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HUMAN GENOME EPIDEMIOLOGY (HuGE) REVIEWS

Genetic Causes of Monogenic Heterozygous Familial Hypercholesterolemia: A HuGE Prevalence Review

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The clinical phenotype of heterozygous familial hypercholesterolemia (FH) is characterized by increased plasma levels of total cholesterol and low density lipoprotein cholesterol, tendinous xanthomata, and premature symptoms of coronary heart disease. It is inherited as an autosomal dominant disorder with homozygotes having a more severe phenotype than do heterozygotes. FH can result from mutations in the low density lipoprotein receptor gene (*LDLR*), the apolipoprotein B-100 gene (*APOB*), and the recently identified proprotein convertase subtilisin/kexin type 9 gene (*PCSK9*). To date, over 700 variants have been identified in the *LDLR* gene. With the exception of a small number of founder populations where one or two mutations predominate, most geographically based surveys of FH subjects show a large number of mutations segregating in a given population. Studies of the prevalence of FH would be improved by the use of a consistent and uniformly applied clinical definition. Because FH responds well to drug treatment, early diagnosis to reduce atherosclerosis risk is beneficial. Cascade testing of FH family members is cost effective and merits further research. For screening to be successful, public health and general practitioners need to be aware of the signs and diagnosis of FH and the benefits of early treatment.

APOB; epidemiology; genetics; hypercholesterolemia, familial; *LDLR*; receptors, LDL

Abbreviations: FH, familial hypercholesterolemia; LDL, low density lipoprotein.

Editor's note: *This article is also available on the website of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/reviews.htm).*

DISEASE

Familial hypercholesterolemia

Familial hypercholesterolemia (FH) has a rich history in the field of genetic epidemiology. In the late 1930s, Müller

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TABLE 1. US MedPed Program diagnostic criteria for familial hypercholesterolemia*

	Total cholesterol cutpoints (mmol/liter)			
	First-degree relative with FH†	Second-degree relative with FH	Third-degree relative with FH	General population
Age (years)				
<20	5.7	5.9	6.2	7.0
20–29	6.2	6.5	6.7	7.5
30–39	7.0	7.2	7.5	8.8
≥40	7.5	7.8	8.0	9.3
Diagnosis (FH is diagnosed if total cholesterol levels exceed the cutpoint)				

* Williams et al. Diagnosing heterozygous familial hypercholesterolemia using new practical criteria validated by molecular genetics. Am J Cardiol 1993;72:171–6 (8).

† FH, familial hypercholesterolemia.

(1) characterized the family clustering of xanthomata, high cholesterol, and myocardial infarctions and postulated a single gene inheritance. In the 1960s, Khachadurian (2) carefully examined the phenotypes segregating in several large families in Lebanon. He clarified the distinction between the heterozygote and homozygote forms of FH and confirmed that the pedigree structures were consistent with the dominant inheritance of a single gene. At about the same time, Fredrickson et al. (3) demonstrated that the FH phenotype is related to improper metabolism of low density lipoproteins (LDLs). In the 1970s, the combined work of Ott et al. (4), Elston et al. (5), and Berg and Heiberg (6) showed genetic linkage between the FH phenotype and the third component of complement (*C3*), a marker known to be located on chromosome 19. Brown and Goldstein (7) built on this work and demonstrated that the clinical FH phenotype can be caused by mutations in the LDL receptor gene (*LDLR*). The clinical phenotype is more severe for homozygotes than heterozygotes. Because homozygotes are so rare and because the more frequent heterozygous condition has greater public health impact, this review will focus on the heterozygous form. Unless otherwise noted, the term "familial hypercholesterolemia" and the abbreviation FH will refer to the heterozygous form.

Diagnostic criteria for FH

Three groups have developed diagnostic tools for FH: The US MedPed Program, the Simon Broome Register Group in the United Kingdom, and the Dutch Lipid Clinic Network. The MedPed criteria use cutpoints for total cholesterol levels specific to an individual's age and family history (8). That is, the cutpoints differ for individuals with first-, second-, or third-degree relatives with FH and for the general population, because individuals with a relative with FH have a higher prior probability of having an FH-causing mutation. For example, as seen in table 1, the cutpoint for an individual under 20 years of age with a second-degree relative with FH would be 5.9 mmol/liter. The levels were derived from mathematical modeling using published cholesterol levels for FH individuals in the United States and Japan (9–12). In a validation study of these criteria using five large Utah families with DNA-verified mutations, the observed specificity was 98 percent and the sensitivity was 87 percent for first-degree relatives (8). The Simon Broome Register criteria for FH include cholesterol levels, clinical characteristics, molecular diagnosis, and family history (table 2) (13). A "definite" diagnosis of FH is made if a patient has elevated cholesterol levels (note that the cutpoint differs for children under the age of 16 years) and tendinous xanthomata, or if the patient has an identified mutation in the *LDLR* gene or the apolipoprotein B-100 gene (*APOB*). A "probable" diagnosis is made if the patient has elevated cholesterol levels and a family history of hypercholesterolemia or heart disease (13, 14). The Dutch Lipid Clinic Network criteria are similar to the Simon Broome Register criteria (table 3) (15). "Points" are assigned for family history of hyperlipidemia or heart disease, clinical characteristics such as tendinous xanthomata, elevated LDL cholesterol, and/or an identified mutation. A total point score of greater than eight is considered "definite" FH, 6–8 is "probable" FH, and 3–5 is "possible" FH. Although the Simon Broome Register criteria consider a molecular diagnosis as evidence for definite FH, the Dutch Lipid Clinic Network requires that at least one other criterion be met in addition to molecular diagnosis.

Although these diagnostic tools do provide a standardization of the FH phenotype, use of these tools will not necessarily result in consistent sensitivity ("true positives") and specificity ("true negatives") of FH diagnosis across populations. For example, cholesterol levels for FH patients overlap with that of the general population, and use of cholesterol levels alone results in false positive and false negative rates of 8–18 percent (16, 17). Sensitivity and specificity can be improved if age-, gender-, and population-specific cutpoints are used (18). In one study, cutoff points were developed based on LDL cholesterol levels in Finnish FH cases with a DNA-verified mutation. The resulting criteria had 98 percent sensitivity and 93 percent specificity for diagnosing Finnish subjects aged 1–25 years (19). Further, the criterion of a family history of premature heart disease used by the Simon Broome Register and Dutch Lipid Clinic Network groups will be influenced by the prevalence of coronary heart

TABLE 2. Simon Broome Familial Hypercholesterolemia Register diagnostic criteria for familial hypercholesterolemia*

	Description
Criteria	
a	Total cholesterol concentration above 7.5 mmol/liter in adults or a total cholesterol concentration above 6.7 mmol/liter in children aged less than 16 years, or
	Low density lipoprotein cholesterol concentration above 4.9 mmol/liter in adults or above 4.0 mmol/liter in children
b	Tendinous xanthomata in the patient or a first-degree relative
c	DNA-based evidence of mutation in the *LDLR* or *APOB* gene
d	Family history of myocardial infarction before age 50 years in a second-degree relative or before age 60 years in a first-degree relative
e	Family history of raised total cholesterol concentration above 7.5 mmol/liter in a first- or second-degree relative
Diagnosis	
A "definite" FH† diagnosis requires either criteria *a* and *b* or criterion *c*	
A "probable" FH diagnosis requires either criteria *a* and *d* or criteria *a* and *e*	

* Risk of fatal coronary heart disease in familial hypercholesterolemia. Scientific Steering Committee on behalf of the Simon Broome Register Group. BMJ 1991;303:893–6 (13); Mortality in treated heterozygous familial hypercholesterolaemia: implications for clinical management. Scientific Steering Committee on behalf of the Simon Broome Register Group. Atherosclerosis 1999;142:105–12 (14).

† FH, familial hypercholesterolemia.

disease in the population. Specificity will be lower in areas such as northern Europe and North America where coronary heart disease is more common (18). In addition, some of the criteria (e.g., tendinous xanthomata and heart disease) are manifest only later in life and, therefore, will have limited clinical utility for diagnosis in younger patients and/or relatives. Further development of a consistent and uniformly applied definition of FH would facilitate interpretation of studies that screen for genetic mutations in patients with FH and would better identify individuals for whom treatment is indicated.

Frequency of the clinical phenotype

The frequency of FH in Caucasian populations is often reported as 1/500 (0.2 percent) (20). This estimate was based on the frequency of FH in survivors of myocardial infarction in the United States, and it is supported by a study of myocardial infarction survivors in the United Kingdom (21) and by a study from the United Kingdom that determined the prevalence of homozygous individuals and then used the Hardy-Weinberg equation to calculate the heterozygous frequency (22). A similar frequency of FH has been estimated in four other countries: three patients with FH were observed among 2,700 consecutive outpatients at a lipid clinic in Japan (0.11 percent) (23); 134 individuals with xanthomata were identified in Ostford County, Norway, in 1969, resulting in an estimated frequency of FH of 0.22 percent (24); 11 infants were diagnosed with FH in a screening of 10,440 Danish newborns (0.11 percent) (25); and 39 FH heterozygotes were identified in a survey of physicians' records for 21,000 individuals in Hungary (0.19 percent) (26). Additionally, Neil et al. (27) estimated the frequency of diagnosed FH in Oxfordshire, United Kingdom, by age. The prevalence was similar to that of other studies for males aged 50–59 years (0.11 percent) and females aged 60–69 years (0.18 percent). However, their overall estimated prevalence (0.054 percent) was much lower because of underdiagnosis in the younger age groups. With the exception of the Danish study (25), each of these studies measured population prevalence rather than birth prevalence. Some of the variation in these estimated frequencies may result from the indirect methods used for estimation or from differences in the criteria used to identify individuals with FH.

As shown in table 4, the frequency of heterozygous FH is considerably higher than 1/500 in some populations, and the elevated frequency is generally attributed to a founder effect. A founder effect occurs when a subpopulation is formed through the immigration of a small number of "founder" subjects, followed by a population expansion. If, by chance, some of the founders had FH, then genetic drift could lead to a high proportion of affected subjects who share specific mutations introduced by the founders. Such founder effects are thought to influence the spectrum of FH mutations in French Canadians (28); South African Afrikaners (29), Jews (30), and Indians (31); Tunisians (32); Christian Lebanese (22); Icelanders (33); and Finns (34) (for review, see the

TABLE 3. Dutch Lipid Clinic Network diagnostic criteria for familial hypercholesterolemia*

	Points
Criteria	
Family history	
First-degree relative with known premature (men: <55 years; women: <60 years) coronary and vascular disease, or	
First-degree relative with known LDLC† above the 95th percentile	1
First-degree relative with tendinous xanthomata and/or arcus cornealis, or	
Children aged less than 18 years with LDLC above the 95th percentile	2
Clinical history	
Patient with premature (men: <55 years; women: <60 years) coronary artery disease	2
Patient with premature (men: <55 years; women: <60 years) cerebral or peripheral vascular disease	1
Physical examination	
Tendinous xanthomata	6
Arcus cornealis prior to age 45 years	4
Cholesterol levels (mmol/liter)	
LDLC, ≥8.5	8
LDLC, 6.5–8.4	5
LDLC, 5.0–6.4	3
LDLC, 4.0–4.9	1
DNA analysis	
Functional mutation in the *LDLR* gene	8
Diagnosis (diagnosis is based on the total number of points obtained)	
A "definite" FH† diagnosis requires more than 8 points	
A "probable" FH diagnosis requires 6–8 points	
A "possible" FH diagnosis requires 3–5 points	

* World Health Organization. Familial hypercholesterolemia—report of a second WHO Consultation. Geneva, Switzerland: World Health Organization, 1999. (WHO publication no. WHO/HGN/FH/CONS/99.2). (15).

† LDLC, low density lipoprotein cholesterol; FH, familial hypercholesterolemia.

article by Goldstein et al. (35)). These founder populations have a frequency of FH ranging from 1/411 (0.24 percent) for North Karelians of Finland (19) to 1/67 (1.5 percent) for Ashkenazi Jews in South Africa (30). Currently, the population frequency has not been estimated for Iceland (33) or for the general Finnish population (34).

GENES

It has been known since the 1970s that the FH phenotype results from mutations in the *LDLR* gene (36, 37). This gene spans 45 kilobases, has 18 exons (38), and maps to the short arm of chromosome 19 at 19p13.1-p13.3 (39). The 860-amino acid LDL receptor protein functions to remove LDL from plasma. It has served as an important model in studies of cell surface receptor molecules (7, 35). For example, Rudenko et al. (40) recently determined the crystal structure of the LDL receptor protein. They showed that, at low pH, the epidermal growth factor precursor domain of the molecule folds back to interact with the binding site, potentially displacing the lipoprotein. This proposed mechanism for ligand release in the endosome may serve as a paradigm for receptor-mediated endocytosis (41).

Research in the late 1980s demonstrated that the same clinical phenotype could also be due to mutations in the *APOB* gene (42, 43). The 29-exon *APOB* gene spans 43 kilobases and is located on chromosome 2p23-24 (44–46). The resulting 4,536-amino acid protein is the only protein component of LDL particles and serves as the ligand for the LDL receptor protein (47). The disorder resulting from mutations in this gene has been termed "familial defective apolipoprotein B-100" (43).

Additional genes are known to contribute to monogenic elevated plasma LDL cholesterol. Research in the last 4 years has identified two loci known to cause recessive forms of hypercholesterolemia (48–51). In 1973, Khachadurian and Uthman (52) first described what is now termed "autosomal recessive hypercholesterolemia" (53). The LDL cholesterol levels of autosomal recessive hypercholesterolemia homozygotes are typically intermediate between

those of FH heterozygotes and FH homozygotes (54, 55). Autosomal recessive hypercholesterolemia heterozygotes have lipid levels similar to those of the general population, but further epidemiologic studies are needed to examine long-term disease risk in this population (55, 56). Autosomal recessive hypercholesterolemia is most frequently found in individuals living on the island of Sardinia, Italy (54). The autosomal recessive hypercholesterolemia gene (*ARH*), which has been localized to chromosome 1p35 (48, 49), codes for a 308-amino acid putative adaptor protein. Sitosterolemia, another rare recessive hypercholesterolemic condition, was also first characterized in the early 1970s (57). It differs from the other hypercholesterolemias described here in that affected individuals have an increased accumulation in the plasma of plant sterols such as sitosterol (58). It is now known that mutations in genes for two adenosine triphosphate-binding cassette transporters *ABCG5* and *ABCG8* (51, 59) localized to 2p21 (50, 51) cause this disorder. Finally, two studies (60, 61) have identified a putative third autosomal dominant locus (designated *FH3*) on chromosome 1p32. Both used a genome-wide scan in families where the *LDLR* locus and the *APOB* locus had been excluded. The gene determining the phenotype has recently been identified as proprotein convertase subtilisin/kexin type 9 (*PCSK9*), and the protein has been identified as neural apoptosis regulated convertase (NARC-I) (62).

Most of the available epidemiologic data on FH focus on the *LDLR* and *APOB* genes, since these genes have been studied the longest and are responsible for the majority of cases of FH. Therefore, this review will focus on variants in these two genes. Although exact proportions are not known, more FH cases are associated with mutations in *LDLR* than with mutations in *APOB* (35).

GENE VARIANTS

LDLR gene and *APOB* gene variants

As of July 1, 2003, over 700 *LDLR* variants have been identified in subjects with FH, and extensive reviews of these gene variants have been previously published (63, 64). In addition, all gene variants for *LDLR* are compiled online at two websites: http://www.ucl.ac.uk/fh/ (65) and www.umd.necker.fr/LDLR/research.html (66). It is worth noting that not all of these variants are known to be functional mutations.

Figure 1 shows the distribution of variants reported in the United Kingdom database (65) across the promoter and 18 exons of *LDLR*. The exon organization corresponds to the LDL receptor protein domain structure (67). Functional *LDLR* mutations have been classified into five classes based on biosynthetic and functional studies of fibroblast cell strains (35, 67). Class 1 mutations are disruptions of the promoter sequence, nonsense, frameshift, or splicing mutations, which result in no protein synthesis (null alleles). Class 2 mutations that primarily occur in the ligand-binding and epidermal growth factor precursor regions disrupt transport of the LDL receptor from the endoplasmic reticulum to the Golgi apparatus. Class 3 mutations interfere with cell surface binding of the receptor to LDL, and these mutations are also primarily found in the ligand-binding and epidermal growth factor precursor regions. Class 4 mutations appear in the cytoplasmic domain or the cytoplasmic and membrane-spanning domains. They inhibit the clustering of LDL receptors on the cell surface, so that the bound LDL particle is not internalized. Class 5 mutations prevent the release of LDL particles in the endosome and, as a result, the LDL receptor is not recycled to the cell surface. Class 5 mutations cluster in the epidermal growth factor precursor region (35, 67).

As seen in figure 1, a large number of variants have been reported in exon 4. This high frequency is partly explained by the large size of the exon, but it is also likely to be due to selection bias. That is, individuals with functional mutations in this region may be overrepresented in the lipid clinic populations surveyed for FH screening (68, 69) because of the high penetrance of these mutations. Exons 2–6 code for the binding domain of the LDL receptor, which comprises seven imperfect repeats of 40 amino acids (35). Exon 4 codes for repeat 5, a repeat required for both LDL binding via apolipoprotein B and very low density lipoprotein binding via apolipoprotein E. Mutations in this region have been shown to be associated with a more severe phenotype than have mutations located in other regions (68), a finding supported by the recent detection of a *LDLR* deletion eliminating exon 4 cosegregating with severe hypercholesterolemia and premature heart disease in a Swiss family (70).

Over 80 deletions and duplications have also been identified in *LDLR*, as tabulated on the two websites. These major rearrangements are thought to comprise 5 percent of FH mutations in genetically heterogeneous populations (71). The breakpoints span the gene, but a majority are located in introns 1–8 and intron 12 through the 3′-untranslated region (65). This pattern corresponds to the distribution of repeat sequences in *LDLR*. That is, the *LDLR* gene has a higher frequency of *Alu* sequences than do other genes, and these repeat sequences are also concentrated in introns 1–8 and intron 12 through the 3′-untranslated region (38).

In contrast to the large number of variants identified in the *LDLR* gene, only a few variants have been characterized in the *APOB* gene (42, 72–75). The *R3500Q*, *R3500W*, and *R3531C* variants have been shown to reduce binding of LDL in vitro (73, 76). However, *R3531C* is not consistently found to be associated with hypercholesterolemia (77–79). *R3500Q* is the result of a G-to-A transition at nucleic acid 10708, resulting in a substitution of glutamine for arginine in codon 3500 (42), whereas *R3500W* is a G-to-T transition at the same location, resulting in a substitution of tryptophan (80). Interestingly, these mutations are not located at the LDL receptor-binding site (residues 3359–3369). Instead, an *R3500-W4369* interaction is necessary to ensure the proper conformational shape of the apolipoprotein B protein, and mutations in these key amino acids result in improper protein folding and reduced receptor binding (81).

Prevalence of *LDLR* and *APOB* variants

Four studies have estimated the frequency of *APOB* variants through population-based screening (table 5). Studies of 5,160 bank employees in California (82), 9,255 participants in the Copenhagen City Heart Survey (78), and 5,000

TABLE 4. Estimated frequency of familial hypercholesterolemia in founder populations by geographic location

Country/ethnicity	FH* subjects and definition of FH	Population	Method of frequency estimation†	Estimated frequency of FH heterozygotes (%)‡	Reference
Africa					
South Africa/Afrikaners	28 homozygotes identified at a lipid disorder clinic of a hospital in Johannesburg who were aged <50 years and alive in 1979. Homozygous FH defined as TC* of >14.3 mmol/liter; xanthomata in first decade of life	Total population of Afrikaners aged <50 years within 150 km of Johannesburg in 1979, reported as 951,000	Heterozygous frequency is estimated from the observed homozygous frequency assuming Hardy-Weinberg equilibrium	1.0 (~1/100 individuals)	Seftel et al., 1980 (29)
South Africa/Afrikaners	18 heterozygotes identified in sample of 187 individuals with TC above the 80th percentile. Heterozygous FH defined as one of three *LDLR* mutations common in Afrikaner populations	1,612 randomly selected participants from a rural Afrikaner community	Assumed background prevalence of FH as 1/500 and estimated that an additional 4.3 participants would be FH heterozygotes with an unidentified mutation. Heterozygous frequency is estimated by dividing the revised estimate of 22.3 heterozygotes by the total sample size of 1,612	1.4 (95% CI*: 0.91, 2.1) (~1/72 individuals)	Steyn et al., 1996 (135)
South Africa/Ashkenazi Jews	6 heterozygotes. Heterozygous FH defined as TC of >7.5 mmol/liter; at least one first-degree relative with TC of >7.5 mmol/liter; no evidence of hypertriglyceridemia in family	403 men (aged 26–44 years); husbands of pregnant women undergoing Tay-Sachs screening	Heterozygous frequency is estimated by dividing the observed no. of heterozygotes by the total population of 403	1.5 (95% CI: 0.55, 3.2) (~1/67 individuals)	Seftel et al., 1989 (30)
Tunisia/Tunisian	26 homozygotes presenting at hospitals in central and southern Tunisia who were aged <50 years and alive in 1992. Homozygous FH defined as LDLC* of >15 mmol/liter; tendinous xanthomata in first decade of life	Total population aged <50 years in central and southern Tunisia given as ~3,000,000	Heterozygous frequency is estimated from the observed homozygous frequency assuming Hardy-Weinberg equilibrium	0.61 (~1/165 individuals)	Slimane et al., 1993 (32)
Asia					
Japan/Japanese	3 heterozygotes. Heterozygous FH defined as TC of >220 mg/ml and tendinous xanthomata	2,700 consecutive outpatients at clinics in the Hokuriku district of Japan	Heterozygous frequency is estimated by dividing the observed no. of heterozygotes by the total population of 2,700	0.11 (95% CI: 0.02, 0.32) (~1/900 individuals)	Mabuchi et al., 1977 (23)
North America					
Canada/French Canadians	19 homozygotes presenting at lipid clinics in Montreal and Quebec City. Homozygous FH defined as TC of >550 mg/dl; xanthomata at an early age	Total French-Canadian population in Quebec Province at the time of the 1981 census, reported as 5.3 million	Heterozygous frequency is estimated from the observed homozygous frequency assuming Hardy-Weinberg equilibrium	0.37 (~1/270 individuals)	Moorjani et al., 1989 (28)

newborns from the Denmark newborn screening program (83) each found a heterozygote frequency of approximately 0.08 percent (1/1,250) for *R3500Q*. In contrast, a study of 728 healthy, randomly selected patients in Switzerland estimated the frequency of *R3500Q* at 0.41 percent (1/209 individuals) (84). The observed increased prevalence of *R3500Q* in Switzerland may be due to chance or methodological differences between studies; however, this pattern is supported by other studies that have extrapolated a frequency of *R3500Q* heterozygotes in the general population from surveys of hypercholesterolemic individuals. These studies typically estimate the frequency of *R3500Q* as 1/500–1/700 with an increased frequency in central Europe (for review, see Miserez and Muller (85)).

As described above, there are a limited number of studies that directly estimate the frequency of homozygosity and/or heterozygosity of variants in *APOB* in population-based samples. However, a large number of studies have examined the frequency of *LDLR* and/or *APOB* variants among patients diagnosed with FH. Web table 1 provides the frequency of *LDLR* variants for FH subjects in the founder populations listed in table 4, and Web table 2 summarizes studies of the frequency of *LDLR* and *APOB* in nonfounder populations. (This information is described in two supple-

TABLE 4. Continued

Country/ethnicity	FH subjects and definition of FH	Population	Method of frequency estimation†	Estimated frequency of FH heterozygotes (%)‡	Reference
United States/ Caucasians	15 heterozygotes. Heterozygous FH defined as TC above the 99th percentile with TG* less than the 99th percentile; TC above the 99th percentile in a first-degree relative or xanthomatosis in a first-degree relative	366 survivors of acute MI* aged <60 years in 13 metropolitan Seattle, Washington, hospitals	Extrapolated frequency from MI survivors to general population assuming the following: 1) the prevalence of CHD* in adults aged 30–59 years is 3%; 2) the frequency of heterozygous FH in MI survivors is the same as the frequency of FH among individuals with other forms of CHD; 3) all FH heterozygotes manifest clinical signs of CHD before they are aged 60 years	0.1–0.2 (~1/1,000–~1/ 500 individuals)	Goldstein et al., 1973 (20)
Europe					
Denmark/Danish	11 heterozygotes. FH defined as a dominantly inherited disease with three-generation vertical transmission of hypercholesterolemia (LDLC or TC above the 95th percentile for age and sex)	10,440 infants born in six obstetric departments in Copenhagen. (Only 85% participated in follow-up necessary for FH diagnosis)	Heterozygous frequency is estimated by dividing the observed no. of heterozygotes by the total population of 10,440. This estimate is conservative, because it includes the 15% who did not participate in follow-up	0.11 (95% CI: 0.05, 0.19) (~1/950 individuals)	Anderson et al., 1979 (25)
Finland/North Karelian	407 heterozygotes identified at all public health centers in the North Karelian region between 1992 and 1996. FH defined as TC of >8 mmol/ liter; tendinous xanthomata or first-degree relative with tendinous xanthomata; TC of >8 mmol/liter in first-degree relative	Total population of North Karelian region reported as ~180,000	Heterozygous frequency is estimated by dividing the observed no. of heterozygotes by the total population of 180,000	0.23 (95% CI: 0.20, 0.25) (~1/441 individuals)	Vuorio et al., 1997 (19)
Hungary/Hungarians	39 heterozygotes identified from family physician registers. FH defined according to the Dutch Lipid Clinic Network criteria (15)	Family physician registers for a random sample of 21,000 individuals. All Hungarian citizens are in the physician registers, regardless of health status	Heterozygous frequency is estimated by dividing the observed no. of heterozygotes by the total population of 21,000	0.19 (95% CI: 0.13, 0.25) (~1/539 individuals)	Kalina et al., 2001 (26)
United Kingdom/British and Welsh	Estimate of 10 homozygotes. Detailed criteria for FH not specified	Population of England and Wales aged <30 years estimated as 1,000,000	Heterozygous frequency is estimated from the observed homozygous frequency assuming Hardy-Weinberg equilibrium	0.16 (~1/623 individuals)	Slack, 1979 (22)
Middle East					
Lebanon/Christian Lebanese	Estimate of 100 homozygotes. Detailed criteria for FH not specified	Population of Christian Lebanese aged <30 years estimated as 1,000,000	Heterozygous frequency is estimated from the observed homozygous frequency assuming 0.2 as the proportion of first-cousin marriages and using a modified Hardy-Weinberg equilibrium formula§	1.2 (~1/85 individuals)	Slack, 1979 (22)

* FH, familial hypercholesterolemia; TC, total cholesterol; CI, confidence interval; LDLC, low density lipoprotein cholesterol; MI, myocardial infarction; TG, triglyceride; CHD, coronary heart disease.

† Under Hardy-Weinberg equilibrium, if p^2 is the estimated frequency of homozygotes, then the frequency of heterozygotes is $2pq$, with $p = 1 - p$.

‡ The 95% confidence interval is not presented for studies that estimated heterozygous frequency based on observed homozygous frequencies.

§ Modified Hardy-Weinberg formula assuming that 0.2 is the proportion of first-cousin marriages; the frequency of heterozygotes is $0.8 \times p^2 + (0.2/16) \times p$.

mentary tables; each is referred to as "Web table" in the text and is posted on the website of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/reviews.htm) as well as on the *Journal*'s website (http://aje.oupjournals.org/).)

Founder populations

As expected, a small number of *LDLR* variants account for the molecular diagnoses of the majority of the patients with FH in each of the founder populations (Web table 1). This is most clearly seen in the North Karelian region of Finland where over 80 percent of FH individuals are heterozygous for the same *LDLR* variant (19, 34). Because there have been no studies that screened 10 or more individuals for *LDLR* or *APOB* variants in Tunisians or Christian Lebanese, they are not included in Web table 1. However, ancillary evidence indicates that each of these populations and the South African Indian population also have only a small number of variants in the *LDLR* gene (86–88). For example, eight

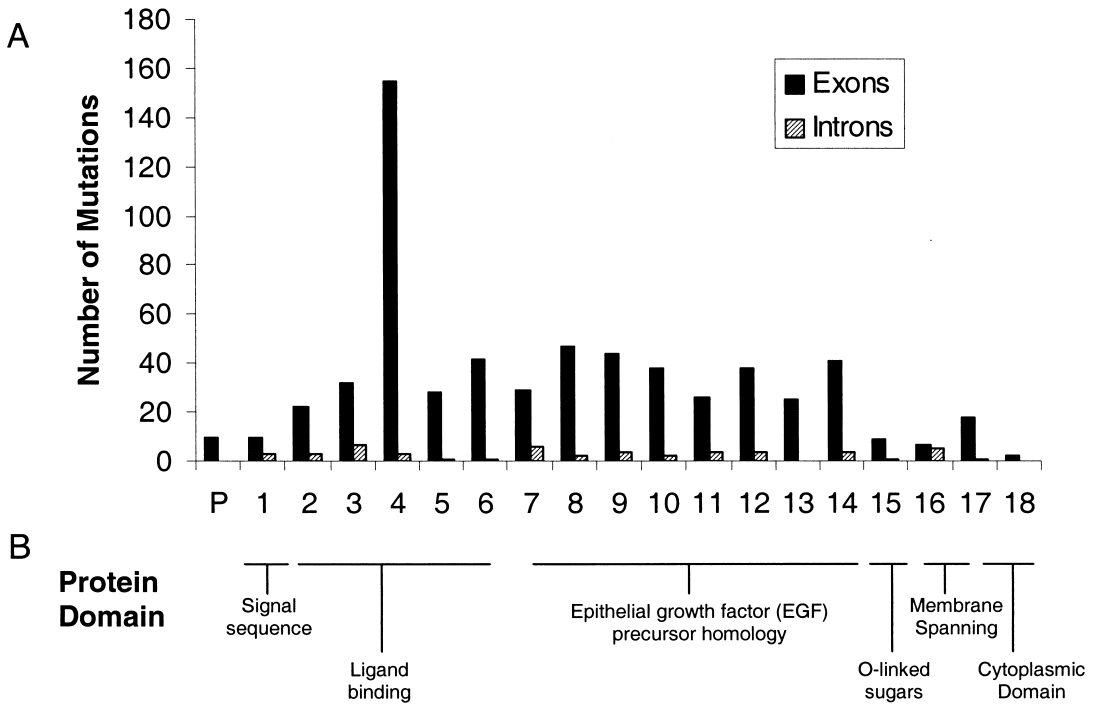

FIGURE 1. A, location of 647 unique mutations (excluding major rearrangements) in the low density lipoprotein receptor gene (*LDLR*) by promoter (P), exon, and intron regions (data were extracted from www.ucl.ac.uk/fh on July 1, 2003); B, correspondence between the *LDLR* gene organization and the low density lipoprotein receptor protein domain structure. O-linked sugars, sugars attached to a hydroxyl (–OH) group on the side chains of serine or threonine.

Christian Lebanese FH homozygotes studied in Dallas were found to be homozygous for the *LDLR C660X* allele (35).

Nonfounder populations

Northern Greece was the only nonfounder population in which an underlying genetic variant was identified for all FH patients (89) (Web table 2). For the other geographic areas, a molecular variant was typically identified in only 60–85 percent of the individuals clinically diagnosed with FH, and the remainder were undetermined by the laboratory approach used. This was true even for studies that examined the entire coding and promoter regions of *LDLR* and that screened for the *APOB* variant *R3500Q*. For example, in Malaysia (90), Israel (91), and the United Kingdom (92), the underlying variant was not identified for a significant proportion of the individuals studied. Thus, the molecular basis for FH in individuals without identified genetic variants remains undetermined. It is possible that these individuals have undetected mutations in *LDLR* or *APOB*. Alternatively, there could be additional monogenic causes (such as *PCSK9*) or polygenic factors interacting with environmental factors that mimic a FH phenotype.

The spectrum of gene variants also differs for founder versus nonfounder populations. Some nonfounder regions, including Japan (93) and Greece (89), have common alleles (i.e., alleles found in over 10 percent of individuals with FH). However, many nonfounder areas have a relatively large number of distinct *LDLR* mutations, each of which is found in only a small number of individuals with FH. For example, a survey of 791 patients with probable or possible FH presenting at lipid clinics in the United Kingdom and America identified 51 different variants in 134 individuals (64). The distinction between a small number of common alleles and a large number of rare alleles is important because it can inform strategies for molecular detection and, thus, the diagnosis of FH. When a small number of mutations predominate, molecular tests can be designed to identify these specific variant alleles. Alternatively, when most variants are unique to a small number of individuals, the entire *LDLR* and *APOB* genes will need to be sequenced to identify an individual's mutation. Thus, molecular testing will be more efficient if it is tailored to the allele frequency distribution of a population (94).

In the comparison of studies of nonfounder populations listed in Web table 2, several limitations should be kept in mind because the criteria used to diagnose FH differ, and the laboratory methods used to screen for mutations vary. The laboratory methods are most notably an issue when comparing studies using restriction digests or other methods to identify a small number of specific alleles (93, 95–97) with more recent studies utilizing techniques to scan the

TABLE 5. Estimated frequency of individuals heterozygous for mutations in the apolipoprotein B gene (*APOB*) in population-based studies by geographic location

Country/ethnicity	Study sample	No. of individuals screened	Screening method for detecting mutation(s)	No. of heterozygotes observed	% heterozygotes (95% CI*)	Reference
Americas						
United States/ multiethnic population	Workers at Wells Fargo banks in California	Total: 5,160	PCR* to detect *R3500Q*	Total: 4	Total: 0.08 (95% CI: 0.01, 0.14)	Bersot et al., 1993 (82)
		Caucasians: 2,859		Caucasians: 3	Caucasians: 0.1 (95% CI: 0.02, 0.30)	
Europe						
Denmark/Danish	Participants in the Copenhagen City Heart Study from 1991 to 1994	9,255	PCR to detect *R3500Q*, *R3531C*, and *R3500W*	7 with *R3500Q*	*R3500Q*: 0.08 (95% CI: 0.03, 0.16)	Tybjaerg-Hansen et al., 1998 (78)
				7 with *R3531C*	*R3531C*: 0.08 (95% CI: 0.03, 0.16)	
				0 with *R3500W*	*R3500W*: 0.0 (95% CI: 0, 0.04)	
Denmark/Danish	Newborns in a PKU*-screening program	5,000	PCR to detect *R3500Q*	5 (2 were twins; zygosity not stated)	0.08 (95% CI: 0.05, 0.13)	Hansen et al., 1994 (136)
Switzerland/Swiss	Unrelated healthy male individuals in military service in August 1991 from German-, French-, and Italian-speaking parts of the country	728	PCR to detect *R3500Q*	3	0.41 (95% CI: 0.08, 1.2)	Miserez et al., 1994 (84)

* CI, confidence interval; PCR, polymerase chain reaction; PKU, phenylketonuria.

entire coding and promoter regions of the genes (65, 90, 94, 98–109). Thus, both the sensitivity and the specificity of the screening method differ across studies. The observed differences in the number and spectrum of identified mutations across populations are likely to be, at least in part, attributable to these variations in study design.

Furthermore, most studies listed in Web table 2 report all genetic variations observed in FH individuals without evaluating the potential functional significance. Not all of the variants reported may actually be the mutation responsible for the observed clinical phenotype (110). Ideally, DNA changes should be evaluated to determine if they are disease causing before they are reported, and criteria have been established for such evaluation (111). Mutations causing a premature stop codon, frameshift mutations, and large deletions/rearrangements generally result in a truncated, nonfunctional LDL receptor protein. Similarly, missense mutations that alter a critical amino acid typically result in a defective LDL receptor protein. Such mutations are likely to be the cause of FH if identified in a clinically diagnosed patient. In contrast, missense mutations that cause a conservative amino acid change, silent mutations, and mutations that occur in noncoding regions of the gene may not be disease causing and require further support, such as in vitro assays demonstrating reduced LDL receptor binding (18). In addition, a mutation can be considered disease causing if it alters an amino acid that is conserved across species, or if it appears to have arisen independently (on different haplotypes) in multiple unrelated FH individuals. The existing databases can be used to identify if a mutation meets these criteria (65, 66). Additionally, since functional mutations should not be present in non-FH individuals, the current recommendation is that 100 normal chromosomes be screened to exclude nonfunctional polymorphisms (111).

In addition to characterizing the frequency of mutations geographically, insight into the evolutionary history of the genes and populations can be gained by comparing mutation frequencies within and between populations (85, 112). For example, a within-population frequency gradient is seen in the *C646Y* (FH-French Canadian 2) allele; thus, the frequency of the allele is 18 percent in northeastern Quebec (113) but only 5 percent in Montreal (114). This gradient indicates heterogeneity within this founder population, and it may reflect more admixture in Montreal. A between-population gradient is seen in *R3500Q*, and this mutation is at high frequency in Poland, Switzerland, and the Czech Republic, at lower frequency in other European populations, and virtually absent from Asian and South African populations (85). Nearly all individuals with this mutation share a rare haplotype defined by eight variable sites in the *APOB* gene and its flanking region (76). On the basis of this distribution and

haplotype analysis, the original *R3500Q* mutation is postulated to have occurred ~6,750 years ago (115).

POPULATION TESTING

Cost effectiveness and screening programs

Familial hypercholesterolemia fulfills the World Health Organization criteria for screening programs (116). That is, clinical endpoint trials of lipid-lowering drug therapy with statins have demonstrated their effectiveness in the primary and secondary prevention of coronary heart disease risk (117–120), especially in the highest risk groups. Although there are no randomized clinical trials specifically in patients with familial hypercholesterolemia, observational studies strongly suggest that statins reduce disease risk in FH individuals (14). However, effective primary prevention requires early diagnosis. Family tracing in a pilot study in the United Kingdom was acceptable and feasible (121), and the success of a program in the Netherlands, based on genetic testing, has recently been reported (122). One paper based on US data has reported the cost-effectiveness of screening for FH (123) and has been subsequently updated (124). Costs and effectiveness were not reported separately, but the analysis supported the benefit of statin treatment. The cost per life-years gained ranged from $3,375 for men aged 20–65 years (based on 100 percent ideal effectiveness) to $6,750 assuming 50 percent effectiveness.

One detailed study from the United Kingdom has reported cost-effectiveness data, comparing the identification and treatment of FH patients by universal screening, opportunistic screening in primary care, screening of premature myocardial infarction admissions, or tracing family members of affected patients ("cascade screening") (125). Cost-effectiveness was calculated as cost per life-year gained (extension of life expectancy resulting from intervention), including estimated screening and treatment costs. Family member tracing was the most cost-effective strategy for the population overall (£3,097 (US $5,752.25) per life-year gained) with 2.6 individuals needing to be screened to identify one case at a cost of £133 (US $246.97) per case detected. If the genetic mutation was known within the family, then the cost per life-year gained (£4,914 (US $9,126.43)) was only slightly increased by genetic confirmation of the diagnosis. Universal population screening was least cost-effective (£13,029 (US $24,196.49) per life-year gained) with 1,365 individuals needing to be screened at a cost of £9,754 (US $18,106.10) per case detected. For each strategy, it was more cost-effective to screen younger people and women (with a 10-fold increase in the cost per life-year gained between the oldest and the youngest age group in the family-tracing strategy), because these groups gained more life-years following treatment. Targeted strategies were more expensive per person screened, but the cost per case detected was lower. Population screening of only persons aged 16 years was as cost-effective as family tracing (£2,777 (US $5,154.46) with a clinical confirmation). However, further study is needed before testing of teenagers would be recommended (18).

This positive view of the cost benefit of cascade screening for FH has been reinforced by a recent analysis of the Dutch FH program (126). The cost per life-year gained ranged between 25,500 euros (US $31,604.91) and 32,000 euros (US $39,655.73). This analysis used the Framingham equation to estimate their effect from the patient cholesterol data and randomized control trial evidence for effectiveness. This modeling assumed 100 percent compliance. As this study did not discount for costs and benefits, it is difficult to compare the results of one modeling exercise with another, although all the studies (124–127) reported that family tracing of relatives of affected FH patients was cost-effective and that it should be piloted on a wider scale. All screening strategies will become cheaper (and therefore more cost-effective) as drug costs fall, which can be expected as the patents for some statins expire soon. The generic equivalent of a preparation can be between one third and two thirds of the cost of the proprietary product (128). As the technology improves (especially DNA diagnostic techniques), the cost-effectiveness of all strategies will benefit.

As cascade screening programs are developed, additional research will be needed to inform about the psychological impact of genetic testing versus traditional screening based on plasma lipid levels and clinical manifestations such as xanthomata. There is evidence that genetic testing is associated with a greater degree of fatalism than trait measurements, and this fatalism may have a negative impact on quality of life. In addition, genetic testing may impact eligibility for health insurance and result in discrimination at work. There has been some preliminary research into these ethical, legal, and social issues in the Netherlands (129, 130) and the United Kingdom (131), and further work is needed in other countries and cultures. Additionally, it is currently unclear to what extent DNA testing will complement traditional testing based on clinical manifestations in terms of false positives and false negatives (132–134).

CONCLUSIONS AND RECOMMENDATIONS FOR RESEARCH

Heterozygous familial hypercholesterolemia is associated with increased coronary heart disease and premature death. Although often sited as a textbook example of an autosomal dominant disorder, the genetic basis of this disorder is actually complex. Over 700 variants have been identified in the *LDLR* gene, and this number is likely to increase as new technology allows for rapid screening of the entire gene at reduced costs. Further understanding of the genetic basis of FH will result from the identification of other potential genes for the FH phenotype, including the *PCSK9* gene on chromosome 1. Variations in all of these genes will likely continue to be reported from screens of individuals with clinical FH, and the functional significance of such variations should be evaluated before concluding that they are causative mutations. Such evaluation should include characterization of allele-specific associations with coronary heart disease, particularly the identification of severe or mild receptor-defective mutations.

Since patients with FH should reduce traditional coronary heart disease risk factors, such as diet and smoking, and

since FH appears to respond well to drug treatment with statins, early diagnosis is beneficial. The current tools for diagnosis range from evaluation of elevated cholesterol levels alone to molecular characterization of mutations. Consistent, uniformly applied, clinically useful definitions are needed. Cascade testing of FH family members does appear to be cost-effective, but additional research is still needed. Furthermore, for screening programs to be successful, awareness by general practitioners, accident and emergency staff, cardiology teams, and the general public of the signs of FH and the benefits of early treatment is important, and extra training of these health professionals is warranted.

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