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 ($P=3\times10^{-5}$) and 53% lower triglyceride level ($P=2\times10^{-4}$). 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 ($P=2\times10^{-7}$), a 30% decrease in triglycerides ($P=5\times10^{-4}$), 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.
APOB (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. 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). 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. 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.

Carriers of PTVs in APOB display lower LDL-C 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 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 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 I in the Data Supplement). Among these individuals, 3 carried PTVs in homozygous state and 13 harbored PTVs in heterozygous form.

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 [Registro Gironí 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 I in the Data Supplement). Among these individuals, 3 carried PTVs in homozygous state and 13 harbored PTVs in heterozygous form.
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 = 2.7×10−5), lower triglyceride levels (−53%; 95% CI, −72 to −21; Mann-Whitney U P = 1.7×10−6), and lower apoB (−43 mg/dL; 95% CI, −53 to −33; Mann-Whitney U P = 2.1×10−3) 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 (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                                |
| N=14,243 N=19,592        | CHD-Free 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)                 | 1938 (46) 3848 (19)                     |
| BMI, kg/m², 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 (%)    | 6307 (48) 4463 (24)                     | 986 (23) 4065 (20)                      |
| Ancestry                |                                         |                                         |
| European                 | 6682 (47) 7201 (37)                     | 4199 (100) 19,939 (100)                 |
| Asian                    | 7180 (51) 11,045 (57)                   | 0 (0) 0 (0)                             |
| African                  | 206 (1) 1128 (6)                        | 986 (23) 3848 (19)                      |
| Other                    | 28 (<0.001)                             | 0 (0) 0 (0)                             |
| Medical history          |                                         |                                         |
| Hypertension, n (%)      | 3212 (31) 5548 (36)                     | 3373 (80) 12,444 (34)                   |
| Type 2 diabetes mellitus, n (%) | 1872 (15) 2056 (12)                  | 1520 (36) 2611 (13)                     |
| Lipid-lowering medication, n (%) | 3463 (35) 538 (4)              | 2494 (59) 3639 (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 mmHg or diastolic blood pressure >90 mmHg.

§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=2.1×10−11), 53 mg/dL lower total cholesterol (95% CI, −72.4 to −34.3; P=4.2×10−8), 4 mg/dL higher HDL-C (high-density lipoprotein cholesterol; 95% CI, −0.39 to 8.8; P=0.47), and 32% lower triglycerides (95% CI, 15%–45%; P=5.0×10−4; Table 2). Additionally, among 37 912 individuals in DiscovEHR, APOB PTV carriers had a 48 mg/dL lower LDL-C (95% CI, −61.9 to −33.4; P=5.6×10−11).

Among the 18 442 individuals with CHD, 7 individuals carried a PTV in APOB (0.038% carrier frequency) compared with 49 of the 39 531 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  | 2.1×10−7 |
| HDL cholesterol, mg/dL | 15283 | −4.20       | 2.34  | 0.07    |
| Total cholesterol, mg/dL | 15466 | −53.31      | 9.72  | 4.2×10−8 |
| Triglycerides, log(mg/dL) | 15787 | −0.38       | 0.11  | 5.0×10−4 |

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

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.](https://example.com/figure2.png)

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 Germany 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 Gironí 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.\(^{10,11}\)

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.\(^{12}\) Also, NPC1L1 (NPC1 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.\(^{13}\)

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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Disclosures

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