# Familial Hypercholesterolemia: Use of Registries, Biobanks, and Cohort Studies To Improve its Diagnosis and Management in Non-Western Populations

Michael D. Shapiro, MD, MBA, FACP, CPE[1] ; Sergio Fazio, MD, PhD[1]

Michael D. Shapiro, MD

Sergio Fazio, MD, PhD

[1] Oregon Health & Science University
Knight Cardiovascular Institute Center for
Preventive Cardiology Portland OR, USA

*Address Correspondence to author:
Michael D. Shapiro, MD
Oregon Health & Science University
Knight Cardiovascular Institute – UHN62
Center for Preventive Cardiology
3181 SW Sam Jackson Park Rd.,
Portland, OR 97239
email: shapirmi@ohsu.edu

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## Abstract

Familial hypercholesterolemia (FH) is the most common monogenic disorder in humans, with an estimated prevalence of 1:200-1:250, based on unbiased genetic screening in Western populations. The vast majority of FH can be explained by mutations in three key genes; LDLR (receptor not synthesized or not functional), APOB (ligand not properly recognizing LDLR), and PCSK9 (gain of function mutations causing excessive elimination of LDLR). Causal mutations in these genes lead to lifelong elevations in low-density lipoprotein-cholesterol, xanthomatosis, and premature atherosclerotic cardiovascular disease. Several large scale patient registries have proliferated around the world and provide real-world data on prevalence and current treatment patterns. In this way, they have highlighted major gaps in the identification, treatment, and follow-up of patients with FH. Regrettably, these registries reveal a consistent and sobering message - patients with FH either remain undiagnosed or receive delayed diagnosis, there are low rates of LDL-C goal attainment even with combination lipid-lowering therapy, and rates of atherosclerotic cardiovascular disease are remarkably higher than the general population. Currently, there are well-developed FH registries in the Netherlands, United Kingdom, Spain, France, Norway, Brazil, Canada, and the United States. Notably absent from this list is the entirety of the Asian continent. As collaborating U.S. investigators who have clinical and research experience with FH and FH registries, we encourage the clinical research leadership of Thailand to design and launch a national FH Registry and genetic biorepository. Besides serving as a tool to advance the science of FH, particularly as it relates to the Thai population, this effort will undoubtedly raise awareness and lead to more efficient diagnosis and treatment, the true role of a registry. The opportunity for Thailand is enormous.

**Keywords :** FH, clinical registry, low-density lipoprotein, atherosclerotic cardiovascular disease, genetic testing, cascade screening, cholesterol, coronary disease

Familial hypercholesterolemia (FH) is the most common monogenic disorder in humans. While numerous genes have been implicated in FH, all known underlying genetic defects lead to impaired clearance of low-density lipoprotein (LDL) particles from the circulation with subsequent severe hypercholesterolemia. Without diagnosis and appropriate treatment, patients are at dramatically increased risk of premature atherosclerotic cardiovascular disease (ASCVD).[1]

This first clinical description of FH was provided by the Norwegian physician, Carl Müller, in 1938 as elevated levels of serum cholesterol together with tendon xanthomas and coronary artery lesions.[2] In 1964, Khachadurian demonstrated the autosomal dominant inheritance pattern of FH.[3] A decade later, Brown and Goldstein made their seminal discovery of LDLR and its feedback regulation. Their work was largely inspired by a young patient with homozygous FH, the more severe form of the condition in which casual mutations are inherited from both parents, who had a heart attack during childhood.[4,5] Building on that work, they identified *LDLR* as the causative gene for FH.[6] As it turned out, the causal mutations in families with pathognomonic clinical presentations such as xanthomas, corneal arcus, and early onset ASCVD most commonly were found to be highly penetrant (co-dominant) mutations that completely abolished or greatly diminished *LDLR* function.[1]

## Pathophysiology and genetics

FH is caused by mutations in genes encoding key proteins involved in the *LDLR* endocytic and recycling pathways and leading to both reduced *LDLR*-mediated endocytosis and severe hypercholesterolemia.[1] Plasma cholesterol is mostly manufactured, exported, and eventually recaptured by hepatocytes. Cholesterol synthesis is a complex, multistep and highly regulated pathway with 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase being the key, rate-limiting enzyme. Statins antagonize the activity of HMG CoA reductase thereby reducing hepatic cholesterol synthesis and upregulating the transcription of *LDLR* via a sensing mechanism linked to the sterol regulatory element binding protein pathway. The main objective of each cell, and a hepatocyte in particular, is to keep membrane cholesterol close to 5% of total cell mass, a critical concentration that assures proper membrane function. It is thus evident that the cell uses a series of quick adjustments to respond to increases and decreases in membrane cholesterol straying from the critical value range. These include synthetic, assembly, secretory, and re-uptake activities. The lipid cargo, mostly triglycerides and cholesterol, is packaged within apolipoprotein B *(APOB)*-containing very low-density lipoproteins (VLDL), the intravascular precursors of LDL, which primarily transport triglycerides from the liver to peripheral tissues, with cholesterol joining for the ride, so to speak. Over 15,000 molecules of triglycerides are packed into one VLDL, which contains over 1,000 cholesterol molecules as well. Receptor mediated endocytosis is facilitated by the binding of APOB on the LDL particle to the *LDLR* and coordinated by an adaptor protein (LDLRAP) that positions *LDLR* on the sinusoidal side of the polarized hepatocyte, clustered in coated pits.[7] After *LDLR* -mediated endocytosis, the LDL/*LDLR* complex is transported to the endosomal pathway to merge with the lysosome. The pH gradient in the descent toward the lysosome induces dissociation between receptor and cargo. In the lysosome, the LDL particle is digested and the cholesterol and triglycerides are de-esterified for transport into the cytosol, where they can take on myriad fates. On the other hand, the *LDLR* is recycled back to the hepatocyte surface to participate in many more rounds of LDL binding and endocytosis. However, when the *LDLR* eventually meets a different ligand, the low abundance proprotein convertase subtilisin/kexin type 9 *(PCSK9),* the normal recycling loop is short-circuited as *PCSK9* disables the *LDLR* from escaping lysosomal digestion, thereby reducing cell surface receptor density. It must be noted that up to half of plasma *PCSK9* is associated with the LDL particle, for a frequency of one *PCSK9* molecule for every 500–1000 LDL particles.[8,9] This introduces the intriguing possibility that the carefully orchestrated cellular regulation of cholesterol concentration is ultimately under the whims of a stochastic extracellular system, where every few hundred encounters with canonical LDL the *LDLR* meets its fate by interacting with a *PCSK9*-carrying LDL that terminates the receptor's life cycle.

The vast majority of FH can be explained by mutations in three key genes; *LDLR* (receptor not synthesized or not functional), *APOB* (ligand not properly recognizing *LDLR*), and *PCSK9* (gain of function mutations causing excessive elimination of *LDLR* LR). In those with detectable mutations, heterozygous *LDLR*, *APOB*, and *PCSK9* mutations are found in >90, ~5, and ~1%, respectively.[10] Clinically homozygous FH results from true homozygous, or more often from compound heterozygous, mutations in either these same genes or in *ARH*, which codes for LDLRAP1, the adaptor that places *LDLR* on the sinusoidal side of the hepatocyte, and is inherited in an autosomal recessive fashion.[11] The prevalence of individual mutations varies geographically, though most studies point to a frequency ranging from 1:200 to 1:500 births, depending on whether a founder effect is in place (i.e., French Canadians, South Africans, and Ashkenazi Jews).[1] Although there have been reports of *LDLR* mutations in Thai patients, the spectrum and prevalence of various mutations associated with FH in Thailand and the whole of South East Asia is not known and should be prioritized as a target of investigation.[12]

## Challenges with diagnosis

The two most widely used clinical diagnostic criteria for identifying FH are the UK Simon Broome and Dutch Lipid Clinic Network (DLCN) criteria (see Table). Both the Simon Broome and DLCN include clinical, laboratory, and genetic variables. Both algorithms heavily weight the results of genetic testing and the presence or absence of tendon xanthomata, and prioritize specificity (true negative rate) over sensitivity (true positive rate), which means they are more useful in the context of cascade screening rather than index case identification (more on this below). Fortunately, the medical community is advancing a more sophisticated view on the diagnosis of FH. First, both secular trends and improvements in therapy have altered the 'classic' presentation of FH. For example, the prevalence of xanthomas today is lower than observed decades ago, perhaps due to improved control of cholesterol from an earlier age. The SAFEHEART registry demonstrated the presence of tendon xanthomas in only 14% of 2,752 individuals with genetically confirmed FH.[13,14] These findings have been recapitulated in FH registries based in both United States and Canada.[15]

Furthermore, both the Simon Broome and the Dutch Lipid Clinic Network Criteria (DLNC) rely greatly on a family history of premature ASCVD. This slavish reliance on family history is now problematic given the statinization of large swaths of the developed world. Entire generations have received statin therapy, thus dramatically attenuating the utility of the family history data. Moreover, family history information is also often difficult to obtain or is unreliable, at least in the western world, due to high divorce rates, which now occurs in approximately 50% of U.S. marriages, and to high rates of adoption. Finally, secular trends in the U.S., including decreased saturated fat intake and the ubiquitous prescription of statins have led to decreases in average LDL-C levels across the population in general, making LDL-C less useful as a diagnostic criterion.[16] Recognizing the significant limitations of the clinical diagnostic algorithms, the American Heart Association sponsored a scientific statement that discusses these issues and endorses a new and more practical


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**Table 1:** Diagnostic criteria for Familial Hypercholesterolemia according to the Dutch Lipid Clinic Network

| Criteria | Score |
|---|---|
| Family History | |
| First-degree relative with premature atherosclerotic cardiovascular disease (men ≤ 55 years, women ≤ 60 years), OR | 1 |
| First-degree relative with known LDL-C ≥ 95$^{th}$ percentile for age and sex | |
| First-degree relative with tendon xanthomata and/or corneal arcus, OR | 2 |
| Child aged ≤ 18 years with known LDL-C ≥ 95$^{th}$ percentile for age and sex | |
| Clinical History | |
| Patient with premature coronary artery disease (age as above) | 2 |
| Patient with premature cerebral or peripheral vascular disease (age as above) | 1 |
| Physical Examination | |
| Tendon Xanthomas | 6 |
| Corneal arcus at age ≤ 45 years | 4 |
| LDL Cholesterol (mg/dL) | |
| LDL-C ≥ 330 | 8 |
| LDL-C 250 – 329 | 5 |
| LDL-C 190 – 249 | 3 |
| LDL-C 155 - 189 | 1 |
| DNA Analysis – functional mutation *LDLR, APOB,* and *PCSK9* | 8 |
| Stratification | Total Score |
| Definite Familial Hypercholesterolemia | > 8 |
| Probable Familial Hypercholesterolemia | 6-8 |
| Possible Familial Hypercholesterolemia | 3-5 |
| Unlikely Familial Hypercholesterolemia | < 3 |

*LDL-C = low-density lipoprotein-cholesterol; *LDLR* = low-density lipoprotein receptor; *APOB* = apolipoprotein B ; *PCSK9* = proprotein convertase/subtilisin kexin type 9

clinical tool for making a diagnosis of FH.[17] These diagnostic criteria are not likely to demonstrate favorable diagnostic characteristics in Asian populations, where the phenotype from the genetic component is less amplified by lifestyle and co-morbid factors compared to patients in the western world. Thus, there is a large unmet need to develop diagnostic criteria for the diagnosis of FH in specific populations, particularly those of South East Asia, due to relative ethnic homogeneity and uniqueness.

## The promise of genetic testing

### *New causal gene identification*

The role of genetic testing in the diagnosis and screening of FH remains controversial. There are some parts of the world (primarily Western Europe), where this is considered standard of care and is covered by payers without patients having to endure part or all of the financial burden. In the U.S., as well as most parts of the world, genetic testing is employed sporadically and inconsistently, and rarely covered by health insurance. Mutations in *LDLR* represent, by far and away, the most common causal defects in FH. However, pathognomonic phenotypic expression in patients and families without mutations in *LDLR* enabled discovery of other less common causal abnormalities in genes of the *LDLR* clearance pathway, including *APOB*, *PCSK9*, APOE, and *LDLRAP.*

Causal mutations are now identified in approximately 70–80% of individuals with a definite phenotypic diagnosis of FH and 20–40% in those with a possible/probable diagnosis of FH.[18,19] However, a negative genetic test does not exclude a diagnosis of FH, especially in those with a strong clinical phenotype.[20] Nevertheless, a sizable proportion of individuals with the severe hypercholesterolemia phenotype who are found to be negative by genetic testing have polygenic hypercholesterolemia.[21] Polygenic hypercholesterolemia is an FH phenocopy that is due to the accumulation of a number of more common LDL-C raising single nucleotide variants at different loci, though its inheritance does not follow a clear Mendelian pattern.[18,21,22]

### *FH is more common than previously recognized*

The original estimates of the prevalence of FH suggested that it occurred in approximately one in 500 individuals among the free-living population in areas where FH-causing mutations are not derived from a recent founder effect. This approximation was based on the original estimate of the prevalence of homozygous FH, which was originally thought to occur in one in a million individuals.[17] Recent unbiased genetic screening of large populations has clearly demonstrated that the prevalence of FH is twice as common as previously thought, with estimates in western populations of 1:200-1:250.[20,23-25] The higher

prevalence noted in these studies is, at least in part, due to the fact that genetic screening detects individuals with milder disease.[24] We do not know the prevalence of FH in Asian countries, and Thailand is well poised to lead the way and produce a formal prevalence study for both genotypic and phenotypic FH, either as unbiased epidemiologic investigation or as a registry.

## International FH registries

Despite its high risk status, FH remains underdiagnosed and undertreated. Fortunately, several large scale patient registries have proliferated around the world. These registries have and continue to provide real-world data on prevalence and current treatment patterns. In this way, they have highlighted major gaps in the identification, treatment, and follow-up of patients with FH. Importantly, longitudinal registry-level data provide insight into how trends in FH diagnosis and management are changing over time. Additionally, country specific registries allow comparison of these findings amongst different social, cultural, and ethnic groups. As in many other conditions, FH is quite heterogeneous and optimal methods for screening, diagnosis, evaluation, and treatment are likely to be different across the globe. Currently, there are well-developed FH registries in the Netherland, United Kingdom, Spain, France, Norway, Brazil, Canada, and the United States. Notably absent from this list is the entirety of the Asian continent. The opportunity for Thailand is enormous.

Despite differences in scale and approach amongst international registries, several common themes emerge. Not surprisingly, there is a strikingly higher risk of ASCVD in individuals with FH patients than in the general population.[15] Consistent with that observation, it was shown that the prevalence of FH among patients with coronary artery disease is 8.3% and, not surprisingly, inversely related to age.[26] Furthermore, the majority of patients with FH fail to attain optimal LDL-C levels despite the use of combination lipid-lowering therapy in the pre-*PCSK9* inhibitor era. Given the current low rate of insurance approvals for *PCSK9* agents in the U.S., the situation overall has not drastically changed for FH patients in the nearly three years since the approval of this new class of agents.[27]

Other noteworthy, country-specific observations from these registries include the following:

1. **Spain** (SAFEHEART): There were 2,752 genetically confirmed FH patients with median age of 44 years old. The prevalence of ASCVD of this FH cohort was 13%, 3–4-fold higher than their unaffected relatives. The majority (71.8%) were on maximal lipid lowering therapy but only 11.2% attained an LDL-C <100 mg/dL.[14,28]
2. **Norway:** The mean age of hospitalization for cardiovascular disease was 45.1 years old compared to 64.9 years old in the general population.[29] Cardiovascular disease was the most common cause of death (42.3%) and significantly higher compared with the general population for those less than 70 years of age. Standardized for age groups, the out-of-hospital risk of CVD deaths was increased by 12-fold among those 20–29 years of age.[30]
3. **CASCADE FH (U.S.):** CASCADE FH demonstrated that individuals with FH received delayed diagnoses (median age 47 years old) and treatment (lipid-lowering therapy initiated at 39 years old). Prevalent ASCVD was reported in 36% of the cases, a 5–7-fold higher prevalence of ASCVD in comparison with the overall U.S. population (NHANES cohort).[15] Only 25% of FH patients in the U.S. achieve an LDL-C <100mg/dL.
4. **Brazil:** This registry included 818 individuals with genetically confirmed FH. Again, prevalence of ASCVD was high. Accordingly, after 1-year follow up, the CVD event rate was 5.7%, and in 29.7% of cases, these were fatal events. There was a doubling of the incidence of nonfatal and fatal CVD events in index cases compared with affected relatives.[31]

In summary, patients with FH either remain undiagnosed or receive delayed diagnosis, there are low rates of LDL-C goal attainment even with combination lipid-lowering therapy, rates of ASCVD are remarkably higher than the general population and unaffected relatives, and genetic testing (in general exquisitely underutilized worldwide) may play a role in early detection, treatment, and cascade screening.

## Genetic registry data

Some, but not all, FH registries collect genetic data. Analysis of these datasets has revealed intriguing relationships between genotype and phenotype. That is, specific mutations convey more precise prognostic information, though the relationship is not wholly deterministic. Complete loss of function mutations in *LDLR* completely abolish *LDLR* activity and are therefore associated with more severe elevations in plasma LDL-C and higher rates of ASCVD. In this regard, recent data has demonstrated that there is a graded response in phenotype between complete loss of function mutations, missense mutations that were predicted to be deleterious by multiple algorithms, and those that were predicted to be less pathogenic or nonpathogenic.[24] A more recent analysis demonstrated that, when compared to a reference group with LDL-C less than 130 mg/dl and no causative FH mutations, individuals with LDL-C at least 190 mg/dl and no causative FH mutations had six-fold higher risk for whereas those with LDL-C at least 190 mg/dl as well as causative FH mutations had a 22-fold increased risk.[23] Thus, the presence of a defined mutation conveys increased risk, likely because it implies certain lifelong exposure to elevated LDL-C. Additionally, they demonstrated that differences in mutation severity impacts the severity of hypercholesterolemia and prognosis. In general, mutations in *APOB* and *PCSK9* mutations are associated with a more modest phenotype and more variable presentation, since the *LDLR* itself is genetically intact and functional.[32]

Large genetic biorepositories linked to FH registry information will be vital to more completely clarify genotype-phenotype correlations. This approach allows evaluation of those who are genotype(-)/phenotype(+), eg, those with the


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severe hypercholesterolemia phenotype but who have no mutation identifiable upon genetic testing. Evaluation of these individuals is critical for identification of additional causal genes and mechanisms for hypercholesterolemia.

It is important to appreciate that for any specific mutation, there is large heterogeneity in the phenotype amongst affected family members. To that end, there are some with known FH mutations who do not manifest severe hypercholesterolemia or ASCVD. On the other hand, there are others with mutations predicted to be less deleterious but sustain severe hypercholesterolemia and early ASCVD. Some of this phenotypic heterogeneity is due to environmental influences. However, there are likely a large number of modifier genes, such as LPA, which codes for lipoprotein(a).[33] Lipoprotein(a) is a highly atherogenic LDL-like particle covalently bound to a truncated form of plasminogen and its levels are, for the most part, genetically determined. Lipoprotein(a) is an independent predictor of ASCVD in those with FH and thus, when elevated, portends a worse prognosis.[33] Currently, there are concerted efforts to identify other modifier genes through large-scale genome wide or exome wide sequencing initiatives. These modifier genes may be ethnically and geographically specific, which again underscores the importance of developing an FH registry and biobank in Thailand.

## The case for genetic testing

Cascade screening (eg, lipid and/or genetic testing all first-degree relatives of an FH proband) has proved to be a cost-effective method for identifying new cases of FH.[26,34-37] This approach allows for family wide diagnosis and initiation of appropriate treatment.[34,38] Cascade genetic testing increases diagnostic accuracy versus strategies that use LDL-C alone since there can be an overlap in LDL-C levels on those with and without FH.[35,39] Detecting an FH mutation provides unequivocal confirmation that the vasculature has been exposed to high LDL-C levels since birth. Cascade testing strategies incorporating genetic testing have been shown to be highly cost-effective when pathogenicity is certain. Countries that have been using genetic cascade screening such as the Netherlands, Norway, and Spain have been successful in identifying large numbers of FH patients.[1] In the Netherlands, those who received a positive test result had lower LDL-C levels compared to those without positive genetic testing due to timely initiation of lipid lowering therapy.[40]

On the basis of these data, cascade screening of close relatives for FH has been recommended in the U.K. by the National Institute for Health and Clinical Excellence. In the U.S., the Center for Disease Control Office of Public Health Genomics classifies FH as a tier 1 condition for cascade genetic screening. In most of the world, large-scale genetic testing and screening has not been performed given economic constraints. However, given dramatic improvements in sequencing technology, the cost of genetic testing has plummeted and continues to decline, even beyond what Moore's law predicts. It is likely that genetic testing/screening for FH will become standard care within the next decade in most parts of the world.

## Conclusion

FH is the most common monogenic disorder in humans. New unbiased genetic data suggest that FH is far more common than previously thought with a prevalence as high as 1:200 births. Left undiagnosed and treated it leads to accelerated ASCVD. Regrettably, most individuals with FH are not aware of their condition and do not receive optimal evaluation and/or treatment, leading to unacceptably high morbidity and mortality. With greater awareness, this readily identifiable condition can be functionally eliminated by early screening and aggressive lipid lowering therapy. International registries have allowed us to greatly enhance our knowledge of FH, and in fact, have been the seed for numerous genetic insights, improved diagnostics, and refined therapeutic approaches. Sadly, most countries do not have a national registry and rely on data derived from populations that are genetically heterogeneous and very distinct. As collaborating investigators in the largest U.S. based FH registry, we encourage the leadership of clinical research in Thailand to take the first steps in bringing a national registry to life. At the practice level, we encourage the clinician to keep in mind the signs of FH and make the appropriate diagnosis based on physical and biochemical data, family history, and consider genetic testing when feasible.

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