FH4=STAP1. Another Gene for Familial Hypercholesterolemia?  
Relevance to Cascade Testing and Drug Development?  
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The average rate of discovery of new genes causing familial hypercholesterolemia (FH) has been ≈1 per decade since the 1970s, a meagre diversity compared with the complexity more recently discovered in familial cardiomyopathies and conduction disorders. In contrast to studies in monogenic FH, genome-wide association studies have unearthed far more than 100 genomic regions, many of unknown functionality, making small contributions to polygenic lipid traits in the general population, with many of these loci influencing low-density lipoprotein (LDL) cholesterol. Nonetheless, the several genes implicated in monogenic pure hypercholesterolemia (Table) have given powerful insight into the role of LDL cholesterol in cardiovascular risk and have opened new avenues in pharmaceutical development. They have also enabled a molecular classification of cardiovascular risk, enabling focused diagnostics in conjunction with cascade testing in mutation-positive families. The apparent identification of a further FH gene (dubbed FH4 by Fouchier et al) should, therefore, be of significant interest to geneticists, lipid clinics, general doctors, and cardiologists with an interest in tracing or treating familial hypercholesterolemics. It may also be of interest to pharmaceutical companies engaged with the development of new approaches to treating hypercholesterolemia.

The early cellular and molecular era in FH was substantially led by Nobel laureates Brown and Goldstein through the 1970s and 1980s. Cellular and biochemical characterization of FH led to the discovery of the LDL receptor, and subsequent gene cloning of LDLR led to the identification of a variety of molecular defects in LDLR causal of FH. The clear-cut chain of causality from defective gene to raised LDL cholesterol level to atheroma and coronary disease offered compelling support that LDL cholesterol is a causal risk factor for coronary disease in the general population and, therefore, worthy of therapeutic intervention. This is an example where an extreme Mendelian genotype–phenotype link affecting a minority was highly informative about a risk factor in the much wider population. Although not labeled as such, LDLR was in effect FH1 and it remains the predominant gene cause of FH.

During the 1980s, binding-defective LDL patients were recognized. Cellular studies showed them to have normal LDL receptor function, but their LDL would not bind to LDL receptors from healthy individuals. This represented a ligand defect and was given the designation familial defective apolipoprotein B (apoB)—FDB—because it turned out that there was a mutation in the LDL receptor binding domain of apoB. A single apoB protein molecule, a very large protein, scaffolds the LDL particle. However, despite a large gene target for mutation (and many reported nonsynonymous mutations), most FDB attributes to a single founder mutation in apoB, R3500Q, common in Western Europeans, suggesting that unlike the LDL receptor, most parts of the protein may be able to tolerate sequence variation without clinical effect. FDB was effectively FH2.

During the 1990s, we sought families with evidence for inconsistency with cosegregation of the FH (hypercholesterolemic) phenotype with either LDLR or APOB (or APOE, see below). One such large family emerged, which provided convincing evidence for an FH3 locus. This family and other
families\textsuperscript{11} led to the identification of FH3 as \textit{PCSK9}.\textsuperscript{12} Both hyper- and hypo-cholesterolemic variants of proprotein convertase subtilisin/kexin type 9, \textit{PCSK9}, have since been found. Although the relative number of FH families accounted for by \textit{PCSK9} mutations is small, the locus has well fulfilled another of the mantras of Mendelian disease gene hunting, namely to identify through hypothesis-free genome-wide linkage scans new and hitherto unsuspected pathways that might offer up new therapeutic targets.\textsuperscript{13} \textit{PCSK9} induces LDL receptor degradation through binding to its epidermal growth factor repeat A domain, so inhibiting \textit{PCSK9} activity should upregulate receptor level and enhance LDL clearance. Several monoclonal antibodies binding the catalytic domain of \textit{PCSK9} are already in clinical trials, and peptide mimics of the EGFA (epidermal growth factor-like repeat A) domain and \textit{PCSK9} antisense oligonucleotides are also being explored.

These then are the 3 autosomal-dominant forms of FH so far discovered. Honorable mention should also go to autosomal recessive hypercholesterolemia, the \textit{LDLRAP1} locus identified at the turn of the millennium through a combination of cellular studies, homozygosity mapping, and positional identification of the causal gene. In addition, \textit{APOE} and \textit{ABCG5/8} can also lead to an FH-like picture, although their phenotypes typically involve, respectively, an additional disturbance of triglyceride levels and different lipoprotein particle picture, or a predominant picture of sitosterolemia in which this plant sterol accumulates to excess. Lysosomal acid lipase deficiency, which causes cholesterol ester storage disease and Wolman syndrome, can also masquerade as autosomal-recessive hypercholesterolemia.\textsuperscript{14}

The expedited report by Fouchier et al\textsuperscript{9} of an FH4 gene, a fourth autosomal-dominant gene in about as many decades, is exciting. How robust are the findings and what might be the implications? The seminal finding was the identification of a FH family in which \textit{LDLR}, \textit{APOB}, and \textit{PCSK9} mutations had been excluded in the proband. It should be noted that the exclusion of mutations can be difficult, given that some mutations may be missed or may be deep in unknown regulatory regions, which are either not searched or would be uninterpretable even if observed. Exclusion of cosegregation with phenotype is more powerful, but requires a large and phenotypically clear-cut family. Uncertain phenotype such as a moderately raised LDL level, or a married in phenocopy, or a polygenic hypercholesterolemic, or an uncertainty about penetrance for a given age, can all contrive to blur the ideal dichotomous classification (affected/unaffected), which should cosegregate with a hypothetical mutation. These factors, therefore, convert what would ideally be a black-and-white linkage analysis into an analysis in shades of grey. The linkage analyses identified 3 promising leads (LOD [logarithm (base 10) of odds] scores of 3.0, representing 1/1000 odds) on 3 chromosomes. Combined with whole exome sequencing in 3 carefully selected family members, just 1 variant emerged, p.Glu97Asp in signal transducing adaptor family member 1 (\textit{STAP1}). Given 3 linkage leads where only 1 locus should emerge, and given a missense rather than stop codon or frameshift variant from whole exome sequencing, one might be concerned about a false-positive finding. There are many missense variants that are effectively private to a family, and missense variants in many instances have no effect, but computational prediction of which ones may be pathogenic is notoriously difficult.\textsuperscript{15} The authors then followed up the \textit{STAP1} linkage discovery by direct sequencing in a large collection of unrelated FH4 probands apparently negative for mutations in the other autosomal-dominant FH genes. In effect, the replication study was of association of cholesterol level (LDL or total) in carriers and noncarriers of \textit{STAP1} mutations—a positive association was found. Taken together, this constellation of evidence that \textit{STAP1} contributes to FH looks promising.

The authors go on to compare \textit{STAP1} carriers and noncarriers with \textit{APOB} and \textit{LDLR} carriers. It seems that the phenotype for \textit{STAP1} carriers is milder than for \textit{APOB}, and both are milder than for \textit{LDLR}. A minor point of note is the very slightly but significantly higher triglyceride levels in \textit{STAP1} carriers compared with controls and \textit{LDLR} and \textit{APOB} mutation carriers. Even for \textit{APOB}, tracing in families will identify mutation-positive but phenotype-negative individuals, and their cholesterol level varies much more over time than in \textit{LDLR} mutation carriers,\textsuperscript{16} despite the average relative risk of coronary disease in FDB being estimated at 7x population risk.\textsuperscript{17} Thus, the identification of \textit{STAP1} by family-based linkage would be expected to present difficulties in phenotypic classification; it seems to be an incompletely penetrant gene. A recent survey of FH patients negative for \textit{LDLR}/\textit{APOB}/\textit{PCSK9} mutations by the UK10K Consortium did not identify

\begin{table}
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\caption{Genes Implicated in Monogenic Pure Hypercholesterolemia}
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Chromosome & Gene & Disease Designation & Discovery Process; Other Comments \\
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1p32 & \textit{PCSK9} & FH3, autosomal-dominant hypercholesterolemia & Kindred cosegregation studies (linkage exclusion of \textit{LDLR} or \textit{APOB} pathogenesis), then genome-wide linkage scan and positional identification, Novel pathway discovered—novel target for drug developments, now in clinical trials. \\
1p35 & \textit{LDLRAP1} & Autosomal-recessive hypercholesterolemia (ARH) & Cellular studies, then homozygosity mapping in consanguineous families, followed by positional identification with direct mutation scanning. \\
4q13.2 & \textit{STAP1} & FH4 & Exclusion of \textit{LDLR}, \textit{APOB}, or \textit{PCSK9} mutations. Discovery by family-based linkage analysis combined with whole exome sequencing. Replication follow-up by resequencing in other unexplained patients, with association analysis of cholesterol levels in those with \textit{STAP1} mutations. \\
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any clear-cut FH4 gene although identifying 18 genes showing an excess of novel functional variations in slight excess in FH patients compared with controls.\textsuperscript{18} STAP1 was not in this list. Although rare mutations show some geographical localizations, there has been significant historical population flow between Holland (source of the Fouchier\textsuperscript{4} study) and the United Kingdom (UK10K study). This seems a surprising difference, but might be reconciled by differences in patient selection, differences in exome sequencing coverage, or possibly just chance if the mutational frequency is relatively low. Crossover analyses between the UK10K FH whole exome data and gene leads and the Fouchier\textsuperscript{4} data sets are clearly warranted for STAP1. A replication of the STAP1 findings in an entirely different population would be valuable. The Dutch report seems to indicate multiple different mutations rather than 1 local founder, so other populations might be expected to show similar effects in STAP1 even if their mutational spectra are different. Fouchier et al\textsuperscript{4} discuss the function of STAP1 in brief; little is known about it.

Beyond the priority further to replicate the STAP1 finding, what practical applications might follow? First, STAP1 would be included into molecular diagnostic repertoires for cascade testing. Second, the function of STAP1 needs to be better defined. Based on the function, it might be possible, as for PCSK9, to consider it, or something in the pathway it represents, as another novel target for therapeutic development for the management of hypercholesterolemia. Fouchier et al\textsuperscript{4} point to its role downstream of a tyrosine kinase in intracellular signaling events, a pathway location that would associate it with an established druggable class of targets. Let us see an independent validation of this promising gene.

Disclosures

None.

References


Key Words: apolipoproteins • cholesterol, LDL • hyperlipoproteinemia type II
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Circ Res. 2014;115:534-536
doi: 10.1161/CIRCRESAHA.114.304801

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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