

# Familial Defective Apolipoprotein B-100 and Increased Low-Density Lipoprotein Cholesterol and Coronary Artery Calcification in the Old Order Amish

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**Background:** Elevated low-density lipoprotein cholesterol (LDL-C) levels are a major cardiovascular disease risk factor. Genetic factors are an important determinant of LDL-C levels.

**Methods:** To identify single nucleotide polymorphisms associated with LDL-C and subclinical coronary atherosclerosis, we performed a genome-wide association study of LDL-C in 841 asymptomatic Amish individuals aged 20 to 80 years, with replication in a second sample of 663 Amish individuals. We also performed scanning for coronary artery calcification (CAC) in 1018 of these individuals.

**Results:** From the initial genome-wide association study, a cluster of single nucleotide polymorphisms in the region of the apolipoprotein B-100 gene (*APOB*) was strongly associated with LDL-C levels ( $P < 10^{-68}$ ). Additional genotyping revealed the presence of R3500Q, the mutation responsible for familial defective apolipoprotein B-100, which was

also strongly associated with LDL-C in the replication sample ( $P < 10^{-36}$ ). The R3500Q carrier frequency, previously reported to be 0.1% to 0.4% in white European individuals, was 12% in the combined sample of 1504 Amish participants, consistent with a founder effect. The mutation was also strongly associated with CAC in both samples ( $P < 10^{-6}$  in both) and accounted for 26% and 7% of the variation in LDL-C levels and CAC, respectively. Compared with non-carriers, R3500Q carriers on average had LDL-C levels 58 mg/dL higher, a 4.41-fold higher odds (95% confidence interval, 2.69-7.21) of having detectable CAC, and a 9.28-fold higher odds (2.93-29.35) of having extensive CAC (CAC score  $\geq 400$ ).

**Conclusion:** The R3500Q mutation in *APOB* is a major determinant of LDL-C levels and CAC in the Amish.

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**E**LEVATED LOW-DENSITY LIPOPROTEIN cholesterol (LDL-C) levels are associated with an increased risk of cardiovascular disease (CVD). Twin and family studies<sup>1-3</sup> suggest that 40% to 80% of the population variation in levels of LDL-C is attributable to genetic factors. The variability in LDL-C levels in the general population is likely polygenic and affected by environmental factors. Rare forms of monogenic hypercholesterolemia have been identified, including familial hypercholesterolemia due to mutations in the LDL receptor gene and familial defective apolipoprotein B-100 due to mutations in the apolipoprotein B (OMIM 107730) (*APOB*).<sup>4,5</sup>

To identify genes associated with variations in LDL-C levels and their relation with subclinical coronary atherosclerosis, we performed a genome-wide association study (GWAS) on LDL-C levels in a socially and

culturally homogeneous Old Order Amish population residing in Lancaster County, Pennsylvania. Almost all the participants descended from fewer than 300 founders who emigrated from Switzerland during the 1700s. Their diet is relatively homogeneous, and their use of cholesterol-lowering medications is low, minimizing the effect of environmental factors that might obscure or modify genetic determinants of LDL-C levels.

## METHODS

### STUDY POPULATION

This study began with a GWAS of LDL-C levels in an initial cohort of 841 individuals (stage 1). Stage 1 individuals were participants in the Heredity and Phenotype Intervention (HAPI) Heart Study,<sup>6</sup> which was initiated in 2003 to identify genes that interact with short-term environmental exposures to

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modify risk factors for CVD. Participants were members of the Old Order Amish community aged 20 years or older and considered to be relatively healthy based on the exclusion criteria of severe hypertension (blood pressure >180/105 mm Hg), malignancy, and kidney, liver, or untreated thyroid disease. Further description of the HAPI Heart Study design is available on request from the authors.

The HAPI Heart Study participants underwent measurement of CVD risk factors and questioning about their history of CVD. Physical examinations were conducted at the Amish Research Clinic in Strasburg, Pennsylvania. Blood samples were collected after an overnight fast. Those taking lipid-lowering medications at enrollment (7 participants) discontinued use 7 days before examination. Serum lipid and high-density lipoprotein cholesterol levels were assayed by Quest Diagnostics (Horsham, Pennsylvania). All the participants had triglyceride levels lower than 400 mg/dL (to convert to millimoles per liter, multiply by 0.0113), and LDL-C levels were calculated according to the formula of Friedewald et al.<sup>7</sup> Lipoprotein subfractions were measured using Vertical Auto Profile technology (VAP; Atherotech, Birmingham, Alabama). Hypertension was defined as a systolic blood pressure of 140 mm Hg or higher, a diastolic blood pressure of 90 mm Hg or higher, or use of prescription blood pressure-lowering medications. Diabetes mellitus was defined as a fasting glucose level of 126 mg/dL or higher (to convert to millimoles per liter, multiply by 0.0555) or current use of prescription medications for diabetes. Smoking habits were recorded; current smoking status included the use of cigarettes, pipes, or cigars. The measurements for coronary artery calcification (CAC) by electron beam computed tomography were available in 355 HAPI Heart Study participants.

In stage 2, we genotyped an independent sample of 663 Old Order Amish individuals aged 30 years or older in whom we had measured serum lipid levels and CAC. These individuals had previously been recruited into the Amish Family Calcification Study between 2002 and 2006 to identify the genetic determinants of CAC.<sup>8</sup> Participants were recruited without regard to their CVD status. The study protocol was approved by the institutional review board at the University of Maryland and participating institutions. Informed consent was obtained from each of the study participants.

## GENOTYPING

Genotyping was performed in all stage 1 study participants using the Affymetrix GeneChip Human Mapping 500K Array Set (Affymetrix, Santa Clara, California), including 500 568 single-nucleotide polymorphisms (SNPs). The Affymetrix GeneChip Genotyping Analysis Software and BRLMM genotype-calling algorithm were used to generate SNP data files. The mean genotype call rate in the stage 1 sample was 98.3%. The SNPs (n=98 806) with minor allele frequencies less than 2% in the overall sample were removed from further analyses. Finally, 369 241 SNPs that passed quality control and Hardy-Weinberg equilibrium checks (at  $P < .001$ ) were retained for analysis.

A region on chromosome arm 2p showed strong evidence of association, implicating the nearby gene, *APOB*, as an attractive positional candidate gene. To determine whether the observed association could be explained by the presence of the *APOB* R3500Q (OMIM 107730.0009) (rs5742904) mutation, which is responsible for familial defective *APOB*-100, we genotyped this variant in stage 1 and 2 participants using Custom TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, California).

## CAC SCORING

Coronary artery calcification was measured by electron beam computed tomography using an Imatron C-150 scanner (Ima-

tron Inc, San Francisco, California) (scanning protocol available on request from the authors). Coronary artery calcification was quantified using the Agatston method, which incorporates density and area.<sup>9</sup> The sum of the Agatston scores in the 4 epicardial arteries was considered the CAC score.

## STATISTICAL ANALYSIS

Association analyses of LDL-C levels and other quantitative traits were performed under a variance component model that assesses the effect of genotype, as an additive effect, on the quantitative trait while simultaneously estimating the effects of age, age<sup>2</sup>, sex, and a polygenic component to account for phenotypic correlation due to relatedness. The polygenic component was modeled using the relationship matrix derived from the complete pedigree structure because all the participants are related. Association analysis using the complete pedigree structure was performed using mixed-model analysis for pedigree software developed by one of us (J.R.O.). We considered a genome-wide significance threshold to be  $P < 10^{-7}$ .

Pairwise linkage disequilibrium correlation statistics ( $r^2$ ) were computed using Haploview software (<http://www.broadinstitute.org/haploview/haploview>). Power calculations using the Genetic Power Calculator program<sup>10</sup> indicated we would have 80% power to detect SNPs in the stage 1 sample (n=841) accounting for approximately 4% of phenotypic variation at  $\alpha = 10^{-7}$ . For example, a SNP accounting for 4% of the trait variance in LDL-C levels that had an allele frequency of 0.20 would be associated with a 13- to 14-mg/dL (to convert to millimoles per liter, multiply by 0.0259) increase in LDL-C per copy of the mutant allele.

Because the distribution of CAC scores was positively skewed, the scores were natural log-transformed after adding 1.<sup>11</sup> Age- and sex-adjusted CAC scores were calculated using linear regression, and standardized residuals were obtained. The residuals, which were approximately normally distributed, were used for analysis of CAC quantity. Odds ratios describing the association between the R3500Q mutation and the presence of CAC and having extensive CAC (CAC score  $\geq 400$ ) were estimated using generalized estimating equations incorporated into SAS version 9.1 (SAS Institute, Inc, Cary, North Carolina), with sibship membership included as a random effect. All reported  $P$  values are 2-sided and are not adjusted for multiple comparisons.

## RESULTS

The clinical characteristics of stage 1 and 2 participants are given in **Table 1**. The mean ages of the stage 1 and 2 samples were 43.7 and 59.6 years, respectively. The mean (SE) heritability of LDL-C, adjusted for age, age<sup>2</sup>, and sex, was 0.60 (0.07) ( $P = 1.2 \times 10^{-20}$ ) in the stage 1 samples and 0.51 (0.08) ( $P = 2.8 \times 10^{-14}$ ) in the stage 2 samples.

In the GWAS, the strongest SNP associations with LDL-C levels were observed for a cluster of 68 SNPs spanning a 12-megabases region on chromosome arm 2p, with  $P$  values ranging from  $P < 10^{-9}$  to a peak association of  $P < 10^{-68}$  for the SNP rs4971516 (300 kilobases [kb] downstream of the *APOB* gene) (additive model, minor allele frequency=0.056). (Detailed GWAS results of LDL-C levels are available on request from the authors.)

One gene in the associated region, *APOB*, located 300 kb downstream of rs4971516, was considered the most compelling positional candidate gene for several reasons. First, the phenotypic distribution of LDL-C and the strength of the observed associations indicated the presence of a highly penetrant allele. Second, rare mutations in *APOB* have been found in patients with familial defective *APOB*-100.<sup>5</sup> Third,

**Table 1. Clinical Characteristics of the Stage 1 and 2 Participants**

Characteristic	Stage 1 (n=841)	Stage 2 (n=663)
Age, mean (SD), y	43.7 (13.9)	59.6 (13.1)
Male, No. (%)	453 (53.9)	290 (43.7)
BMI, mean (SD)	26.5 (4.5)	28.3 (5.4)
Blood pressure, mean (SD), mm Hg		
Systolic	121.1 (14.7)	119.9 (17.5)
Diastolic	76.5 (8.7)	70.8 (9.0)
Cholesterol, mean (SD), mg/dL		
Total	208 (46)	214 (43)
LDL-C	139 (43)	139 (40)
HDL-C	56 (14)	56 (15)
Triglycerides, median (IQR), mg/dL	56 (42-80)	78 (56-114)
Hypertension, No. (%)	115 (13.7)	154 (23.2)
Diabetes mellitus, No. (%)	5 (0.6)	27 (4.1)
Current smoker, No. (%)	90 (10.7)	49 (7.4)
Lipid medication use, No. (%)	7 (0.8)	45 (6.8)
Self-reported history of CVD, No. (%)	22 (2.6)	81 (12.2)
Presence of CAC, score $\geq 1$ , No. (%) <sup>a</sup>	137 (38.6)	394 (59.4)
Extensive CAC, score $\geq 400$ , No. (%) <sup>a</sup>	24 (6.8)	122 (18.4)
CAC score, median (IQR) <sup>a</sup>	0 (0-24.6)	11.3 (0-233.1)

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CAC, coronary artery calcification; CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol.

SI conversion factors: To convert HDL-C, LDL-C, and total cholesterol to millimoles per liter, multiply by 0.0259; triglycerides to millimoles per liter, multiply by 0.0113.

<sup>a</sup>Available for 355 participants in stage 1 and 663 participants in stage 2.

examination of haplotypes among presumed carriers suggested the presence of an extended region of haplotype sharing that included *APOB* compatible with a single mutation transmitted through the population from a common ancestor. Thus, we hypothesized that a mutation in the *APOB* gene may have entered the Amish population and increased in frequency through genetic drift. We then genotyped *R3500Q* (dbSNP designation: rs5742904) in the stage 1 sample.

*R3500Q* was in near-complete linkage disequilibrium with rs4971516 ( $D' = 0.99$  and  $r^2 = 0.96$ ) and was strongly associated with total cholesterol ( $P = 1.60 \times 10^{-40}$ ) and LDL-C ( $P = 1.84 \times 10^{-52}$ ) levels and with the presence ( $P < .001$ ) and quantity ( $P = 6.67 \times 10^{-7}$ ) of CAC comparing *R3500Q* heterozygote carriers with noncarriers (**Table 2**). The frequency of the *R3500Q* allele was 0.064; the 12% of the population that carried the mutant allele had LDL-C levels approximately 61 mg/dL higher than those of noncarriers. We performed an additional GWAS that included *APOB R3500Q* in the model as a covariate but did not identify any further SNPs significantly associated with LDL-C concentrations (data not shown).

In stage 2 participants, the frequency of the *R3500Q* allele was 0.06. *R3500Q* was strongly associated with LDL-C levels ( $P < 10^{-30}$ ) and accounted for an approximately 55-mg/dL increase in LDL-C levels. The mutation was associated with a higher prevalence ( $P < .001$ ) and quantity ( $P = 9.2 \times 10^{-7}$ ) of CAC in this independent replication sample. *R3500Q* genotypes were not associated with LDL-C subclass particle patterns (data not shown) or appreciably with high-density lipoprotein cholesterol levels (Table 2).

Because of the consistency of effect (61- and 55-mg/dL increases in LDL-C levels per allele in stages 1 and 2, respectively), stage 1 and 2 samples were combined to permit estimation of age- and sex-specific effects of the *R3500Q* mutation on LDL-C levels. *R3500Q* carriers had higher LDL-C levels in each age group and in both sexes (**Figure 1**). In the combined sample, the *R3500Q* mutation accounted for 26% of the variation in age- and sex-adjusted LDL-C levels.

The *R3500Q* mutation also accounted for 7% of the variation in adjusted CAC quantity in the combined sample. The prevalence of any detectable CAC and of extensive CAC (defined as a CAC score  $\geq 400$ )<sup>11</sup> by age in the combined sample is shown in **Figure 2** according to carrier status. Before age 40 years, the prevalence of CAC was low in carriers and noncarriers. After age 50 years, *R3500Q* carriers had greater prevalence of both any CAC and extensive CAC in every group. The *R3500Q* mutation accounted for 4.5% of CAC presence and 12.8% of extensive CAC presence in this population. After adjusting for age, sex, lipid-lowering medication use, and sibship, *R3500Q* carriers had a 4.41-fold higher odds (95% confidence interval [CI], 2.69-7.21) of having detectable CAC compared with noncarriers. Moreover, carriers had an increased risk of having a CAC score of at least 400 compared with noncarriers (odds ratio [OR], 9.28; 95% CI, 2.93-29.35) (**Table 3**).

To determine whether the increase in CAC in *APOB R3500Q* carriers could be explained by increased LDL-C levels measured at a single time point, we assessed the effects of *R3500Q* on the degree of CAC after additional adjustment for LDL-C levels in the combined sample. After this adjustment, the *R3500Q* mutation explained 4.1% of the residual variance in the degree of CAC. Even with LDL-C in the model, *R3500Q* remained a strong independent predictor of CAC presence (OR, 3.18; 95% CI, 1.81-5.59) and of extensive CAC (5.43; 1.54-19.18) (Table 3).

Results of the association analyses were confirmed using the family-based association test (FBAT).<sup>12</sup> These analyses revealed excess *R3500Q* transmission to be associated with higher LDL-C levels ( $P = 4.0 \times 10^{-11}$ ) and a greater degree of CAC ( $P = 10^{-5}$ ).

## COMMENT

The surprising finding from this study was the identification of the *R3500Q* mutation in *APOB* as a major determinant of LDL-C levels and CAC in the Old Order Amish. This mutation accounted for approximately 13% of extensive CAC in the population, a substantial effect given that CAC is strongly associated with the risk of future cardiovascular events. The present sample included 181 carriers of the *R3500Q* allele (ie, approximately 1 of every 8-9 participants), a much higher frequency for this mutation than in any other single population previously reported.<sup>13</sup> Because the Old Order Amish in Lancaster County are descendants of approximately 300 founders who emigrated to Pennsylvania from Switzerland in the latter part of the 1700s,<sup>14</sup> we speculate that the *R3500Q* mutation was introduced into the population by a single founding ancestor and has been maintained in the population at relatively high frequency through genetic drift.

**Table 2. Characteristics of the Study Sample According to *APOB R3500Q* Genotype in Stages 1 and 2<sup>a</sup>**

Characteristic	<i>R3500Q</i> Genotype							
	Stage 1				Stage 2			
	<i>RR</i> (n=731)	<i>RQ</i> (n=106)	<i>QQ</i> (n=2)	<i>P</i> Value <sup>b</sup>	<i>RR</i> (n=585)	<i>RQ</i> (n=75)	<i>QQ</i> (n=3)	<i>P</i> Value <sup>b</sup>
Age, mean (SD), y	43.9 (13.9)	41.8 (14.5)	59.0 (5.7)	.16	59.8 (12.9)	57.3 (14.0)	70.0 (17.8)	.12
Male, No. (%)	395 (54.0)	58 (54.7)	0	.90	260 (44.4)	29 (38.7)	1 (33.3)	.34
BMI, mean (SD)	26.6 (4.5)	26.0 (4.6)	21.6 (2.7)	.21	28.3 (5.6)	28.6 (4.5)	27.4 (3.7)	.90
Blood pressure, mean (SD), mm Hg								
Systolic	121.1 (14.9)	120.7 (13.5)	143.0 (19.8)	.65	119.7 (17.8)	121.1 (15.7)	124.3 (23.2)	.48
Diastolic	76.6 (8.6)	75.3 (9.4)	76.0 (2.8)	.31	70.8 (9.1)	70.6 (8.9)	68.0 (2.6)	.41
Cholesterol, mean (SD), mg/dL								
Total	200 (40)	260 (49)	391 (18)	$1.60 \times 10^{-40}$	208 (37)	259 (55)	296 (83)	$1.48 \times 10^{-23}$
LDL-C	130 (35)	193 (43)	327 (5)	$1.84 \times 10^{-52}$	133 (32)	187 (52)	221 (88)	$2.37 \times 10^{-37}$
HDL-C	56 (15)	54 (14)	56 (15)	.37	57 (15)	52 (16)	55 (13)	.06
Triglycerides, median (IQR), mg/dL	59 (43-88)	51 (40-73)	43 (37-48)	.07	77 (56-114)	88 (54-121)	74 (68-145)	.37
Hypertension, No. (%)	105 (14.4)	9 (8.5)	1 (50.0)	.10	134 (22.9)	19 (25.3)	1 (33.3)	.64
Diabetes mellitus, No. (%)	5 (0.7)	0	0	.39	23 (3.9)	4 (5.3)	0	.56
Current smoker, No. (%)	79 (10.8)	11 (10.4)	0	.89	44 (7.5)	5 (6.7)	0	.79
Lipid medication use, No. (%)	4 (0.6)	3 (2.8)	0	.02	32 (5.5)	12 (16.0)	1 (33.3)	<.001
Self-reported history of CVD, No. (%)	18 (2.5)	3 (2.8)	1 (50.0)	.82	72 (12.3)	8 (10.7)	1 (33.3)	.48
Presence of CAC, score $\geq 1$ , No. (%) <sup>c</sup>	114 (35.2)	21 (72.4)	2 (100)	<.001	340 (58.1)	52 (69.3)	2 (66.7)	<.001
Extensive CAC, score $\geq 400$ , No. (%) <sup>c</sup>	16 (4.9)	7 (24.1)	1 (50.0)	<.001	98 (16.8)	22 (29.3)	2 (66.7)	<.001
CAC score, median (IQR) <sup>c</sup>	0 (0-8.9)	72.2 (0-315.5)	763.1 (106.0-1420.1)	$6.67 \times 10^{-7}$	8.2 (0-203.2)	99.2 (0-587.4)	585.0 (0-1997.9)	$9.23 \times 10^{-7}$

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CAC, coronary artery calcification; CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol.

SI conversion factors: To convert HDL-C, LDL-C, and total cholesterol to millimoles per liter, multiply by 0.0259; triglycerides to millimoles per liter, multiply by 0.0113.

<sup>a</sup>The size of the subsamples does not total 1504 because 2 participants in stage 1 failed the follow-up *R3500Q* genotype.

<sup>b</sup>Based on comparison of *RR* genotype with *RQ* genotype groups because of the small number in the *QQ* genotype group. All the comparisons were adjusted for age, age<sup>2</sup>, sex, and family structure. Lipid levels were further adjusted for the use of lipid-lowering medications. The *P* values for triglycerides are based on natural logarithm transformation. Percentage comparisons are based on the  $\chi^2$  test.

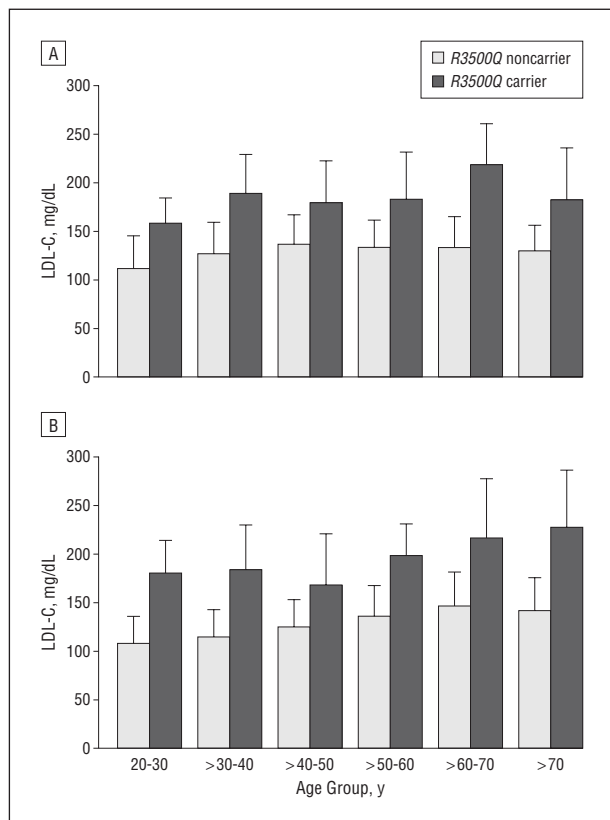
<sup>c</sup>The CAC comparisons are based on 324, 29, and 2 patients with the *RR*, *RQ*, and *QQ* genotypes, respectively, in stage 1 and on 585, 75, and 3 patients with the *RR*, *RQ*, and *QQ* genotypes, respectively, in stage 2. Patients with the *QQ* genotype were excluded as in the previous footnote because of the small sample size in this group. The *P* values for presence of (extensive) CAC were adjusted for age and sex. The *P* values for CAC score based on age- and sex-adjusted residuals were adjusted for use of lipid-lowering medication and family structure.

The *R3500Q* mutation, which is responsible for familial defective apolipoprotein B-100,<sup>5</sup> is believed to prevent the proper folding of apolipoprotein B by altering the interactions between 2 amino acids, thereby reducing the ability of the LDL-C particle to bind to the LDL receptor.<sup>15</sup> The precise effects of this mutation on CVD risk have been hard to quantify because previous studies have either included few participants sampled from the general population or have focused on samples enriched with prevalent ischemic heart disease. In the largest published population sample, the *APOB R3500Q* mutation was associated with total cholesterol levels 100 mg/dL higher and LDL-C levels 82 mg/dL higher based on only 7 carriers identified through a population screen of 9255 Danes<sup>16</sup> compared with the present estimated effect sizes of 55 mg/dL for total cholesterol and 58 mg/dL for LDL-C based on a sample of 181 carriers. Previous studies<sup>16-18</sup> based on identification of individuals with hypercholesterolemia or ischemic heart disease have reported even larger effect sizes. *R3500Q* has been associated with ischemic heart disease, although a clear effect of this mutation on early coronary artery atherosclerosis has not been established. These findings reinforce the idea that the *APOB R3500Q* mutation may increase ischemic heart disease risk by increasing LDL-C levels and CAC. This view is supported

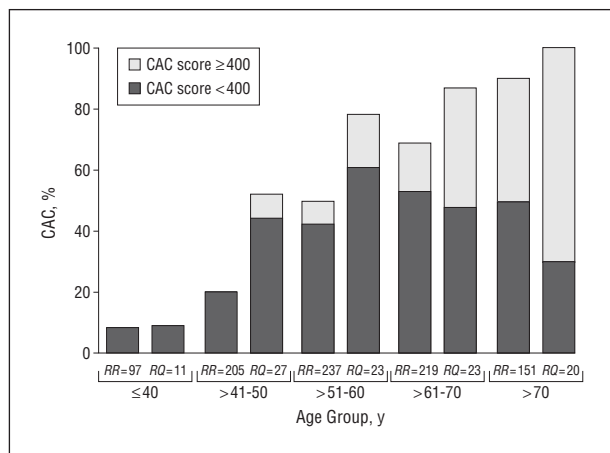
by evidence that the *APOB R3500Q* mutation is a direct cause of elevated LDL-C levels, the known associations of *APOB R3500Q* and LDL-C levels with ischemic heart disease, and previous studies,<sup>8,19-21</sup> including from this population,<sup>8</sup> showing that LDL-C concentrations are associated with the degree and presence of CAC.

The association between the *R3500Q* mutation and CAC could not be accounted for entirely by a 1-time measure of LDL-C levels because the *R3500Q* mutation remained strongly associated with CAC even after adjusting for or stratifying by LDL-C. A possible explanation is that *R3500Q* carriers have been exposed to a lifelong increase in LDL-C levels, which cannot be captured by a single cross-sectional LDL-C measurement. Alternatively, the mutation may be associated with features related to LDL-C metabolism that affect CAC development independently of circulating LDL-C levels. However, through this analysis of LDL subparticle distributions, we found no evidence to suggest that the mutation was associated with more atherogenic LDL.

Controlling for *R3500Q* in the GWAS abolished almost all other associations with SNPs in the chromosome 2 region (data not shown). However, many SNPs in this region are in high-linkage disequilibrium, and carriers of the *R3500Q* mutation share a common extended haplotype. We,



**Figure 1.** Mean low-density lipoprotein cholesterol (LDL-C) levels in *APOB* *R3500Q* noncarriers and carriers in the combined sample by age in men (A) and women (B). To convert LDL-C to millimoles per liter, multiply by 0.0259. Error bars represent standard deviation.



**Figure 2.** Prevalence of detectable and extensive coronary artery calcification (CAC) in *APOB* *R3500Q* noncarriers (RR) and carriers (RQ) in the combined sample by age group.

thus, cannot exclude the possibility that there might be other variants in linkage disequilibrium with *R3500Q* that are functional and contribute to elevated LDL-C levels and increased CAC levels. However, given what is known about the *R3500Q* mutation,<sup>15,22</sup> there does not seem to be a strong rationale for positing the presence of additional functional variants in the region that co-segregate with the *R3500Q* mutation. Even if such variants did exist, it would be difficult to detect and attribute functional conse-

**Table 3.** ORs for the Presence of CAC and Extensive CAC Among *APOB* *R3500Q* Carriers Compared With Noncarriers in the Combined Sample<sup>a</sup>

Variable	Model 1		Model 2: Model 1 + LDL-C	
	OR (95% CI)	P Value	OR (95% CI)	P Value
Presence of CAC <sup>b</sup>	4.41 (2.69-7.21)	<.001	3.18 (1.81-5.59)	<.001
Presence of extensive CAC <sup>c</sup>	9.28 (2.93-29.35)	<.001	5.43 (1.54-19.18)	.009

Abbreviations: CAC, coronary artery calcium; CI, confidence interval; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio.

<sup>a</sup>The model 1 covariates were age, sex, use of lipid-lowering medication, and sibships. Additional adjustment for body mass index, systolic and diastolic blood pressure, high-density lipoprotein cholesterol, and smoking did not appreciably change the results.

<sup>b</sup>A CAC score  $\geq 1$  vs  $< 1$ .

<sup>c</sup>A CAC score  $\geq 400$  vs  $< 1$ .

quences to them because of the limited recombination along the at-risk haplotype.

The *R3500Q* mutation is relatively uncommon in non-Amish populations. Population-based surveys have reported carrier frequencies of 1 per 500 to 1 per 1250,<sup>23</sup> and a carrier frequency of 1 per 209 has been reported in a Swiss population.<sup>13</sup> Of 1840 individuals genotyped in the Baltimore-Washington Stroke Prevention in Young Women Study,<sup>24</sup> we identified 2 heterozygotes, for a carrier rate of 0.11% (range, 0.02%-0.44%), in line with prior estimates of 0.08% to 0.41% reported from population-based studies.<sup>23</sup> Importantly, even this low rate corresponds to a large number of carriers at the population level. For example, a carrier rate of 0.1% in the United States (2008 estimated population size of 305 million<sup>25</sup>) would translate into an estimated 305 000 *R3500Q* carriers. The markedly increased level of LDL-C and degree of CAC due to this single mutation have potentially important implications for personalized medicine in the full US population and in the Amish population, in whom this mutation is enriched. Because *R3500Q* carriers are more likely than noncarriers to have an increased burden of subclinical coronary atherosclerosis even at the same LDL-C levels, such individuals may benefit from earlier and more aggressive treatment with lipid-lowering medications. Is there value in screening for *R3500Q* carriers at the population level? If so, how would this be most effectively accomplished, for example, by screening for the mutation or by screening for LDL-C levels? Prospective clinical trials are necessary to address these questions and to determine the most efficacious CAC treatment strategies for carriers of this mutation, including at what age to start treatment.

In summary, we demonstrated that carriers of the *R3500Q* mutation in *APOB* are frequent in the Old Order Amish. In this population, the *R3500Q* mutation is a major determinant of LDL-C levels and CAC.

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