Abstract We previously identified a highly consanguineous familial hypercholesterolemia (FH) family demonstrating segregation of the JD Bari mutation in the LDL receptor as well as a putative cholesterol-lowering trait. We aimed to identify genes related to the latter effect. LDL cholesterol (LDLc) values were normalized for FH affection status, age, and gender. Using genome-wide SNP data, we examined whether known SNPs gleaned from a genome-wide association study could explain the variation observed in LDLc. Four individuals with markedly reduced LDL levels underwent whole exome sequencing. After prioritizing all potential mutations, we identified the most promising candidate genes and tested them for segregation with the lowering trait. We transfected a plasmid carrying the top candidate mutation, microsomal triglyceride transfer protein (MTTP) R634C, into COS-7 cells to test enzymatic activity. The SNP showed reduced activity (49.1 nmol/ml) compared with the wild type (185.8 nmol/ml) (P = 0.0012) and was marginally associated with reduced LDLc in FH patients (P = 0.05). Phenotypic variability in a FH pedigree can only partially be explained by a combination of common SNPs and a rare mutation and a rare variant in the MTTP gene. LDLc variability in FH patients may have nongenetic causes.—Winther, M., S. Shpitzen, O. Yaacov, J. Landau, L. Oren, L. Foroozan-Rosenberg, N. Lev Cohain, D. Schurr, V. Meiner, A. Szalat, S. Carmi, M. R. Hayden, E. Leitersdorf, and R. Durst.

In search of a genetic explanation for LDLc variability in an FH family: common SNPs and a rare mutation in MTTP explain only part of LDL variability in an FH family

Michael Winther,* Shoshi Shpitzen,† Or Yaacov,‡ Jakob Landau,* Limor Oren,† Linda Foroozan-Rosenberg,† Naama Lev Cohain,** Daniel Schurr,† Vardiela Meiner,†‡ Auryan Szalat,†§ Shai Carmi,§ Michael R. Hayden,* Eran Leitersdorf,† and Ronen Durst†‡###

Centre for Molecular Medicine and Therapeutics,* University of British Columbia, Vancouver, Canada; Center for Research, Prevention, and Treatment of Atherosclerosis,† Radiology Department,** Department of Genetics and Metabolic Diseases,†† Internal Medicine Ward,§§ and Cardiology Division,### Hadassah-Hebrew University Medical Center, Jerusalem, Israel; and Hebrew University-Hadassah Braun School of Public Health and Community Medicine,§ Jerusalem, Israel

ORCID ID: 0000-0001-5159-1419 (M.R.H.)

Familial hypercholesterolemia (FH) is an autosomal dominant disorder characterized by a high LDL cholesterol (LDLc) content in the serum. FH occurs in one in 250–500 in heterozygous (HTZ) form and one in a million in homozygous (HMZ) form (1). In most cases, the disorder results from mutations in the LDL receptor (LDLR) gene. Mutations in the APOB-100 gene and the recently identified proprotein convertase subtilisin/kexin type 9 (PCSK9) gene result in phenocopies of the disease (2). Over a thousand different mutations in LDLR have been recorded to date. Owing to the high content of LDL in the blood, this disorder predisposes patients to early atherosclerosis, premature coronary heart disease, cerebrovascular accidents, calcification of cardiac valves leading to their dysfunction, and even early death. It is thought that at least 5% of all early myocardial infarctions are in FH individuals.

Although FH is caused by mutations in single genes, individuals with FH show striking phenotypic variability.

Supplementary key words low density lipoprotein • genetics • cholesterol metabolism • diseases/dyslipidemia • gene expression/polymorphisms • genes in lipid dysfunction • low density lipoprotein/assembly • low density lipoprotein cholesterol • familial hypercholesterolemia • single nucleotide polymorphism • microsomal triglyceride transfer protein

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Abbreviations: FH, familial hypercholesterolemia; GCTA, Genome-wide Complex Trait Analysis; GWAS, genome-wide association study; HMZ, homozygous; HTZ, heterozygous; LDLc, LDL cholesterol; LDLR, LDL receptor; MTTP, microsomal triglyceride transfer protein.

1To whom correspondence should be addressed.

2e-mail: durst@hadassah.org.il

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LDLc levels and age of onset of cardiovascular disease are highly variable (3). The mutational heterogeneity of FH explains some of the phenotypic variation found among FH homozygotes in whom a strong correlation is found between residual receptor activity and severity of the disease (4, 5). However, the correlation between mutation type and LDL levels is complex. Significant variations in LDLc levels that cannot be explained by gender and age differences are seen even in individuals carrying exactly the same mutation. Studies have shown that while the mutation type and residual activity of the mutated LDLR can explain some of the variability in cholesterol and LDL levels, factors such as gender, age, and apoE genotype also have significant effects (6, 7). In a study of 177 untreated FH patients in the United Kingdom, Humphries et al. (8) demonstrated a large range of total cholesterol levels ranging from just above 5 mmol/l to over 15 mmol/l. Mutations in genes that are known to reduce LDLc, particularly APOB mutations known to cause hypobetalipoproteinemia but also mutations in PCSK9, APOE, and ANGPTL3 were found to reduce LDLc in some FH patients (9–12). These and other studies (13) demonstrate that the large variability in phenotypic FH is influenced by both genetic and environmental modifiers.

Several large pedigrees segregating LDLR mutations have been described in which some carriers of an LDLR mutation have untreated normal cholesterol levels, suggesting the existence of a gene with a cholesterol-lowering effect. Hobsb et al. (14), the first to suggest the existence of such a gene, described a 9-year-old male who was HMZ for a point mutation changing Ser156 to Leu, and whose plasma cholesterol level was higher than 500 mg/dl. The proband’s mother, although HTZ for this mutation, had an LDLc level in the 28th percentile for the population. Further genotyping of the family identified the mutant gene in HTZ form in 17 of the mother’s relatives, five of whom had normal LDLc values. The inheritance pattern of the cholesterol-suppressing trait was consistent with single locus, and apoB-100 were excluded as APOE genes that are known to reduce LDLc, particularly LDLR-reducing mutation in family 408. To exclude common variants causing cholesterol reduction, we used SNP arrays to genotype the entire pedigree and then calculated a score for each individual based on prior genome-wide association studies. After this analysis failed to explain most of the observed variability in LDLc, we carried out exome sequencing to search for rare variants that might explain the observed cholesterol-lowering effect.

MATERIALS AND METHODS

Participants

All participants were members of family 408, of whom 143 individuals were identified through the MedPed project, and for 119 of whom high-quality DNA was available (19). The study was approved by the institutional review board of the Hadassah-Hebrew University Medical Center in accord with the Helsinki Declaration. All participants signed their informed consent to take part in the study. Patients who underwent MRI signed an additional consent form for the procedure.

SNP array analysis

All members of the pedigree were genotyped using the PsychArray-24 kit (v1.1; Illumina iScan). Quality control was performed using PLINK (21, 22) to assure a genotyping rate greater than 95% per person, a missingness rate per variant lower than 2%, and minor allele frequency above 1%. We also removed variants with suspected Mendelian errors and variants deviating from Hardy-Weinberg equilibrium (P < 10^-7). LDLc values were adjusted to account for the known Y828C LDLR mutation by dividing the individuals into three subgroups based on the genotype (9.5% HMZ carriers, 46.7% HTZ carriers, and 43.8% WT), and subtracting from each individual the mean LDLc of the respective subgroup.

Association was analyzed by the software, Genome-wide Complex Trait Analysis (GCTA) (23), which employs a linear mixed model that can account for any degree of relatedness between study subjects. We used default parameters, except that to increase power we opted to use the LOCO “leave one chromosome out” method. Association summary figures were prepared in R using the qqman package (https://CRAN.R-project.org/web/packages/qqman/index.html). We also ran an association analysis using PLINK’s qfam command, with similar results to those obtained by GCTA.

We calculated a polygenic score using SNPs found to affect LDLc in a large recent genome-wide association study (GWAS) termed Y828C) (supplemental Fig. S2) (18). The LDLR mutation in this family resides in the receptor’s cytoplasmic tail and causes defective receptor internalization with virtual absence of LDLR activity (19, 20). Because several FH mutation carriers in family 408 had normal or even low LDL concentrations, it was assumed that this particular family segregates for one or more genes that lower LDLc, thus compensating for the high cholesterol expected in carriers of the FH mutation (18). The mutation causing the cholesterol-lowering effect in this family has not yet been discovered but was putatively linked, using tandem repeat analysis, to the long arm of chromosome 13. Subsequently we failed, however, to find a cholesterol-lowering gene within this locus.

The aim of the present study was to identify the putative LDLc-reducing mutation in family 408. To exclude common variants causing cholesterol reduction, we used SNP arrays to genotype the entire pedigree and then calculated a score for each individual based on prior genome-wide association studies. After this analysis failed to explain most of the observed variability in LDLc, we carried out exome sequencing to search for rare variants that might explain the observed cholesterol-lowering effect.
using regression analysis in R.

The score was correlated to the adjusted LDLc level as the sum of the SNP effects for all effect alleles carried by that individual. The score for each individual was calculated in PLINK.

Whole exome sequencing

Using whole exome sequencing, we analyzed DNA samples from four of our FH family members. All four were HTZ for the JD Bari mutation, but three had normal LDLc levels, suggesting that they had a cholesterol-lowering trait (herein: “affecteds”), and one (“control”) had a high cholesterol level as anticipated for an FH family member. Genomic DNA was randomly fragmented, and the fragments were amplified, purified, and hybridized to Agilent’s SureSelect Biotinylated RNA Library (BAITS) for enrichment. Each captured library was then loaded onto an HiSeq2000 platform (Illumina), and we performed high-throughput sequencing to ensure that each sample met the desired average sequencing depth.

RNA extraction and RT-qPCR analysis

RNA was extracted using TRI-Reagent (Sigma-Aldrich) according to the manufacturer’s instructions and resuspended in 10 μl of diethylpyrocarbonate water. From each sample we extracted 1 μg of RNA for reverse transcription with the qScript cDNA kit (Quanta), as described by the manufacturer. RT-quantitative (q) PCR reactions using software 2.3 of the StepOnePlus™ real-time PCR (Applied Biosystems) were run in triplicate using PerfeCTa SYBR Green FastMix (Quanta). Relative quantification of the large MTTP subunit transcript was performed in relation to a standard curve. Quantified values for MTTP were normalized against the input determined by a housekeeping gene (HPRT1) (Quanta).

Plasmid transfection

COS-7 and HepG2 cells were transfected using the jetPRIME® kit (Polyplus-transfection®) as described by the manufacturer. Three types of constructs were used for the transfection: i) pcDNA3.1 (+) plasmid containing WT (N) MTTP; ii) pcDNA3.1 (+) plasmid containing mutated MTTP (Keyclone Technologies, San Diego, CA); and iii) GFP plasmid to evaluate transfection success. The coding sequence of MTTP cDNA constructs was verified by Sanger sequencing.

For assessing transfer activity by measuring the increase in fluorescence that occurs as a labeled neutral lipid substrate is transferred from donor to acceptor. Student’s t-test was used to compare MTTP activity between preparations.

MRI analysis

All patients underwent MRI of the liver, scanned on a 1.5T (Avanto, Siemens Healthcare, Germany) fitted with an eight-channel body array surface coil. Chemical shift-encoded spoiled gradient-echo acquisitions provided the T1-weighted in-phase and out-of-phase sequences. Acquisition parameters were 400 cm FOV, 6 mm slice thickness, 25 slices, 70 flip angle (to minimize T1-related bias), 390 kHz receiver bandwidth; TR = 115 ms, TE/ΔTE = 2.38/1.98 ms (total of four echos). Separate in-phase and out-of-phase images were used for the evaluation. A region of interest on the liver was marked on each image for signal intensity evaluation. The results were then inserted into the formula to calculate the fat-signal fraction (FF = liver SIIP – liver SIOP)/2 × liver SIIP (SI, signal intensity; IP, in-phase; OP, out-of-phase).

RESULTS

SNP array analysis

After adjusting the LDLc values according to age and gender, we performed a genome-wide association analysis using the linear mixed model of GCTA (23). The results showed many associations with LDLc at genome-wide significant P values (supplemental Fig. S1A). On further inspection, the associated loci were found to be concentrated on chromosome 19 linked to the known LDLR mutation. When we adjusted the LDLc phenotype to the known LDLR genotype, no genome-wide associated SNPs could be detected (q-q plot in supplemental Fig. S1B).

To determine whether the variance in LDLc values could be explained, at least in part, by the combined effect of multiple genes (a polygenic model), we computed the polygenic score for each individual in the pedigree according to the effect sizes reported in a recent large-scale GWAS (24). Regression of the LDLR mutation-corrected LDLc values on the polygenic score revealed minimal correlation between SNP scores and corrected LDLc values (adjusted $r^2 = 0.002$, $P = 0.26$). However, when we repeated the analysis after omitting two outlier individuals with an apparently strong cholesterol-lowering effect, the model explained approximately 3% of the variance (Fig. 1) (adjusted $r^2 = 0.028$, $P = 0.045$). This may suggest that whereas the model...
can explain part of the variability in LDLc, those results are offset by the strong LDLc-lowering effects of some individuals within the pedigree. This effect may be coded by a rare variant in a gene causing a robust cholesterol-lowering effect. We therefore performed whole exome sequencing of those individuals with extreme phenotypes in an attempt to find a rare variant that could explain the cholesterol-lowering effect.

**Whole exome sequencing analysis**

We performed whole exome analysis on four FH family members. SNPs passing filtering criteria as described in the Materials and Methods (Whole exome sequencing section) were further filtered based on the following assumptions to account for both Mendelian and non-Mendelian inheritance patterns: i) Homozygote assumption: The three affected individuals carry HMZ variation at the same position, whereas the control does not. Under this assumption, six tolerated SNPs [based on SIFT and PolyPhen scores (25, 26)] and no damaging SNPs were obtained. ii) Heterozygote assumption: The three affected individuals carry HTZ variation at the same position, whereas the control carries no variation at that position. Under this assumption, we obtained a total of 168 SNPs of which 78 were predicted to be damaging. iii) Additive model assumption: The three affected individuals have either type of variation (HTZ or HMZ) at the same position, while the control has no variation. Under this assumption, we obtained 186 SNPs of which 84 were predicted to be damaging. iv) Polygenic model assumption: Two of the three affected individuals have any type of variation at the same position, while neither the third affected individual nor the control carries the variation. A total of 951 SNPS were obtained. Of these, it was predicted by SIFT or PolyPhen software that 480 would be damaging.

The next step required manual curation of each result to obtain candidate genes that would fit the predetermined model of inheritance and would likely play a role in cholesterol homeostasis. A total of 271 LDLc homeostasis-related genes were listed from PubMed and OMIM and were cross-referenced with the list of genes from the sequencing analysis. Of the 180 variations of interest that appeared in both lists, 86 were predicted to be damaging. Among these variants, 16 were shared between at least two affected individuals but not with the control. All variations were nonsynonymous. Finally, five variants met the criteria of manual Integrative Genomics Viewer quality control (27) (Table 1). Among these variants, a variant in the *MTTP* was the likeliest to be cholesterol lowering. *MTTP* codes for a key protein in the assembly and secretion of apoB-containing lipoproteins in the liver and intestine. Mutations in *MTTP* are the molecular basis of abetalipoproteinemia, a rare autosomal recessive disorder characterized by the absence of circulating apoB-containing lipoproteins (such as VLDL) of both intestinal and hepatic origin (28). A recent reanalysis of a LDLc GWAS with >100,000 individuals also demonstrated strong association with *MTTP* (29). It should be noted that this signal was not seen in smaller GWAS studies, suggesting that the SNP effect is modest.

The p.Arg634Cys mutation (Table 1) (herein termed R634C) was reported only once in gnomAD with an allele frequency of 0.000008955 and 0.000004062 and the entire gnomAD population respectively. Multiple lines of computational evidence support a deleterious effect on the gene or gene product (https://varsome.com/variant/hg19/MTTP%20R634C). We further tested 127 FH individuals from other pedigrees sharing similar ancestry, representing 254 alleles. None carried R634C.

**Linkage analysis of the MTTP mutation**

R634C in *MTTP* was genotyped in 119 family members. LDLc concentrations for all family members were normalized relative to the 50th percentile in the same FH affection status, age, and gender. To account for FH affection status, the family members were divided into two groups: HTZ for the p.Tyr828Cys (Y828C) *LDLR* mutation and WT for the *LDLR*. Each of these groups was further divided into two subgroups (Fig. 2): R634C-mutated *MTTP* (both HTZ and HMZ) and nonmutated (N) *MTTP*. Statistical analysis for the effect of *MTTP* mutation on LDLc levels in the various groups was carried out twice: once for all family
SNPs and MTTP mutation explain LDL FH variability

Members (Fig. 2) and once excluding children under the age of 8 years (for whom the correlation between age and cholesterol is nonlinear). The P values for a difference in LDLc levels between carriers and noncarriers of the R634C MTTP mutation (for LDLR mutation carriers) were P = 0.05 and P = 0.04 for the entire cohort and when excluding children under 8, respectively. While these P values do not survive Bonferroni correction (0.05/4 = 0.0125), possibly due to the small sample size, they are suggestive of an association. Thus, an assay to show the pathogenicity of the MTTP R634C mutation was carried out.

**MTTP activity assay**

As shown in Fig. 3, MTTP activity in transfected COS cell plasmids was significantly reduced by the R634C mutation (49.1 nmol/ml vs. 185.8 nmol/ml for mutated and

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TABLE 1. A list of the candidate mutations that met the criteria of manual Integrative Genomics Viewer quality control

| Coordinates | 12 | 35 | 60 | 30 | Substitution | dbSNP Identification | Gene Name |
|-------------|----|----|----|----|--------------|---------------------|-----------|
| 4,100532349,1.C/T | 1/1 | 0/1 | 0/1 | 0 | R634C | Novel | MTTP |
| 4,110638824,1.C/T | 0/1 | 0/1 | 0 | 0 | D111N | rs114816312:T | PLA2G12A |
| 9,46632521.A/C | 0/1 | 0 | 0/1 | 0 | S202R | rs72695803:C | PPAPDC2 |
| 17,41063102,1.C/A | 0 | 0/1 | 0/1 | 0 | L265M | Novel | G6PC |
| 19,11240282,1.A/G | 0/1 | 0/1 | 0/1 | 0/1 | Y828C | rs28942085:G | LDLR |

Individuals with IDs 12, 35, and 60 are affected; 30 is the control. Analysis of candidate mutations suggested five potential LDLc-lowering mutations. Of these, two (in MTTP and G6PC) are novel. MTTP is a likely candidate because it is known to cause marked lowering of LDLc. LDLR is the JD Bari mutation known to cause FH. PLA2G12A, phospholipase A2 group XIIA; PPAPDC2, phospholipid phosphatase 6; G6PC, glucose-6-phosphatase catalytic subunit.

**Fig. 2.** LDLc levels in carriers and noncarriers of the R634C MTTP mutation stratified by LDLR mutation status in all 119 family members. The results suggest generally higher levels of LDLc in the HTZ LDLR group, but which can be lowered in carriers of the MTTP mutation. Similar results were obtained when children under the age of 8 are excluded. N, noncarriers.
nonmutated MTTP, respectively; P = 0.0012). Mutated MTTP activity did not differ significantly from that in nontransfected COS cells. In nontransfected COS cells, the level of activity was lower than in HepG2 cells, which possess endogenous MTTP activity (Fig. 3). Transcript mRNA levels were similar between the mutated and nonmutated cells, suggesting that there was no difference in transfection efficacy between the cultures. MTTP mutations in humans may cause significant phenotype, only reported in the homozygote state. To test the hypothesis that our mutation might have a dominant negative effect, we transfected COS cells with WT and mutated alleles. The activity of the mutated allele-transfected cells was similar to the nontransfected Hep2G cell line. Thus, we could not demonstrate a dominant negative effect with the mutation (supplementary Fig. S3).

MRI analysis

Drugs that inhibit MTTP are known to cause hepatosteatosis. To test for the effect or the R634C MTTP mutation on liver fat content, liver fat was analyzed by MRI spectroscopy (Table 2) (30) in eight HTZ LDLR mutation carriers. Half were HTZ for the R634C MTTP mutation and half were WT. ANOVA revealed no significant difference between the two groups, pointing to a lack of a significant difference in transfection efficacy between the cultures. MTTP mutations in humans may cause significant phenotype, only reported in the homozygote state. To test the hypothesis that our mutation might have a dominant negative effect, we transfected COS cells with WT and mutated alleles. The activity of the mutated allele-transfected cells was similar to the nontransfected Hep2G cell line. Thus, we could not demonstrate a dominant negative effect with the mutation (supplementary Fig. S3).

**DISCUSSION**

FH is a genetic condition in which the cellular function of LDLR is damaged, preventing evacuation of LDLc particles from the blood into the cells. LDLc concentrations in some members of the FH family described in this study were lower than expected. In attempting to explain this observed variability, we first looked at the SNP-based polygenic score for LDLc in the pedigree. After individuals with very strong cholesterol-lowering effects were omitted from the analysis, the score could explain ~3% of the observed variability in LDLc. To determine whether a rare variant was causing a major change in LDLc, we employed exome sequencing in a search for a rare variant with a strong LDLc-reducing effect. A novel mutation (R634C) in the MTTP gene, not previously described in dbsNP, was identified. FH patients carrying this MTTP mutation demonstrated lower LDLc values than noncarriers. Together, both SNP score and the rare variant search could explain, at most, only part of the variability in LDLc observed in the pedigree. LDLc variability in our pedigree may only be partly explained by SNP score or rare variants. It is therefore assumed that LDLc variability in FH patients may be more complex and also involve epigenetic and environmental influences.

MTTP is located in the endoplasmic reticulum and plays a central role in lipoprotein assembly, catalyzing the transport of triglyceride, cholesteryl ester, and phospholipid between vesicles (31). MTTP is predicted to have three major structural domains: an N-terminal β-barrel (amino acid residues 22–297); a middle α-helical domain (residues 298–563); and a C-terminal β-sheet (residues 604–894) (32, 33). The N-terminal β-barrel domain mediates interaction with the N-terminus of apoB; the middle α-helical domain associates with both protein disulfide isomerase and apoB; and the C-terminal β-sheet domain contains both the lipid-binding and the lipid-transfer activities of MTTP (32, 34). The R634C mutation is located in the lipid-binding and lipid-transfer activity domains. Thus, we speculate that the replacement of a positively charged amino acid (R) by a neutral/negatively charged amino acid (D) may cause a change in MTTP activity, leading to reduced LDLc concentrations.

**TABLE 2.** Liver fat content by MRI and fibro tests in four MTTP mutation carriers and four noncarriers

| Sample Number | Age at MRI | LDLR Mutation | MTTP Mutation | BMI  | MRI Results (%) |
|---------------|------------|----------------|---------------|------|-----------------|
| 408-15        | 55         | HTZ            | N             | 33.2 | 40.4            |
| 408-23        | 49         | HTZ            | N             | 23.5 | 24.2            |
| 408-50        | 50         | HTZ            | N             | 34.3 | 24.3            |
| 408-37        | 41         | HTZ            | N             | 24.9 | 0.8             |
| 408-14        | 43         | HTZ            | HTZ           | 28.5 | 18              |
| 408-74        | 60         | HTZ            | HTZ           | 28.9 | 24.4            |
| 408-95        | 50         | HTZ            | HTZ           | 19.9 | 0.4             |
| 408-9         | 47         | HMZ            | HTZ           | 0.35 | 0.4             |

N, noncarrier.

*MRI results in percentage of liver fat infiltration.
acid (C), combined with the location of the mutation, damages the protein’s activity. An in vitro assay in transfected cells demonstrated a significant reduction in the mutated allele compared with the WT gene. The similar mRNA levels indicate that the difference in activity was not due to lower levels of mRNA in the transfected cells but was rather the result of either lower enzyme activity or reduced protein level. Human heterozygotes for MTTP mutation were not reported to have a significant phenotype. We tested the option that the R634C mutation may have a dominant negative effect but could not demonstrate such an effect. Our assumption is that in FH mono- and heterozygotes given the markedly elevated LDLc at baseline, even a small haploinsufficiency effect will have a statistically significant effect on LDLc.

Drugs that inhibit MTTP activity are used to treat FH. These drugs, however, have problematic side effects, particularly fatty liver changes (35). An analysis of liver fat contents in four FH patients carrying the MTTP mutation and four controls revealed no significant differences in either MRI or plasma biomarkers. It is possible that the inhibitory effect of the mutation is not associated with changes in liver fat content, suggesting that such changes do not necessarily result from inhibition of MTTP activity.

In conclusion, in this study of a large consanguineous FH family exhibiting a cholesterol-lowering trait, we sought to better understand genetic modifiers of the FH trait. SNP score could explain only 3% of the observed variability in LDLc. Exome sequencing performed in the search for rare LDLc-reducing variants identified an R634C MTTP mutation that reduces LDLc when present in HTZ carriers. We showed that this mutation reduces MTTP activity in transfected COS-7 cells, and that this reduction is not associated with a tendency toward an increase in liver fat. Our data thus support the concept that an FH phenotype can be modified by MTTP mutations as well as by common SNPs. However, these at best explain only part of the observed variability in LDLc among FH patients. Other causes may be relevant for the observed LDLc variability. These may include epigenetic as well as environmental factors.

REFERENCES

1. Otani, K., M. Takeuchi, K. Kakui, N. Haruki, H. Yoshitani, M. Eto, M. Tamura, M. Okazaki, H. Abe, Y. Fujino, et al. 2012. Evidence of a vicious cycle in mitral regurgitation with prolapse: secondary tethering attributed to primary prolapse demonstrated by three-dimensional echocardiography exacerbates regurgitation. Circulation. 121 (1 Suppl. 1): S214–S221.

2. Austin, M. A., C. M. Hutter, R. L. Zimmerm, and S. E. Humphries. 2004. Genetic causes of monogenic heterozygous familial hypercholesterolemia: a HuGE prevalence review. Am. J. Epidemiol. 160: 407–420.

3. Jansen, A. C. M., S. van Wissen, J. C. Defesche, and J. J. P. Kastelein. 2002. Phenotypic variability in familial hypercholesterolemia: an update. Curr. Opin. Lipidol. 13: 165–171.

4. Kozer, M., J. W. De Vlillers, K. Steyn, J. A. Krick, A. D. Marais, E. Langenhoven, J. S. Herbert, J. F. Graaf Van Roggen, D. R. Van der Westhuizen, and G. A. Coetzer. 1993. Phenotypic variation among familial hypercholesterolemics heterozygous for either one of two Afrikaner founder LDL receptor mutations. Arterioscler. Thromb. 13: 1460–1468.

5. Sprecher, D. L., J. M. Hoeg, E. J. Schaefer, L. A. Zech, R. E. Gregg, E. Lakatos, and H. B. Brewer, Jr. 1985. The association of LDL receptor activity, LDL cholesterol level, and clinical course in homozygous familial hypercholesterolemia. Metabolism. 34: 294–300.

6. Huigen, R., S. W. Fouquier, M. Denoon, B. A. Hutton, M. N. Vissers, G. Lambert, and J. J. Kastelein. 2012. Plasma levels of PCSK9 and phenotypic variability in familial hypercholesterolemia. J. Lipid Res. 53: 979–983.

7. Bertolini, S., A. Cantaforda, M. Averna, C. Cortese, C. Mottu, S. Martini, G. Pes, A. Postiglione, C. Stefanelli, I. Blotta, et al. 2000. Clinical expression of familial hypercholesterolemia in families of mutations of the LDL receptor gene that cause a receptor-defective or receptor-negative phenotype. Arterioscler. Thromb. Vasc. Biol. 20: E41–E52.

8. Humphries, S. E., R. A. Whittall, C. S. Hubbart, S. Maplebeck, J. A. Cooper, A. K. Soutar, R. Naoumova, G. R. Thompson, M. Seed, P. N. Durrington, et al. 2006. Genetic causes of familial hypercholesterolemia in patients in the UK relation to plasma lipid levels and coronary heart disease risk. J. Med. Genet. 43: 943–949.

9. Huigen, R., B. Sjouke, K. Vis, J. S. E. de Randamie, J. C. Defesche, J. J. P. Kastelein, G. K. Hovingh, and S. W. Foucsher. 2012. Genetic variation in APOB, PCSK9, and ANGPTL3 in carriers of pathogenic autosomal dominant hypercholesterolemogenic mutations with unexplained low LDLc. Hum. Mutat. 33: 550–555.

10. Leren, T. P., and K. E. Berge. 2008. Identification of mutations in the apolipoprotein B-100 gene and in the PCSK9 gene as the cause of hypocholesterolemia. Clin. Chim. Acta. 397: 92–95.

11. Slimani, A., A. Jelassi, I. Jguirim, M. Najah, L. Rebbi, A. Omezzine, F. Maatouk, K. B. Hamda, M. Kacem, J-P. Rabes, et al. 2012. Effect of mutations in LDLR and PCSK9 genes on phenotypic variability in Tunisian familial hypercholesterolemia patients. Atherosclerosis. 222: 158–166.

12. Rabacchi, C., F. Bigazzi, M. Puntoni, F. Shrana, T. Sampietro, P. Tarugi, S. Bertolini, and S. Calandra. 2016. Phenotypic variability in 4 homozygous familial hypercholesterolemia siblings compound heterozygous for LDLR mutations. J. Clin. Lipidol. 10: 944–952.e1.

13. Arca, M., and E. Jokinen. 1998. Low density lipoprotein receptor mutations in a selected population of individuals with moderate hypercholesterolemia. Atherosclerosis. 136: 187–194.

14. Hobbs, H. H., E. Leitersdorf, C. C. Leffert, D. R. Cryer, M. S. Brown, and J. L. Goldstein. 1989. Evidence for a dominant gene that suppresses hypercholesterolemia in a family with defective low density lipoprotein receptors. J. Clin. Invest. 84: 656–664.

15. Vega, G. L., H. H. Hobbs, and S. M. Grundy. 1991. Low density lipoprotein kinetics in a family having defective low density lipoprotein receptors in which hypercholesterolemia is suppressed. Arterioscler. Thromb. 11: 578–585.

16. Sass, C., L. M. Giroux, Y. Ma, M. Roy, J. Lavigne, S. Lussier-Cacan, J. Davignon, and A. Minnich. 1995. Evidence for a cholesterol-lowering gene in a French Canadian kindred with familial hypercholesterolemia. Hum. Genet. 96: 21–26.

17. Ma, Y. H., C. Betard, M. Roy, J. Davignon, and A. M. Kessling. 1989. Identification of a second “French Canadian” LDL receptor gene deletion and development of a rapid method to detect both deletions. Clin. Genet. 36: 219–228.

18. Knoblauch, H., B. Muller-Myhsok, A. Busjahn, L. Ben Avi, S. Bahring, H. Baron, S. C. Heath, R. Uhlmann, H. D. Faulhaber, S. Shpitzen, et al. 2000. A cholesterol-lowering gene maps to chromosome 13q. Am. J. Hum. Genet. 66: 157–166.

19. Reshef, A., H. Nissen, L. Triger, T. S. Hansen, O. Eliav, D. Schurr, R. Safadi, M. Gare, and E. Leitersdorf. 1996. Molecular genetics of familial hypercholesterolemia in Israel. Hum. Genet. 98: 581–586.

20. Brown, M. S., and J. L. Goldstein. 1976. Analysis of a mutant strain of human fibroblasts with a defect in the internalization of receptor-bound low density lipoprotein. Cell. 9: 663–674.

21. Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M. A. Ferreira, D. Bender, J. Maller, P. Sklar, P. I. de Bakker, M. J. Daly, et al. 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81: 559–575.

22. Chang, C. C., C. C. Chow, L. C. Tellier, S. Vattikuti, S. M. Purcell, and J. J. Lee. 2015. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience. 4: 7.

23. Yang, J., S. H. Lee, M. E. Goddard, and P. M. Visscher. 2011. GCTA: a tool for genome-wide complex trait analysis. Am. J. Hum. Genet. 88: 76–82.

24. Willer, C. J., E. M. Schmidt, S. Sengupta, G. M. Peloso, S. Gustafsson, S. Kanoni, A. Ganna, J. Chen, M. L. Buchkovich, S. Mora, et al.; SNPs and MTTP mutation explain LDL FH variability 1739
Global Lipids Genetics Consortium. 2013. Discovery and refinement of loci associated with lipid levels. *Nat. Genet.* **45**: 1274–1283.

25. Adzhubei, I. A., S. Schmidt, L. Peshkin, V. E. Ramensky, A. Gerassimova, P. Bork, A. S. Kondrashov, and S. R. Sunyaev. 2010. A method and server for predicting damaging missense mutations. *Nat. Methods.* **7**: 248–249.

26. Kumar, P., S. Henikoff, and P. C. Ng. 2009. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat. Protoc.* **4**: 1073–1081.

27. Robinson, J. T., H. Thorvaldsdottir, W. Winckler, M. Guttman, E. S. Lander, G. Getz, and J. P. Mesirov. 2011. Integrative genomics viewer. *Nat. Biotechnol.* **29**: 24–26.

28. Berriot-Varoqueaux, N., L. P. Aggerbeck, M. E. Samson-Bouma, and J. R. Wetterau. 2000. The role of the microsomal triglyceride transfer protein in abetalipoproteinemia. *Annu. Rev. Nutr.* **20**: 663–697.

29. Zhu, X., and M. Stephens. 2018. A large-scale genome-wide enrichment analysis identifies new trait-associated genes, pathways and tissues across 51 human phenotypes. *Nat Commun.* **9**: 4361.

30. Imbert-Bismut, F., V. Ratziu, L. Pieroni, F. Charlotte, Y. Benhamou, T. Poynard, and M. Group. 2001. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet.* **357**: 1069–1075.

31. Wetterau, J. R., M. C. Lin, and H. Jamil. 1997. Microsomal triglyceride transfer protein. *Biochim. Biophys. Acta.* **1345**: 136–150.

32. Hussain, M. M., J. Shi, and P. Dreizen. 2003. Microsomal triglyceride transfer protein and its role in apoB-lipoprotein assembly. *J. Lipid Res.* **44**: 22–32.

33. Mann, C. J., T. A. Anderson, J. Read, S. A. Chester, G. B. Harrison, S. Köchl, P. J. Ritchie, P. Bradbury, F. S. Hussain, J. Amey, et al. 1999. The structure of vitellogenin provides a molecular model for the assembly and secretion of atherogenic lipoproteins. *J. Mol. Biol.* **285**: 391–408.

34. Hussain, M. M., J. Iqbal, K. Anwar, P. Rava, and K. Dai. 2003. Microsomal triglyceride transfer protein: a multifunctional protein. *Front. Biosci.* **8**: s500–s506.

35. Cuchel, M., and D. J. Rader. 2013. Microsomal transfer protein inhibition in humans. *Curr. Open. Lipidol.* **24**: 246–250.