

ASSOCIATION OF MUTATIONS IN THE APOLIPOPROTEIN B GENE WITH HYPERCHOLESTEROLEMIA AND THE RISK OF ISCHEMIC HEART DISEASE

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ABSTRACT

Background Familial hypercholesterolemia leads to premature ischemic heart disease and is often caused by mutations in the gene for the low-density lipoprotein receptor. Mutations in the apolipoprotein B gene, which encodes a ligand for this receptor, may also result in this phenotype.

Methods We studied the genotypes of 9255 women and men from the general population, 948 patients with ischemic heart disease, and 36 patients with familial hypercholesterolemia, all from Denmark, for three mutations in the apolipoprotein B gene: Arg3500Gln, Arg3531Cys, and Arg3500Trp.

Results The prevalence of heterozygotes in the general population was 0.08 percent (95 percent confidence interval, 0.03 to 0.16 percent) for both the Arg3500Gln and the Arg3531Cys mutations, and 0.00 percent (95 percent confidence interval, 0.00 to 0.18 percent) for the Arg3500Trp mutation. Among carriers of the Arg3500Gln mutation, cholesterol levels were significantly higher than among noncarriers in the general population — by 100 mg per deciliter (2.6 mmol per liter) among carriers in the general population, 154 mg per deciliter (4.0 mmol per liter) among patients with ischemic heart disease, and 172 mg per deciliter (4.5 mmol per liter) among patients with familial hypercholesterolemia. Heterozygous carriers of the Arg3500Gln mutation were significantly more common among patients with ischemic heart disease (odds ratio, 7.0; 95 percent confidence interval, 2.2 to 22; $P=0.003$) and patients with familial hypercholesterolemia (odds ratio, 78; 95 percent confidence interval, 16 to 388; $P=0.001$) than in the general population. Heterozygous carriers of the Arg3531Cys mutation in the general population did not have higher-than-normal plasma cholesterol levels or an increased risk of ischemic heart disease (odds ratio; 1.4; 95 percent confidence interval, 0.2 to 11; $P=0.54$).

Conclusions The Arg3500Gln mutation in the apolipoprotein B gene, which is responsible for familial defective apolipoprotein B-100 and is present in approximately 1 in 1000 persons in Denmark, causes severe hypercholesterolemia and increases the risk of ischemic heart disease. (N Engl J Med 1998;338:1577-84.)

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ELEVATED plasma low-density lipoprotein (LDL) cholesterol levels are causally related to ischemic heart disease,^{1,2} and a reduction in these levels reduces the incidence of ischemic heart disease³⁻⁵ and the associated mortality rate.^{3,4} Elevated LDL cholesterol levels may be caused by decreased removal of LDL from plasma, as a result of either mutations in the LDL-receptor gene, as in classic familial hypercholesterolemia,⁶ or mutations in the apolipoprotein B (*APOB*) gene that affect the binding of LDL to the LDL receptor. Apolipoprotein B is the chief protein component of LDL and serves as the ligand for the removal of LDL from the circulation by the LDL receptor.

Despite intensive research in this field,⁷⁻¹⁵ only three such mutations in *APOB* have been identified: Arg3500Gln, which is responsible for familial defective apolipoprotein B-100^{7,10}; Arg3531Cys⁸; and Arg3500Trp.⁹ However, since familial defective apolipoprotein B-100 has with few exceptions been identified only among patients with hyperlipidemia or familial hypercholesterolemia¹¹⁻¹³ and not in the general population, the estimate of the frequency of this mutation in the adult general population is only approximate, the effect of the mutation on cholesterol levels is probably overestimated, and the risk of ischemic heart disease associated with this mutation is not known. Neither the frequencies of Arg3531Cys^{8,14,15} and Arg3500Trp⁹ in the general population nor their effect on hyperlipidemia and the risk of ischemic heart disease is known.

From a practical point of view, in order to be of clinical value for screening, a mutation in *APOB* must be both relatively frequent and associated with hypercholesterolemia, and thus with an increased risk of ischemic heart disease. We studied the three mutations in *APOB* to see whether they fulfill these criteria.

METHODS**Subjects**

To obtain subjects from the general population, we recruited 5121 women and 4134 men who had participated in the Co-

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penhagen City Heart Study from 1991 through 1994.¹⁶⁻¹⁸ We screened participants for manifestations of ischemic heart disease by reviewing all hospital admissions and diagnoses entered in the Danish National Hospital Discharge Register. The diagnoses were based on the *International Classification of Diseases*, eighth revision (ischemic heart disease codes 410 through 414).

Patients with ischemic heart disease were identified among 992 consecutive female and male patients from the greater Copenhagen area who were referred for coronary angiography from 1991 through 1993.^{16,17} All patients were evaluated by experienced cardiologists at Rigshospitalet, National University Hospital, Copenhagen. Among these, 948 patients (248 women and 700 men) had ischemic heart disease with characteristic symptoms of stable angina according to the guidelines of the European Society of Cardiology¹⁹ on the basis of the location, character, and duration of pain and the relation of pain to exercise, plus at least one of the following: severe stenosis on coronary angiography (≥ 70 percent stenosis of at least one coronary artery or ≥ 50 percent stenosis of the left main coronary artery), a previous myocardial infarction, or a positive exercise electrocardiography test.

Thirty-six apparently unrelated patients (4 women and 32 men) with a clinical diagnosis of familial hypercholesterolemia were selected from a larger population of patients attending the lipid clinic at Rigshospitalet. The diagnosis was based on the following criteria: a plasma total cholesterol level above 309 mg per deciliter (8.0 mmol per liter) and a plasma LDL cholesterol level above 232 mg per deciliter (6.0 mmol per liter), tendinous xanthomas in the patient or a first-degree relative, and a history of hypercholesterolemia, ischemic heart disease, or both in a first-degree relative before the age of 60 years.

In all groups, more than 99 percent were white and nearly 99 percent were of Danish descent. All the participants gave informed consent. The studies were approved by the relevant ethics committees.

DNA Analyses

The Arg3500Gln and Arg3531Cys mutations are caused by the substitution of adenine for guanine at position 10,699 of complementary DNA²⁰ and thymine for cytosine at position 10,791, respectively, in exon 26 of the *APOB* gene.^{7,8} The presence of either mutation was determined in pooled samples²¹ by the polymerase chain reaction (PCR): there was a common band of 334 bp and a mutation-specific band of 167 bp in the case of Arg3500Gln and 111 bp in the case of Arg3531Cys.

Three primers were used in the PCR for the detection of Arg3500Gln (5'GACCACAAGCTTAGCTTGG3', 1 μ mol per liter; 5'GGGTGGCTTTGCTTGTATG3', 0.025 μ mol per liter; and 5'TGCAGCTTCACTGAACACT3', 1 μ mol per liter) and Arg3531Cys (5'GACCACAAGCTTAGCTTGG3', 0.025 μ mol per liter; 5'GGGTGGCTTTGCTTGTATG3', 1 μ mol per liter; and 5'GAGAAGCCACACTCAAAT3', 1 μ mol per liter); mutations in allele-specific primers are printed in *italics*, and mismatches in primers are underlined. Annealing temperatures for the two procedures were 48°C and 55°C, respectively, and all reactions were performed in a total volume of 50 μ l, with the concentrations of primers as indicated, 1 U of *Taq* polymerase, 200 μ mol of each deoxynucleoside triphosphate per liter, 1.5 mmol of magnesium chloride per liter, 1 \times buffer (200 mM TRIS-hydrochloride, pH 8.4, and 500 mM potassium chloride), and 0.1 to 0.2 μ g of DNA. When a mutation was identified in a pooled sample, DNA from each of the 20 subjects in the sample was again subjected to PCR, followed by digestion with *MspI*²² or *NsiI*, to confirm the diagnosis and to determine zygosity. The Arg3500Trp mutation is caused by the substitution of thymine for cytosine at position 10,698²⁰ in exon 26 of the *APOB* gene.⁹ Two primers were used in the PCR (5'GTGTCAGTGGCAAAAACCA3', 1 μ mol per liter; and 5'CAGAGGGAATATATGCGTTGG3', 1 μ mol per liter), and the annealing temperature was 57°C. The other reagents and conditions of the PCR were as described above. All patients with familial hypercholesterolemia or ischemic

heart disease, as well as the 2021 subjects from the general population who had plasma cholesterol and apolipoprotein B levels above the 70th percentile for age and sex, were screened for the Arg3500Trp mutation. PCR, followed by digestion with *Hsp92II*, revealed common bands of 108 bp and 314 bp and a mutation-specific band of 206 bp.

Other Analyses

Colorimetric and turbidimetric assays were used to measure plasma levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and apolipoproteins B and AI (all Boehringer Mannheim, Mannheim, Germany) and lipoprotein(a) (DAKO, Glostrup, Denmark); LDL cholesterol levels were calculated with the equation of Friedewald et al.²³

Statistical Analysis

Prevalence estimates of the three mutations were expressed as percentages with exact binomial 95 percent confidence intervals.²⁴ Remaining data were analyzed with the SPSS program.²⁵ To examine the effect of the Arg3500Gln and Arg3531Cys mutations on the phenotype in carriers identified in the general population, we converted values for continuous variables for carriers to their respective percentiles¹¹ and compared them with the values for the general population as a whole, using z-scores, as described previously.^{21,26} In the general-population sample, phenotypes of subjects who were heterozygous for either Arg3500Gln or Arg3531Cys were compared by the Mann-Whitney U test with an exact two-tailed probability. Categorical variables were compared by Fisher's exact test. We calculated the absolute increase in a given variable in heterozygotes by subtracting the 50th percentile value for age and sex in the general population from the value for the individual subject; the median increases or decreases were compared by the Mann-Whitney U test with an exact two-tailed probability. The ability of the presence of the mutations to predict ischemic heart disease and familial hypercholesterolemia was expressed as an odds ratio with approximated 95 percent confidence intervals; Fisher's exact test was used as the test of independence. All P values were two-sided. A P value of less than 0.05 was considered to indicate statistical significance.

RESULTS

Frequencies of *APOB* Mutations

The Arg3500Gln and Arg3531Cys mutations were each identified in 7 of the 9255 subjects in the general population (carrier frequency, 0.08 percent). All 14 subjects were heterozygous for the mutations. Five patients with ischemic heart disease and two patients with familial hypercholesterolemia were heterozygous for the Arg3500Gln mutation, and one patient with ischemic heart disease was heterozygous for the Arg3531Cys mutation. The Arg3500Trp mutation was not identified among the 2021 subjects with hypercholesterolemia in the general population, the 948 patients with ischemic heart disease, or the 36 patients with familial hypercholesterolemia.

Phenotypic Characteristics of Heterozygous Carriers of *APOB* Mutations in the General Population

The characteristics of the 22 heterozygous carriers of Arg3500Gln or Arg3531Cys are shown in Table 1. Figure 1 shows the age-adjusted and sex-adjusted percentiles of lipid, lipoprotein, and apolipoprotein values for heterozygous subjects in the general population. Subjects who were heterozygous for Arg3500Gln had

TABLE 1. CHARACTERISTICS OF THE SUBJECTS WHO WERE HETEROZYGOUS FOR Arg3500Gln OR Arg3531Cys.*

CHARACTERISTIC	Arg3500Gln			Arg3531Cys	
	PATIENTS WITH FH (N = 2)	PATIENTS WITH IHD (N = 5)	GENERAL POPULATION (N = 7)	PATIENTS WITH IHD (N = 1)	GENERAL POPULATION (N = 7)
Sex (F/M)	0/2	1/4	3/4	0/1	6/1
Median age (yr)	46, 45	52	54	77	73
Range		38–58	27–70		38–86
Median cholesterol (mg/dl)	394, 398	367	344	347	235†
Range		305–417	243–405		205–266
Median LDL cholesterol (mg/dl)	317, 290	286	220	255	154‡
Range		224–347	178–328		131–158
Median HDL cholesterol (mg/dl)	54, 31	42	42	62	62§
Range		35–58	31–58		39–89
Median triglycerides (mg/dl)	124, 389	230	97	159	106
Range		115–301	80–451		89–133
Median apolipoprotein B (mg/dl)	ND	117	116	127	85¶
Range		108–168	96–153		78–93
Symptoms of IHD (no. of patients)	1	5	3	1	0
Age at onset of symptoms of IHD (yr)	34	44	51	67	
Range		34–54	34–55		
Family history of IHD <60 yr of age, HC, or both (no. of patients)	2	4	6	0	1**

*For subjects with hyperlipidemia, lipids were measured when they were not being treated. Triglycerides were not measured during fasting in the general population. P values are for comparisons between Arg3500Gln heterozygotes and Arg3531Cys heterozygotes in the general population by the Mann-Whitney U test and Fisher's exact test. FH denotes familial hypercholesterolemia, IHD ischemic heart disease, LDL low-density lipoprotein, HDL high-density lipoprotein, HC hypercholesterolemia, and ND not determined. To convert values for cholesterol to millimoles per liter, multiply by 0.0259. To convert values for triglycerides to millimoles per liter, multiply by 0.0113.

†P=0.007.

‡P=0.001.

§P=0.04.

¶P=0.003.

||Data were missing on one patient.

**P=0.03.

significantly higher plasma cholesterol levels (mean percentile, 93rd; 95 percent confidence interval, 84th to 100th), LDL cholesterol levels (mean percentile, 95th; 95 percent confidence interval, 89th to 100th), and apolipoprotein B levels (mean percentile, 95th; 95 percent confidence interval, 88th to 100th) than either the general population as a whole ($P < 0.001$ for all comparisons) or carriers of the Arg3531Cys mutation ($P = 0.001$, $P = 0.001$, and $P = 0.003$, respectively). Although the percentiles for HDL cholesterol ($P = 0.09$) and apolipoprotein AI ($P = 0.10$) tended to be lower among Arg3500Gln heterozygotes than in the general population, the percentiles for triglycerides, lipoprotein(a), and age (Fig. 1) as well as the mean fibrinogen levels, glucose levels, body-mass index, and ratio of waist to hip circumference (data not shown) in this group were not significantly different from values in the general population.

Subjects from the general population who were heterozygous for the Arg3500Gln mutation had a significantly increased frequency of ischemic heart disease (odds ratio, 13; 95 percent confidence interval, 3 to 56; $P = 0.005$), hypertension (odds ratio, 10;

95 percent confidence interval, 2 to 51; $P = 0.005$), and peripheral arterial disease (odds ratio, 10; 95 percent confidence interval, 2 to 43; $P = 0.01$) as compared with noncarriers in the general population, whereas sex distribution, smoking habits, frequencies of xanthelasma and arcus corneae, diabetes mellitus, and ischemic stroke were similar in the two groups (data not shown).

There were no significant differences in any of the above-mentioned variables, continuous or categorical, between carriers of Arg3531Cys in the general population sample and noncarriers (Fig. 1, Table 1, and data not shown).

Phenotypic Characteristics of Heterozygous Carriers of APOB Mutations in the General Population and of the Patients

Heterozygous carriers of the Arg3500Gln mutation in the general population had higher median plasma cholesterol, LDL cholesterol, and apolipoprotein B levels, by 100 mg per deciliter (2.6 mmol per liter), 82 mg per deciliter (2.1 mmol per liter), and 43 mg per deciliter, respectively, than mutation

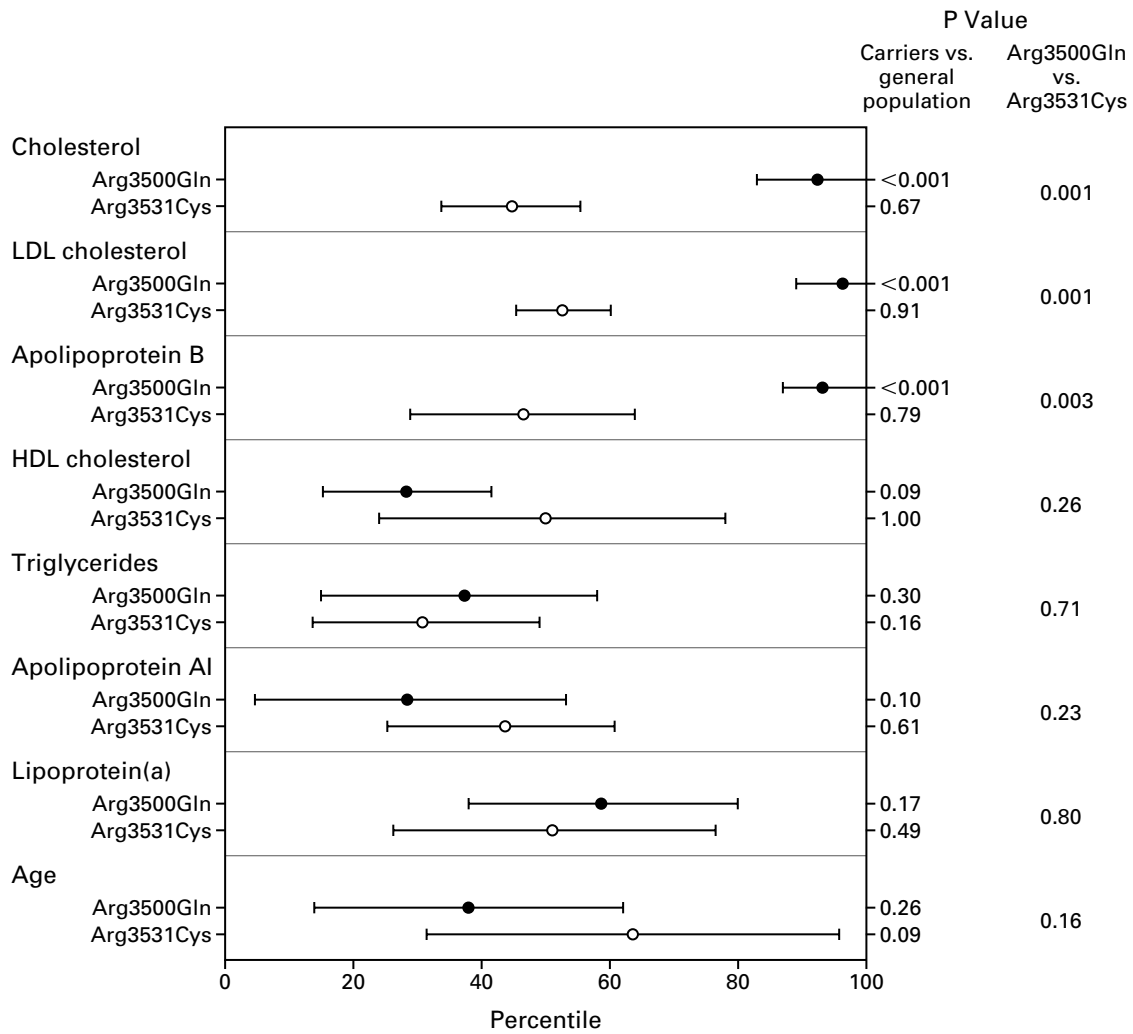


Figure 1. Mean Percentiles for Plasma Lipids, Lipoproteins, Apolipoproteins, and Age in Heterozygous Carriers of Arg3500Gln or Arg3531Cys in the General Population.

Mean values and 95 percent confidence intervals are given relative to age-matched and sex-matched subjects (for lipids, lipoproteins, and apolipoproteins) or sex-matched subjects (for age) in the total general-population sample. Comparisons of heterozygous carriers of either mutation with the total sample were made with the z-test.^{21,26} Comparisons between carriers were made with the Mann-Whitney U test. LDL denotes low-density lipoprotein, and HDL high-density lipoprotein.

noncarriers of the same age and sex (Table 2). Median plasma cholesterol levels were even higher in patients with ischemic heart disease or familial hypercholesterolemia who had the mutation than in carriers in the general population ($P=0.03$). A similar trend was observed for LDL cholesterol levels ($P=0.16$).

There was no such increase in plasma cholesterol, LDL cholesterol, or apolipoprotein B levels in carriers of Arg3531Cys in the general population (Table 2). However, the one patient with ischemic heart disease with this mutation had increases in plasma

cholesterol and LDL cholesterol that were similar to those of carriers of Arg3500Gln.

APOB Mutations and the Risk of Ischemic Heart Disease and Familial Hypercholesterolemia

The Arg3500Gln mutation was significantly more frequent among patients with ischemic heart disease than in the general population (odds ratio, 7.0; 95 percent confidence interval, 2.2 to 22; $P=0.003$) (Table 3) and, as mentioned above, was also significantly more common among patients with ischemic heart disease in the general-population sample than

TABLE 2. EFFECT OF THE Arg3500Gln AND Arg3531Cys MUTATIONS ON CHOLESTEROL, LDL CHOLESTEROL, AND APOLIPOPROTEIN B LEVELS IN HETEROZYGOUS CARRIERS.*

VARIABLE	Arg3500Gln			P VALUE	Arg3531Cys	
	GENERAL POPULATION (N=7)†	PATIENTS WITH IHD (N=5)†	PATIENTS WITH FH (N=2)		GENERAL POPULATION (N=7)	PATIENTS WITH IHD (N=1)
	median (range)				median (range)	
Cholesterol (mg/dl)	100 (23 to 147)	154 (81 to 174)	172 (170 to 174)	0.03	0 (-23 to +8)	116
LDL cholesterol (mg/dl)	82 (34 to 181)	149 (88 to 201)	165 (152 to 178)	0.16	0 (-5 to +19)	112
Apolipoprotein B (mg/dl)†	43 (25 to 69)	36 (24 to 82)	ND	0.90	-2 (-13 to +18)	39

*Values are the median differences between values for carriers and the 50th-percentile values for persons of the same age and sex in the general population. P values are for the comparison of carriers in the general population with all patients with either ischemic heart disease (IHD) or familial hypercholesterolemia (FH) by the Mann-Whitney U test. To convert values for cholesterol to millimoles per liter, multiply by 0.0259. LDL denotes low-density lipoprotein, and ND not determined.

†Apolipoprotein B values were not measured in the absence of treatment for hyperlipidemia in two carriers in the general population and one patient with ischemic heart disease.

TABLE 3. FREQUENCIES OF AND ODDS RATIOS FOR ISCHEMIC HEART DISEASE AND FAMILIAL HYPERCHOLESTEROLEMIA AMONG SUBJECTS HETEROZYGOUS FOR Arg3500Gln, Arg3500Trp, OR Arg3531Cys.*

GROUP	NO. OF PROBANDS	FREQUENCY OF HETEROZYGOTES (95% CI)†	ODDS RATIO (95% CI)‡	P VALUE§
percent				
Arg3500Gln				
General population (n=9255)	7	0.08 (0.03-0.16)		—
Patients with IHD (n=948)	5	0.53 (0.17-1.23)	7.0 (2.2-22)	0.003
Patients with FH (n=36)	2	5.6 (0.7-19)	78 (16-388)	0.001
Arg3500Trp				
General population (n=2021)¶	0	0.00 (0.00-0.18)		
Patients with IHD (n=948)	0	0.00 (0.00-0.39)	ND	ND
Patients with FH (n=36)	0	0.00 (0.00-9.7)	ND	ND
Arg3531Cys				
General population (n=9255)	7	0.08 (0.03-0.16)		
Patients with IHD (n=948)	1	0.11 (0.00-0.59)	1.4 (0.2-11)	0.54
Patients with FH (n=36)	0	0.00 (0.00-9.7)	ND	ND

*CI denotes confidence interval, IHD ischemic heart disease, FH familial hypercholesterolemia, and ND not determined.

†Exact binomial confidence intervals are given.

‡The reference group is the general population. Approximate confidence intervals are given.

§Comparisons were made with Fisher's exact test.

¶Only subjects with cholesterol and apolipoprotein B values above the 70th percentile for age and sex were screened.

in those without ischemic heart disease in the general population. The odds ratio for familial hypercholesterolemia for the same mutation was also significant (odds ratio, 78; 95 percent confidence interval, 16 to 388; $P=0.001$) (Table 3).

The Arg3531Cys mutation was not more frequent among patients with ischemic heart disease than in the general population sample (odds ratio, 1.4; 95 percent confidence interval, 0.2 to 11; $P=0.54$) (Ta-

ble 3) and was not identified among subjects with ischemic heart disease in the general-population sample or among patients with familial hypercholesterolemia.

DISCUSSION

Among Danes, as among most other white populations, the frequency of clinically diagnosed heterozygous familial hypercholesterolemia is about 0.2

percent.^{6,27} Approximately 6 percent of patients with clinical familial hypercholesterolemia are carriers of the Arg3500Gln mutation (Table 3),¹¹ which is responsible for familial defective apolipoprotein B-100,¹⁰ whereas the rest have different LDL-receptor mutations.⁶ Our data indicate that Arg3500Gln occurs at a frequency of 0.08 percent (approximately 1 in 1000) in a general population of whites, and is associated with considerably higher than normal plasma cholesterol levels (by 100 mg per deciliter), and an increased risk of ischemic heart disease (odds ratio, 7.0). In contrast, the Arg3531Cys mutation, which is just as common, is not in itself associated with hypercholesterolemia or an increased risk of ischemic heart disease.

Arg3500Gln

The frequency of Arg3500Gln was slightly lower in our study than in studies of patients with familial hypercholesterolemia or type IIa hyperlipidemia.^{11,13} In probands in the general population sample, the average effect of this substitution on plasma cholesterol and LDL cholesterol levels after correction for the effect of age and sex was substantial: the respective levels were 100 and 82 mg per deciliter higher than in the general population. These values were similar to those reported in carriers identified among patients with moderate hypercholesterolemia from the United States, Canada, and Austria,²⁸ though lower than the value of 116 mg per deciliter (3 mmol per liter) for both variables reported earlier, which were measured in 135 carriers identified mainly among patients with hypercholesterolemia from eight countries.¹³ This finding is in keeping with further results of our study, which suggest that the effect of this mutation on cholesterol levels was overestimated among patients with ischemic heart disease and familial hypercholesterolemia — most likely reflecting the fact that high cholesterol levels are a risk factor for ischemic heart disease as well as one of the clinical criteria used in the diagnosis of familial hypercholesterolemia. Thus, as a consequence of the study design, carriers identified in a group of patients with ischemic heart disease and familial hypercholesterolemia may have higher cholesterol levels than carriers identified in the general population.

We also found that the risk of ischemic heart disease in carriers of Arg3500Gln was seven times that of subjects in the general population as a whole. This finding was substantiated by the finding of a greatly increased risk of ischemic heart disease in Arg3500Gln carriers (odds ratio, 13), when the frequencies of this mutation in subjects with ischemic heart disease and those without it in the general-population sample were compared. In addition, carriers had an increased risk of peripheral arterial disease (odds ratio, 10) and hypertension (odds ratio, 10) when carriers and noncarriers in the general

population were compared. A possible explanation of the increased risk of hypertension in carriers could be the presence of widespread atherosclerosis.

Arg3531Cys

The Arg3531Cys mutation has previously been identified in eight subjects,^{8,14,15} six of whom had hypercholesterolemia. However, all eight carriers were identified among either patients with hyperlipidemia who were attending lipid clinics^{8,15} or patients with coronary artery disease¹⁴; therefore, the presence of hyperlipidemia could be a consequence of the study design. In support of this theory is the fact that there was an absence of simple cosegregation of this mutation with the hypercholesterolemia in two of the studies^{8,15}; no family data were available in the third study.¹⁴ The one patient in our study who had ischemic heart disease and the Arg3531Cys mutation, a 77-year-old man, was the only one of eight carriers who also had hypercholesterolemia. He did not have a family history of either hypercholesterolemia or premature ischemic heart disease. It therefore seems likely that this patient may have had another cause of hypercholesterolemia. Taken together, the absence of simple cosegregation in families, the absence of a family history in our patient with coronary artery disease, and the complete absence of hypercholesterolemia in any carriers in the general population in our study suggest that this mutation is not sufficient to cause hypercholesterolemia.

Although a previous study suggested a borderline overrepresentation of the Arg3531Cys mutation in patients with coronary artery disease as compared with healthy controls,¹⁵ our results suggest that this mutation, in keeping with the absence of an association with hypercholesterolemia, is also not associated with an increased risk of ischemic heart disease. However, it is possible that this mutation, when present with other factors such as hypertension, low HDL cholesterol levels, the apolipoprotein E ϵ 4 allele, and hypertriglyceridemia or factors affecting the binding of apolipoprotein B to the LDL receptor, slightly increases susceptibility to hypercholesterolemia and ischemic heart disease.

Arg3500Trp

The Arg3500Trp mutation has been identified in 2 of 907 subjects with hyperlipidemia who were attending a lipid clinic.⁹ We did not identify this mutation among 36 patients with familial hypercholesterolemia, 948 patients with ischemic heart disease, or 2021 subjects in the general population with cholesterol and apolipoprotein B levels above the 70th percentile for age and sex. This implies that in Denmark this mutation is not a common cause of either hypercholesterolemia or ischemic heart disease.

A potential limitation of our study was that all the subjects from the general population were 20 years

of age or older; any effect of the mutations on early mortality would therefore bias the results toward a lower prevalence of a given mutation and thus perhaps lead to an underestimate of the odds ratio for ischemic heart disease. Such an effect does not seem very likely for the Arg3500Gln mutation, since the frequency of 0.08 percent in this study is very similar to the frequency of 5 in 5000 reported in Danish newborns.²⁹ Given that the other two mutations studied have a similar⁹ or much less severe^{8,14} effect on apolipoprotein B dysfunction in vitro than the Arg3500Gln mutation and that neither segregates with cholesterol levels in families, an effect on early mortality of these mutations seems very unlikely.

In most white populations, the estimated frequency of Arg3500Gln ranges from 1 in 500 to 1 in 1000 and has been associated with hypercholesterolemia in studies both in vitro and in vivo.^{11,13} The generalizability of our data on Arg3531Cys and Arg3500Trp to other white populations is not completely clear. The available data suggest that the frequency of Arg3531Cys is similar to that in the general population in our study and that this mutation is not associated with hypercholesterolemia, whereas Arg3500Trp is probably very rare and also not associated with hypercholesterolemia.^{8,9,11,14,15,30}

Most studies searching for new mutations in the *APOB* gene potentially associated with hypercholesterolemia have concentrated on screening the presumed receptor-binding region^{8,9,14,15,30} in patients with hyperlipidemia and have used either reduced binding to the LDL receptor or other in vitro measures of apolipoprotein B dysfunction as an indicator of probable hypercholesterolemia in vivo. With the use of these methods only three such rare mutations have been identified, and these are the ones we studied. It is possible that mutations contributing to defective binding of apolipoprotein B may lie elsewhere in the gene or that there are common genetic variations in *APOB* that have only relatively small effects on cholesterol levels and are not detectable by these methods.

In conclusion, our results suggest that the Arg3500Gln mutation is at present the only known *APOB* mutation worth screening for in white patients with hypercholesterolemia or ischemic heart disease and their relatives.

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