *LDLR*, this estimate is similar to one made more than 40 years ago using an analysis of total cholesterol. Among controls, about one in 217 carried an *LDLR* coding-sequence mutation and had plasma LDL cholesterol greater than 190 mg/dl.

5. Besseling J, Kindt I, Hof M, *et al.* Severe heterozygous familial hypercholesterolemia and risk for cardiovascular disease: a study of a cohort of 14 000 mutation carriers. *Atherosclerosis* 2014; 233:219–223.

Data on 14 283 patients with molecularly defined HeFH, identified by the national familial hypercholesterolaemia screening programme in The Netherlands.

6. Wierzbicki AS, Humphries SE, Minhas R. Familial hypercholesterolaemia: summary of NICE guidance. *BMJ* 2008; 337:a1095.
7. Humphries SE. Guidelines for the identification and management of patients with familial hypercholesterolaemia (FH): are we coming to a consensus? *Atheroscler Suppl* 2011; 12:217–220.
8. Nherera L, Marks D, Minhas R, *et al.* Probabilistic cost-effectiveness analysis of cascade screening for familial hypercholesterolaemia using alternative diagnostic and identification strategies. *Heart* 2011; 97:1175–1181.
9. Usifo E, Leigh SE, Whittall RA, *et al.* Low density lipoprotein receptor gene familial hypercholesterolaemia variant database: update and pathological assessment. *Ann Hum Genet* 2012; 76:387–401.
10. 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; 43:943–949.
11. Futema M, Plagnol V, Whittall RA, *et al.* Use of targeted exome sequencing as a diagnostic tool for Familial Hypercholesterolaemia. *J Med Genet* 2012; 49:644–649.

12. Vandrovcova J, Thomas ER, Atanur SS, *et al.* The use of next-generation sequencing in clinical diagnosis of familial hypercholesterolemia. *Genet Med* 2013; 15:948–957.
13. 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; 51:537–544.

Exomes of 75 unrelated DFH no *LDLR/APOB/PCSK9* patients with a low-polygenic LDL-C SNP score were sequenced. None of the genome-wide association studies identified genes whereas common variants that determine LDL-C showed evidence for having rare familial hypercholesterolaemia-causing mutations. Using a gene-based burden test for an excess of rare (frequency  $< 0.005$ ) or novel variants in cases versus 1926 controls, no major novel locus for familial hypercholesterolaemia was detected, with no gene having a functional variant in more than three patients. Suggests that the genetic cause of familial hypercholesterolaemia in these unexplained cases is likely to be very heterogeneous, which complicates the diagnostic and novel gene discovery process.

14. Braenne I, Reiz B, Medack A, *et al.* Whole-exome sequencing in an extended family with myocardial infarction unmasks familial hypercholesterolemia. *BMC Cardiovasc Dis* 2014; 14:108.
15. 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; 46:60–68.

The index patient (LDL-C 188 mg/dl) referred for molecular-genetic investigations. He had coronary artery bypass graft at the age of 59 years; 12 out of 15 first, second and third degree relatives were affected with CAD and/or premature MI. Whole-exome sequencing identified an *LDLR* variant in the acceptor splice site of exon 10 (c.1359-1G>A), which cosegregated with CAD (LOD 3.0) and increased *LDLC* ( $> 190$  mg/dl). Even in families with unusual clustering of CAD familial hypercholesterolaemia remain to be underdiagnosed, which underscores the need for implementation of systematic screening programs.

16. Yarram-Smith L, Dean P, *et al.* The impact of routine next generation sequencing testing for familial hypercholesterolaemia – 5 months service experience. *Atherosclerosis* 2014; 236:3304.

Targeted genetic testing for familial hypercholesterolaemia using NGS, a population-based study.

17. Norsworthy PJ, Vandrovcova 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.

Using NGS pathogenic mutations were found in 2.1% of 193 hypercholesterolaemic individuals, in 2.2% of 232 individuals on cholesterol-lowering therapy and in 42% of their available first-degree relatives. Variants of uncertain clinical significance were detected in 1.4% of the hypercholesterolaemic and cholesterol-lowering therapy groups. No pathogenic variants or variants of uncertain clinical significance were detected in controls.

18. Maglio C, Mancina RM, Motta BM, *et al.* Genetic diagnosis of familial hypercholesterolaemia by targeted next-generation sequencing. *J Intern Med* 2014; 276:396–403.

Seventy seven individuals with a familial hypercholesterolaemia clinical diagnosis analysed by NGS using SEQPRO LIPO RS, a kit that detects mutations in the *LDLR/APOB/PCSK9/LDLRAP1* genes along with copy-number variations in the *LDLR* gene. Twenty six mutations detected in 50 individuals (65% success rate) (23 *LDLR*, two *APOB* gene and one *PCSK9*). Four mutations with unknown pathogenicity were detected in *LDLR*. Of these, three mutations (*Gly505Asp*, *Ile585Thr* and *Gln660Arg*) have been previously reported in individuals with familial hypercholesterolaemia, but their pathogenicity has not been proved. The fourth, a mutation in *LDLR* affecting a splicing site (exon 6-intron 6) has not previously been reported; it was found to segregate with high cholesterol levels in the family of the proband.

19. Thomas ER, Atanur SS, Norsworthy PJ, *et al.* Identification and biochemical analysis of a novel APOB mutation that causes autosomal dominant hypercholesterolemia. *Mol Genet Genomic Med* 2013; 1:155–161.
20. Alves AC, Etxebarria A, Soutar AK, *et al.* Novel functional APOB mutations outside LDL-binding region causing familial hypercholesterolaemia. *Hum Mol Genet* 2014; 23:1817–1828.

In 65 familial hypercholesterolaemia patients without mutations in *LDLR/PCSK9* or R3527Q in *APOB*, whole sequencing of *APOB* performed by pyrosequencing and 10 putative mutations identified. Flow cytometry with fluorescently labelled LDL from patients and relatives showed that p.Arg1164Thr and p.Gln4494del had 40% lower internalization in lymphocytes and HepG2 cells, very similar to LDL-Q3527, whereas all others were of neutral effect. Results show the need to study the whole *APOB* gene to improve patient identification.

21. 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; 381:1293–1301.

Key article describing the use of an LDL-C SNP score to show that the majority of mutation-negative familial hypercholesterolaemia patients are likely to have a polygenic cause.

22. 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; 61:231–238.
- Reduced the 12-SNP score used in Talmud *et al.* to the six best SNPs. Meta-analysis of the weighted 6-SNP score in the independent familial hypercholesterolaemia/mutation negative cohorts showed a consistently higher score in comparison with the Whitehall II study population ( $P < 2.2 \times 10^{-16}$ ). Modelling in individuals with a 6-SNP score in the top three-fourths of the score distribution indicated a greater than 95% likelihood of a polygenic explanation of their increased LDL-C.
23. Starr B, Hadfield SG, Hutten BA, *et al.* Development of sensitive and specific age- and gender-specific low-density lipoprotein cholesterol cutoffs for diagnosis of first-degree relatives with familial hypercholesterolaemia in cascade testing. *Clin Chem Lab Med* 2008; 46:791–803.
24. Huijgen R, Hutten BA, Kindt I, *et al.* Discriminative ability of LDL-cholesterol to identify patients with familial hypercholesterolemia: a cross-sectional study in 26 406 individuals tested for genetic FH. *Circ Cardiovasc Genet* 2012; 5:354–359.
25. Datta BN, McDowell IF, Rees A. Integrating provision of specialist lipid services with cascade testing for familial hypercholesterolaemia. *Curr Opin Lipidol* 2010; 21:366–371.
26. Ho CK, Stirling D, Hannant W, Walker SW. Genetic mutations in patients with possible familial hypercholesterolaemia in South East Scotland. *Scott Med J* 2012; 57:148–151.
27. Ademi Z, Watts GF, Juniper A, Liew D. A systematic review of economic evaluations of the detection and treatment of familial hypercholesterolemia. *Int J Cardiol* 2013; 167:2391–2396.
28. Neil A, Cooper J, Betteridge J, *et al.* Reductions in all-cause, cancer, and coronary mortality in statin-treated patients with heterozygous familial hypercholesterolaemia: a prospective registry study. *Eur Heart J* 2008; 29: 2625–2633.
29. Versmissen J, Oosterveer DM, Yazdanpanah M, *et al.* Efficacy of statins in familial hypercholesterolaemia: a long term cohort study. *BMJ* 2008; 337: a2423.
30. Ademi Z, Watts GF, Pang J, *et al.* Cascade screening based on genetic testing is cost-effective: evidence for the implementation of models of care for familial hypercholesterolemia. *J Clin Lipidol* 2014; 8:390–400.
- Used a Markov model with a 10-year time horizon to simulate the onset of first-ever CHD and death in close relatives of probands with genetically confirmed familial hypercholesterolaemia. Model estimated that screening for familial hypercholesterolaemia would reduce the 10-year incidence of CHD from 50.0 to 25.0% among people with familial hypercholesterolaemia. Of every 100 people screened, there was an overall gain of approximately 25 life-years and 29 QALYs (discounted). The ICER was in Australian dollars, \$4155 per years of life saved and \$3565 per QALYs gained.
31. Pears R, Griffin M, Watson M, *et al.* The reduced cost of providing a nationally recognised service for familial hypercholesterolaemia. *Open Heart* 2014; 1:e000015.
- Three alternative models of care for familial hypercholesterolaemia were examined – specialist led based on referral to a tertiary referral lipid clinic, primary care led where patients were only seen and managed by primary care practitioners and a dual care model in which the majority of patients in the cascade testing pathway were managed in primary care but with hard to manage patients by lipidologists. Costs for all three models were now less than 50% of the cost of the original estimates undertaken by NICE.