

ORIGINAL ARTICLE



# Rare Protein-Truncating Variants in *APOB*, Lower Low-Density Lipoprotein Cholesterol, and Protection Against Coronary Heart Disease

**BACKGROUND:** Familial hypobetalipoproteinemia is a genetic disorder caused by rare protein-truncating variants (PTV) in the gene encoding *APOB* (apolipoprotein B), the major protein component of LDL (low-density lipoprotein) and triglyceride-rich lipoprotein particles. Whether heterozygous *APOB* deficiency is associated with decreased risk for coronary heart disease (CHD) is uncertain. We combined family-based and large scale gene-sequencing to characterize the association of rare PTVs in *APOB* with circulating LDL-C (LDL cholesterol), triglycerides, and risk for CHD.

**METHODS:** We sequenced the *APOB* gene in 29 Japanese hypobetalipoproteinemia families, as well as 57 973 individuals derived from 12 CHD case-control studies—18 442 with early-onset CHD and 39 531 controls. We defined PTVs as variants that lead to a premature stop, disrupt canonical splice-sites, or lead to insertions/deletions that shift reading frame. We tested the association of rare *APOB* PTV carrier status with blood lipid levels and CHD.

**RESULTS:** Among 29 familial hypobetalipoproteinemia families, 8 families harbored *APOB* PTVs. Carrying 1 *APOB* PTV was associated with 55 mg/dL lower LDL-C ( $P=3\times 10^{-5}$ ) and 53% lower triglyceride level ( $P=2\times 10^{-4}$ ). Among 12 case-control studies, an *APOB* PTV was present in 0.038% of CHD cases as compared to 0.092% of controls. *APOB* PTV carrier status was associated with a 43 mg/dL lower LDL-C ( $P=2\times 10^{-7}$ ), a 30% decrease in triglycerides ( $P=5\times 10^{-4}$ ), and a 72% lower risk for CHD (odds ratio, 0.28; 95% CI, 0.12–0.64;  $P=0.002$ ).

**CONCLUSIONS:** Rare PTV mutations in *APOB* which are associated with lower LDL-C and reduced triglycerides also confer protection against CHD.

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**Key Words:** cholesterol ■ genetics  
■ human ■ hypobetalipoproteinemia  
■ triglycerides

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**A**POB (apolipoprotein B) is a structural component of lipoproteins with a functional role as a ligand that binds to cell-surface receptors, including the LDL (low-density lipoprotein) receptor.<sup>1</sup> Rare protein-truncating variants (PTVs) that truncate *APOB* lead to familial hypobetalipoproteinemia (FHBL, OMIM no. 107730), an autosomal dominant genetic disorder characterized by low levels of plasma LDL-C (LDL cholesterol).<sup>2,3</sup> Those affected by FHBL display not only lower LDL-C but also nonalcoholic fatty liver disease.

Mipomersen is an antisense drug approved by the US Food and Drug Administration that targets the mRNA for *APOB* and inhibits the synthesis of the apoB protein. Mipomersen is approved to lower cholesterol in individuals with homozygous familial hypercholesterolemia.<sup>4</sup> Mipomersen leads to a significant decrease in LDL-C levels in individuals with homozygous familial hypercholesterolemia; however, similar to *APOB* PTVs, mipomersen also leads to fatty liver and elevated liver function test abnormalities.<sup>5</sup>

Carriers of PTVs in *APOB* display lower LDL-C<sup>6</sup> and triglyceride levels and as such, might be expected to have reduced risk for coronary heart disease (CHD). However, to date, there is little evidence as to whether loss of *APOB* function will affect CHD risk,<sup>7,8</sup> and a pharmacological test of this hypothesis with mipomersen seems unlikely because of the adverse effects of this therapy. As such, here, we took a human genetics approach to address the following: (1) the extent to which *APOB* PTV carrier status is associated with serum lipid levels using 29 Japanese FHBL families; and (2) whether PTVs in the *APOB* gene are associated with lipid levels and CHD among ≈58 000 individuals from large case-control studies.

## METHODS

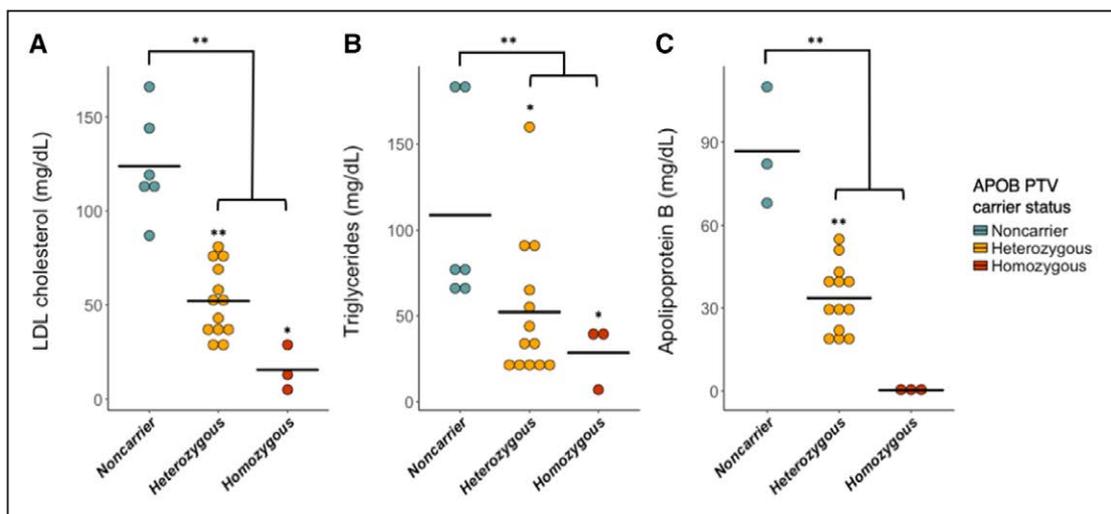
All participants in the study provided written informed consent for genetic studies. The institutional review boards at the Broad Institute and each participating institution approved the study protocol. To minimize the possibility of unintentionally sharing information that can be used to reidentify private information, a subset of the data generated for this study are available at dbGaP (The database of Genotypes and Phenotypes) and can be accessed at through dbGaP Study Accessions: phs000814.v1.p1 (ATVB [Italian Atherosclerosis, Thrombosis, and Vascular Biology]), phs001398.v1.p1 (BRAVE [Bangladesh Risk of Acute Vascular Events study]), phs000279.v2.p1 (EOMI [Exome Sequencing Project Early-Onset Myocardial Infarction]), phs001098.v1.p1 (JHS [Jackson Heart Study]), phs001000.v1.p1 (Leicester [Leicester Myocardial Infarction]), phs000990.v1.p1 (North German MI [North German Myocardial Infarction]), phs000916.v1.p1 (South German MI [South German Myocardial Infarction]), phs000806.v1.p1 (OHS [Ottawa Heart Study]), phs000883.v1.p1 (PROCARDIS [Precocious Coronary Artery Disease]), phs000917.v1.p1 (PROMIS [Pakistan Risk of Myocardial Infarction Study]), phs000902.v1.p1 (Regicor [Registre Gironi del COR (Gerona Heart Registry)]).

The full methods are available in the [Data Supplement](#).

## RESULTS

### Hypobetalipoproteinemia Families

In FHBL pedigrees, we tested whether *APOB* PTVs were associated with serum lipids and apolipoproteins. We recruited 29 Japanese FHBL families and sequenced the exome in 69 participants from the families. Of those, 12 individuals in 4 families and 4 single probands harbored *APOB* PTVs that appeared causative (Figure 1 in the [Data Supplement](#)). Among these individuals, 3 carried PTVs in homozygous state and 13 harbored PTVs in heterozygous form. Identi-



**Figure 1.** Serum lipid levels among Japanese individuals.

LDL-C (low-density lipoprotein cholesterol; (A), triglyceride (B), and apoB (C) are compared among heterozygous (n=13) and homozygous (n=3) *APOB* protein truncating variant carriers and noncarriers (n=6). Each dot represents an individual's lipid level. Each horizontal line indicates mean value of the lipid level for each genotype. *P* values were calculated using Mann-Whitney *U* test. PTV indicates protein truncating variant. \**P*<0.05, \*\**P*<0.01 compared with noncarriers.

fied causative variants were confirmed through Sanger sequencing (primers shown in Table I in the [Data Supplement](#)). Five of these *APOB* PTVs had not been previously described in FHBL families (Table II in the [Data Supplement](#)). The *APOB* PTVs cosegregated with serum LDL-C and apoB levels. Both homozygote and heterozygous carriers exhibited reduction of serum LDL-C, triglyceride, and apoB levels (Figure 1, Table III in the [Data Supplement](#)). Based on linear regression for effect size (95% CI), carrying a PTV in *APOB* was associated with lower LDL-C (−55 mg/dL; 95% CI, −68 to −42; Mann-Whitney  $U$   $P=2.7\times 10^{-5}$ ), lower triglyceride levels (−53%; 95% CI, −72 to −21; Mann-Whitney  $U$   $P=1.7\times 10^{-4}$ ), and lower apoB (−43 mg/dL; 95% CI, −53 to −33; Mann-Whitney  $U$   $P=2.1\times 10^{-3}$ ) after adjusting for age and sex.

In the set of Japanese FHBL individuals, *APOB* PTV carriers had higher hepatobiliary enzymes compared

with noncarriers (Table III in the [Data Supplement](#)). The 3 individuals homozygous for *APOB* PTV were all >40 years old with evidence of fatty liver on imaging and associated elevation in hepatobiliary enzymes (Table IV in the [Data Supplement](#)).

## Association of *APOB* PTVs With Lipids and CHD

We sequenced the *APOB* gene in a total of 57 973 participants from the MIGen (Myocardial Infarction Genetics Consortium) of African, European, and South Asian ancestries (N=33 835) and from participants of European ancestry (N=24 138) in the Geisinger Health System and Regeneron Genetics Center DiscovEHR study who were recruited as part of the MyCode Community Health Initiative<sup>9</sup> (Table 1). Across a total of 57 973 individuals in 12 studies (Table V in

**Table 1. Baseline Characteristics of Myocardial Infarction Genetics Consortium and DiscovEHR Study Participants**

	Myocardial Infarction Genetics Consortium		Geisinger Health System DiscovEHR Cohort	
	CHD	CHD-Free	CHD	CHD-Free
	Cases	Controls	Cases*	Controls
	N=14 243	N=19 592	N=4199	N=19 939
Age, y, mean (SD)	46.2 (8.0)	56.5 (12.1)	51.8 (7.3)†	45.0 (12)†
Male gender, n (%)	10 930 (77)	14 556 (74)	1 938 (46)	3 848 (19)
BMI, kg/m <sup>2</sup> , median (IQR)	26.8 (24.1–30.1)	26.2 (23.8–29.0)	32.3 (28–38)	31.0 (26–37)
Current smoker, n (%)	6 307 (48)	4 463 (24)	986 (23)	4 065 (20)
Ancestry				
European	6 682 (47)	7 201 (37)	4 199 (100)	19 939 (100)
Asian	7 180 (51)	11 045 (57)	0 (0)	0 (0)
African	206 (1)	1 128 (6)	0 (0)	0 (0)
Other	28 (<0.001)	0 (0)	0 (0)	0 (0)
Medical history				
Hypertension,‡ n (%)	3 212 (31)	5 548 (36)	3 373 (80)	12 444 (34)
Type 2 diabetes mellitus,§ n (%)	1 872 (15)	2 056 (12)	1 520 (36)	2 611 (13)
Lipid-lowering medication,   n (%)	3 463 (35)	538 (4)	2 494 (59)	3 639 (18)
Lipid profile, mg/dL				
LDL cholesterol,   mean (SD)	142 (53.9)	119 (43)	130 (40)	122 (37)
HDL cholesterol, mean (SD)	37 (12)	41 (14)	46 (13)	52 (15)
Triglycerides, median (IQR)	167 (117–247)	151 (102–222)	154 (112–215)	119 (85–167)
Total cholesterol,   mean (SD)	219 (58)	194 (49)	214 (43)	203 (42)

BMI indicates body mass index; CHD, coronary heart disease; DiscovEHR, DiscovEHR partnership of the Regeneron Genetics Center and Geisinger Health System; ICD-9, *International Classification of Diseases, Ninth Revision*; IQR: interquartile range; HDL, high-density lipoprotein; and LDL, low-density lipoprotein.

\*Participants were considered to have early-onset (men <55 y, women <65 y) CHD if they had a history of coronary revascularization in the electronic health records, or history of acute coronary syndrome, ischemic heart disease, or exertional angina (ICD-9 codes 410\*, 411\*, 412\*, 413\*, 414\*) with angiographic evidence of obstructive coronary atherosclerosis (>50% stenosis in at least 1 major epicardial vessel from catheterization report).

†At the time of median lifetime lipid measurement.

‡Participants were considered to have hypertension if they had a history of hypertension in the electronic health records, antihypertensive medication use, or systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg.

§Participants were considered to have diabetes mellitus if they had a history of type 2 diabetes mellitus in the electronic health records, antidiabetic medication use, or fasting glucose >126 mg/dL or hemoglobin A1c >6.5%.

||Total and LDL cholesterol values were divided by 0.8 and 0.7, respectively, in those on lipid-lowering medication to estimate untreated values.

the [Data Supplement](#)), we observed 37 *APOB* PTVs. Thirty-two (86%) of these PTVs were only seen in a single individual (Table VI in the [Data Supplement](#)). These mutations included 19 nonsense single-nucleotide substitutions, 3 single-nucleotide substitutions that were predicted to disrupt splicing, and 15 frame-shift indels. In aggregate, these 37 mutations were seen in a total of 56 individuals in heterozygous form. No homozygotes or compound heterozygotes were observed.

Among MIGen individuals free of CHD, we found that *APOB* PTV carriers had 43 mg/dL lower LDL-C (95% CI,  $-59.4$  to  $-26.9$ ;  $P=2.1 \times 10^{-7}$ ), 53 mg/dL lower total cholesterol (95% CI,  $-72.4$  to  $-34.3$ ;  $P=4.2 \times 10^{-8}$ ), 4 mg/dL higher HDL-C (high-density lipoprotein cholesterol; 95% CI,  $-0.39$  to  $8.8$ ;  $P=0.07$ ), and 32% lower triglycerides (95% CI, 15%–45%;  $P=5.0 \times 10^{-4}$ ; Table 2). Additionally, among 37912 individuals in DiscovEHR, *APOB* PTV carriers had a 48 mg/dL lower LDL-C (95% CI,  $-61.9$  to  $-33.4$ ;  $P=5.6 \times 10^{-11}$ ).

Among the 18442 individuals with CHD, 7 individuals carried a PTV in *APOB* (0.038% carrier frequency) compared with 49 of the 39531 controls (0.092% carrier frequency; Figure 2). Carriers of *APOB* PTVs had 72% lower risk of CHD when compared with noncarriers (odds ratio, 0.28; 95% CI, 0.12–0.64;  $P=0.002$ ). In a sensitivity analysis, we found similar results (odds ratio, 0.29; 95% CI, 0.12–0.71;  $P=0.006$ ) in the MIGen study after adjusting for sex, principal components (PCs) of ancestry, and cohort.

**Table 2.** Associations of *APOB* Protein Truncating Variant Carrier Status With Plasma Lipids in the Myocardial Infarction Genetics Consortium

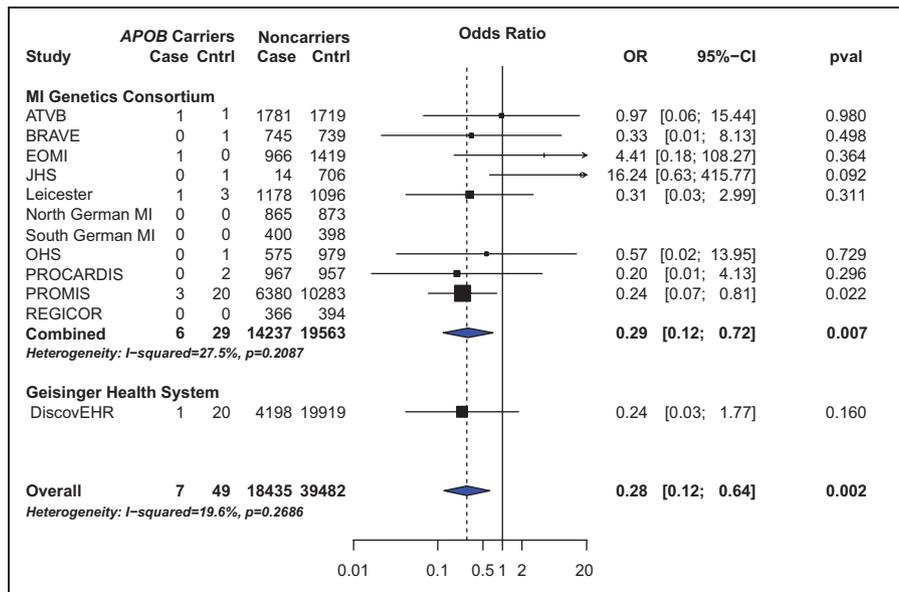
Lipid level	N	Effect Size	SE	P Value
LDL cholesterol, mg/dL	14754	−43.14	8.30	$2.1 \times 10^{-7}$
HDL cholesterol, mg/dL	15283	4.20	2.34	0.07
Total cholesterol, mg/dL	15466	−53.31	9.72	$4.2 \times 10^{-8}$
Triglycerides, log(mg/dL)	15787	−0.38	0.11	$5.0 \times 10^{-4}$

Results are adjusted for the first 5 principal components of ancestry, cohort, and sex. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; and PC, .

## DISCUSSION

In this study, we assessed whether rare PTVs in *APOB* were associated with lower lipid levels and reduced CHD. Among Japanese FHBL families, we found that carrying an *APOB* PTV in heterozygous form was associated with lower apoB, LDL-C, and triglycerides. Among >57 000 participants with and without CHD, *APOB* PTV carrier status also linked to lower total cholesterol, LDL-C, triglycerides, and a 72% lower risk for CHD when compared with noncarriers. These results permit several conclusions.

First, we demonstrate that *APOB* PTVs are a frequent cause of FHBL among the Japanese in this study. By analyzing 29 pedigrees with an extreme LDL-C phenotype, we identified 13 heterozygous carriers and 3 homozygous carriers. Identification of such individuals can enable deep phenotyping to understand the consequences of lifelong perturbation. For example, we note



**Figure 2.** Association of *APOB* protein truncating variant carrier status with risk of coronary heart disease (CHD) among 57 973 individuals.

In each study, the relationship of protein truncating variants in *APOB* with risk of CHD was determined. Exact methods were used to calculate *P* values for association tests and CI. Cochran-Mantel-Haenszel statistics for stratified 2-by-2 tables was performed for meta-analysis. Odds ratio in the North German MI study (North German Myocardial Infarction) and South German MI study (South Germ Myocardial Infarction) were not available due to a lack of observed *APOB* protein truncating variant carriers. ATVB indicates Italian Atherosclerosis, Thrombosis, and Vascular Biology; BRAVE, Bangladesh Risk of Acute Vascular Events study; Cntrl, control; DiscovEHR, DiscovEHR partnership of the Regeneron Genetics Center and Geisinger Health System; EOMI, Exome Sequencing Project Early-Onset Myocardial Infarction; JHS, Jackson Heart Study; Leicester, Leicester Myocardial Infarction; MI, myocardial infarction; OHS, Ottawa Heart Study; OR, odds ratio; PROCARDIS, Precocious Coronary Artery Disease; PROMIS, Pakistan Risk of Myocardial Infarction Study; REGICOR, Registre Gironi del COR (Gerona Heart Registry).

that each of the 3 homozygotes had not only extremely low LDL-C but also evidence of fatty liver. The presence of fatty liver is consistent with previous reports of adverse effects of using *APOB* inhibitors.<sup>10,11</sup>

Second, we provide evidence that, despite an increased risk of fatty liver, carriers of *APOB* PTVs are at substantially reduced risk of CHD. These findings are of particular importance because clinical trials of mipomersen for CHD outcomes are highly unlikely to be undertaken because of the associated adverse liver effects of mipomersen. These results emphasize the dominant role of apoB-containing lipoproteins in protection from CHD.

Third, our results add to a growing body of evidence demonstrating that rare variants associated with reduced circulating apoB-containing lipoproteins are associated with reduced risk of CHD. Rare nonsense mutations in the *PCSK9* (proprotein convertase subtilisin/kexin type 9) gene was noted in 2.6% of blacks and associated with a 88% reduction in risk for CHD.<sup>12</sup> Also, *NPC1L1* (NPC 1 like intracellular cholesterol transporter 1) rare inactivating variants are observed in 1 in 650 individuals and linked to a 53% relative risk reduction for CHD.<sup>13</sup>

Strengths of this study include the large sample size and the evaluation of family-based and population-based samples. However, we were not able to assess hepatic enzymes in the population-based samples, we did not functionally validate PTVs, and we were unable to compare effects stratified by ancestry groups given the small number of individuals carrying PTVs within each study.

## CONCLUSIONS

Rare PTVs in the *APOB* gene associated with lower LDL-C, lower triglycerides, and decreased risk for CHD.

## ARTICLE INFORMATION

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