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# Exome sequencing and directed clinical phenotyping diagnose cholesterol ester storage disease presenting as autosomal recessive hypercholesterolemia

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## Abstract

**Objective—**Autosomal recessive hypercholesterolemia (ARH) is a rare inherited disorder characterized by extremely high total and low-density lipoprotein cholesterol levels that has been previously linked to mutations in *LDLRAP1*. We identified a family with ARH not explained by mutations in *LDLRAP1* or other genes known to cause monogenic hypercholesterolemia. The aim of this study was to identify the molecular etiology of ARH in this family.

**Approach and Results—**We used exome sequencing to assess all protein coding regions of the genome in three family members and identified a homozygous exon 8 splice junction mutation (c. 894G>A, also known as E8SJM) in *LIPA* that segregated with the diagnosis of hypercholesterolemia. Since homozygosity for mutations in *LIPA* is known to cause cholesterol ester storage disease (CESD), we performed directed follow-up phenotyping by non-invasively measuring hepatic cholesterol content. We observed abnormal hepatic accumulation of cholesterol in the homozygote individuals, supporting the diagnosis of CESD. Given previous suggestions of cardiovascular disease risk in heterozygous *LIPA* mutation carriers, we genotyped E8SJM in >27,000 individuals and found no association with plasma lipid levels or risk of myocardial infarction, confirming a true recessive mode of inheritance.

**Conclusions—**By integrating observations from Mendelian and population genetics along with directed clinical phenotyping, we diagnosed clinically unapparent CESD in the affected

individuals from this kindred and addressed an outstanding question regarding risk of cardiovascular disease in *LIPA* E8SJM heterozygous carriers.

### Keywords

hypercholesterolemia; genetics; myocardial infarction

## Introduction

Monogenic hypercholesterolemia is a disorder of lipid metabolism in which extremely elevated levels of total and low-density lipoprotein cholesterol (LDL-C) are caused by a single gene mutation. Mutations in *LDLR*[1], *APOB*[2], and *PCSK9*[3] cause autosomal dominant hypercholesterolemia, a disease affecting at least 1 in 500 individuals. Autosomal recessive hypercholesterolemia (ARH) occurs much less frequently – estimated to occur in 1:1,000,000 live births – and has been linked to mutations in *LDLRAP1*[4]. In some families with apparent monogenic hypercholesterolemia, an underlying molecular defect cannot be identified in any of these known genes.

We identified a family with apparent Mendelian inheritance of high LDL-C levels that was not caused by mutations in any of the above genes known to affect LDL-C. The small size of the family pedigree precluded use of traditional linkage mapping. Next-generation sequencing (NGS), a rapid and low-cost method to perform large-scale DNA sequencing[5], has emerged as an important tool for uncovering the cause of inherited diseases[6]. In this study, we used exome sequencing, a technique in which NGS is used to assess all protein-coding regions of the genome, in three individuals from this family to search for a rare genetic variant that co-segregated with high LDL-C levels.

## Materials and Methods

Materials and Methods are available in the online-only Supplement.

## Results

### Subject recruitment

The proband (Figure 1; individual II-2) presented to the Lipid Clinic at the Academic Medical Center, University of Amsterdam, the Netherlands at the age of 23. Her LDL-C level exceeded the 99$^{th}$ percentile when adjusted for age and gender. She had two siblings (one of which was a monozygotic twin), both of whom shared LDL-C levels exceeding the 99$^{th}$ percentile. Her father and mother, a non-consanguineous union, had LDL-C levels at the 25$^{th}$ and 78$^{th}$ percentile, respectively, when adjusted for age and gender (Figure 1). The proband and both siblings lacked hepatosplenomegaly on abdominal examination. Based on the pedigree, an autosomal recessive mode of inheritance appeared to be the most likely explanation for the family's phenotype.

## Exome Sequencing

To identify the molecular basis of hypercholesterolemia in this family, exome sequencing was performed in the proband, the proband's father, and the proband's brother (Figure 1; individuals II-2, I-1, and II-1, respectively). A total of 32,950,014 bases across the exome were targeted and each sample was sequenced with an average of 126-fold coverage across the target. Across the exome, 82% of targeted bases were covered with >30-fold coverage. This yielded a mean of 36,986 single nucleotide variants per individual. The average ratio of heterozygous to homozygous alleles (1.6) and ratio of transitions to transversions (2.7) per individual were expected and similar to contemporary large-scale population sequencing projects[7].

## Exome Sequencing Analysis

To exclude genetic variation unlikely to be responsible for this family's hypercholesterolemia, we relied on three main assumptions: (1) the causal variant(s) alters the gene's corresponding protein product; (2) the causal variant(s) is inherited in an autosomal recessive fashion; and (3) the causal variant(s) exhibits complete penetrance. For the first assumption, we only included single nucleotide substitutions and short insertions or deletions that were predicted to alter the protein sequence.

We next included either 1) compound heterozygous changes (a heterozygous variant in both affected siblings and the father located in a gene that also contained a separate heterozygous variant in both affected siblings not found in the father) or 2) variants that were homozygous in both affected siblings and heterozygous in the unaffected father. Finally, we excluded variants from further consideration if they were present in the general population at a frequency of greater than 1%, or if they were present in either heterozygous or homozygous form in the exome sequences of 235 individuals with very low LDL-C levels.

After applying this analysis, the number of variants shared among the three family members was reduced from 54,301 to two candidate single nucleotide substitutions. One was a synonymous variant predicted to alter the splice donor site of the eighth exon in the gene lipase A, lysosomal acid, cholesterol esterase (*LIPA*) (c.894G>A, in the last nucleotide of exon 8) and the other was a missense change predicted to result in the substitution of Alanine for Proline at residue 384 in the gene ATP/GTP binding protein-like 2 (*AGBL2*).

Since previous reports showed a link between the c.894G>A mutation – also known as the Exon 8 Splice Junction Mutation (E8SJM) – in *LIPA* and cholesterol ester storage disease (CESD)[8], a disorder with mixed hyperlipidemia as part of the phenotypic presentation, we focused on a potential diagnosis of CESD as the most likely cause for this family's apparent autosomal recessive hypercholesterolemia.

## Functional assessment of E8SJM

Sanger sequencing was performed and confirmed the presence of the E8SJM allele in the homozygous state in affected individuals and in the heterozygous state in both unaffected parents. Haplotype analysis revealed that both maternal and paternal E8SJM alleles were on the same haplotype as previously reported for this mutation ("Haplotype 1" from Fasano et

al.[9]). This does not appear to be a result of consanguinity as the proband was found to share 53% and 49% of her exome identical-by-descent with her brother and father, respectively, eliminating cryptic consanguinity. The skipping of exon 8 was confirmed in all individuals carrying the mutated allele (Figure 2).

Although the affected individuals did not present with clinically apparent hepatic disease, given the previous reports linking mutations in *LIPA* with CESD, we reassessed the affected individuals for the level of hepatic cholesterol ester using magnetic resonance spectroscopy (MRS), a technique shown to correlate well with histologic lipid distribution[10]. In individuals II-1, II-2 and II-3, MRS demonstrated a distinct cholesterol peak separate from the larger and expected triglyceride peak at 1.25 ppm. The ratios between triglyceride at 1.25 ppm and cholesterol at 0.9 ppm were 0.57, 0.34 and 0.40 for individuals II-1, II-2 and II-3 respectively, indicating the presence of an excess of hepatic cholesterol deposition (Figure 3). The elevated cholesterol peak at 0.9 ppm was not identified in individual I-1. Individuals II-1, II-2, and II-3 had normal hepatic size as measured on the magnetic resonance imaging portion of the study.

### Population impact of E8SJM

Given previous reports suggesting that serum lipids levels are increased in heterozygous E8SJM carriers[11], we genotyped the E8SJM variant in 13,194 individuals of European ancestry. Triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and LDL-C levels were available in 13,194, 13,144, and 12,805 individuals, respectively. In these individuals, the E8SJM was present with an allele frequency of 0.16% and no association was observed with any of these three lipid fractions (Table 1).

Furthermore, to also assess the impact of partial loss of *LIPA* function on risk for myocardial infarction (MI) or coronary artery disease (CAD) in the population, we genotyped the E8SJM variant in 27,472 individuals of European ancestry (12,747 cases with MI/CAD, 14,725 controls free of MI and CAD). In these individuals, the E8SJM was present with an allele frequency of 0.11% and there was no association of E8SJM with risk for MI or CAD (odds ratio for MI or CAD in carriers = 0.85; p-value = 0.6).

## Discussion

Traditional Mendelian genetic analyses have relied on positional cloning and sequencing the genetic regions under linked peaks to identify causal defects responsible for monogenic disorders. These techniques are unfortunately of limited utility in small families such as the one presented in the current study. NGS, however, now allows for the potential identification of candidate genes underlying Mendelian disorders in families regardless of the pedigree size. In this study, we performed NGS across the exome in three individuals from a family with suspected ARH and identified homozygous E8SJM alleles in *LIPA* that co-segregated with the clinical diagnosis of hypercholesterolemia.

Lysosomal acid lipase (LAL), encoded by the gene *LIPA*, is responsible for hydrolyzing cholesterol esters and triglycerides that are delivered to lysosomes. Mutations in *LIPA* that completely inactivate LAL have previously been identified as the molecular cause of

Wolman disease, a rapidly lethal disease of infancy, characterized by hepatosplenomegaly, abdominal distension, adrenal calcification, and steatorrhea with extensive storage of cholesterol esters and triglycerides in the liver, spleen, and other organs in the first weeks of life[12, 13].

A related disorder, cholesterol ester storage disease (CESD), is associated with a less severe phenotype[14, 15]. Characterized by massive hepatic accumulation of cholesterol esters, hepatomegaly, steatosis, and mixed hyperlipidemia, CESD is caused by mutations in *LIPA* that result in near complete loss of LAL activity with enough residual enzymatic activity to hydrolyze triglycerides but not cholesterol esters.

The identification of homozygous E8SJM alleles in *LIPA* was surprising in this family, as it has been previously identified as a cause of CESD[16]. E8SJM has been shown to cause sub-total loss of gene function resulting in only 2–4% normally spliced *LIPA* mRNA transcripts and LAL activity[16]. Homozygosity for E8SJM has previously been reported in individuals with hepatic disease and mixed hyperlipidemia, characterized by elevated levels of LDL-C and TG with decreased HDL-C levels (Table 2).

The homozygous individuals in the current study presented with a very different phenotype and would not have been clinically diagnosed with CESD. Their lipid profile is characterized by extremely elevated LDL-C with normal to high HDL-C and normal TG levels while previously described E8SJM homozygotes have been noted to have increased LDL-C with low HDL-C and elevated TG levels (Table 2). In addition, the hepatic phenotype in the homozygous individuals from the current study appears to consist of only a subtle elevation in ALT (Table 2) without the typical hepatosplenomegaly (hepatomegaly and splenomegaly are present in >99% and 74% of patients with CESD, respectively[17]).

Given the previous associations between *LIPA* E8SJM and CESD (for homozygous carriers) and polygenic hypercholesterolemia and potentially increased risk of MI/CAD (for heterozygote carriers)[11], we performed directed phenotypic and genetic follow-up analyses to address two questions: 1) Do the homozygous carriers within this pedigree have hepatic hallmarks of CESD?; and 2) Are the heterozygote parents at increased risk for MI/CAD? Using non-invasive hepatic MRS, we demonstrated the presence of abnormal quantities of hepatic cholesterol in the homozygous E8SJM carriers of this family. This finding is entirely consistent with previously reported hepatic MRS findings in patients with LAL deficiency (previously reported MRS ratios 0.24–0.5)[18] and confirms the diagnosis of CESD in the three offspring. A liver biopsy was not thought to be clinically indicated given the absence of increased transaminase levels combined with previous reports surrounding the causal role of *LIPA* E8SJM in CESD and the confirmatory MRS findings. While a seemingly subtle distinction, this diagnosis is clinically important as the offspring should be followed for the progression of hepatic disease and may be potential candidates in the future for enzyme replacement therapy that is currently in development[19]. In addition, this finding illustrates that CESD may have a more variable phenotypic presentation than previously appreciated.

To assess the potential of increased cardiovascular disease risk in the heterozygous parents, we genotyped *LIPA* E8SJM in the population. The population frequency of *LIPA* E8SJM

has previously been estimated to be between 0.21% and 0.25% in individuals of European descent[20,21], and has been associated with a polygenic hypercholesterolemia phenotype[11], prompting the hypothesis that it may be associated with increased risk of MI/CAD. We now firmly establish that this variant is rarer than previously estimated (allele frequency = 0.11%). We consider our estimate of the carrier frequency for European individuals to be more accurate than previous reports given the larger numbers of individuals assessed (27,472 in the current study compared with 4,112 in a previous report[21]).

In this large genetic study, we observed no association of heterozygosity with plasma lipid levels or risk for MI/CAD. Although we cannot definitively exclude a weak association with MI/CAD or serum lipid levels, we had 93% power to detect a 2-fold increased risk of MI/CAD at an alpha of 0.05 and 94% power to detect a variant explaining 0.1% of the phenotypic variance in LDL-C at an alpha of 0.05. These findings suggest that the E8SJM acts in a truly recessive fashion and that heterozygous loss of function does not result in a distinct lipid or MI phenotype.

It is uncertain why the presentation of CESD in this family differed from those described in previous reports. The E8SJM in this family occurs on the same haplotype as previously reported for this mutation, supporting a common founder ancestor for this mutation and suggesting that the milder-than-expected phenotype is not explained by a simple difference of local genetic background in *LIPA*. In addition to the E8SJM in *LIPA*, we identified rare homozygous alleles in *AGBL2* carried by all three affected offspring. At this time it is unclear what, if any, phenotypic effect this confers. There may be a genetic factor (in *AGBL2* or elsewhere) conferring a protective hepatic effect; however, given the lack of family members with hepatic disease as a comparator, we are underpowered to discover such a variant.

In summary, we report homozygosity for E8SJM in *LIPA* as a cause of clinically unapparent CESD presenting as autosomal recessive hypercholesterolemia. The discovery of E8SJM in *LIPA* in this family highlights both the blessing and the curse of using NGS in genetic discovery studies; along with the potential unbiased discovery of the causal variant comes tens of thousands of additional variants unrelated to the phenotype of interest and the possibility of unexpected findings. We suggest integrating Mendelian and population genetics with directed clinical testing as a powerful way to discern signal from noise in the next generation of genetic discovery studies.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

| | |
|---|---|
| **ARH** | Autosomal recessive hypercholesterolemia |
| **CAD** | Coronary artery disease |
| **CESD** | Cholesterol ester storage disease |
| **E8SJM** | Exon 8 splice junction mutation |
| **HDL-C** | High-density lipoprotein-cholesterol |
| **LDL-C** | Low-density lipoprotein-cholesterol |
| **MI** | Myocardial infarction |
| **NGS** | Next generation sequencing |
| **TG** | Triglycerides |

## References

1. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. Science. 1986; 232:34–47. [PubMed: 3513311]
2. Innerarity TL, Weisgraber KH, Arnold KS, Mahley RW, Krauss RM, Vega GL, Grundy SM. Familial defective apolipoprotein B-100: Low density lipoproteins with abnormal receptor binding. Proc Natl Acad Sci U S A. 1987; 84:6919–6923. [PubMed: 3477815]
3. Abifadel M, Varret M, Rabes JP, et al. Mutations in *PCSK9* cause autosomal dominant hypercholesterolemia. Nat Genet. 2003; 34:154–156. [PubMed: 12730697]
4. Garcia CK, Wilund K, Arca M, Zuliani G, Fellin R, Maioli M, Calandra S, Bertolini S, Cossu F, Grishin N, Barnes R, Cohen JC, Hobbs HH. Autosomal recessive hypercholesterolemia caused by mutations in a putative LDL receptor adaptor protein. Science. 2001; 292:1394–1398. [PubMed: 11326085]
5. Shendure J, Ji H. Next-generation DNA sequencing. Nat Biotechnol. 2008; 26:1135–1145. [PubMed: 18846087]
6. Ku CS, Naidoo N, Pawitan Y. Revisiting mendelian disorders through exome sequencing. Hum Genet. 2011; 129:351–370. [PubMed: 21331778]
7. Tennessen JA, Bigham AW, O'Connor TD, et al. Evolution and functional impact of rare coding variation from deep sequencing of human exomes. Science. 2012; 337:64–69. [PubMed: 22604720]
8. Lohse P, Maas S, Lohse P, Elleder M, Kirk JM, Besley GT, Seidel D. Compound heterozygosity for a Wolman mutation is frequent among patients with cholesteryl ester storage disease. J Lipid Res. 2000; 41:23–31. [PubMed: 10627498]
9. Fasano T, Pisciotta L, Bocchi L, Guardamagna O, Assandro P, Rabacchi C, Zanoni P, Filocamo M, Bertolini S, Calandra S. Lysosomal lipase deficiency: Molecular characterization of eleven patients with Wolman or cholesteryl ester storage disease. Molecular genetics and metabolism. 2012; 105:450–456. [PubMed: 22227072]
10. Ruberg FL, Viereck J, Phinikaridou A, Qiao Y, Loscalzo J, Hamilton JA. Identification of cholesteryl esters in human carotid atherosclerosis by ex vivo image-guided proton MRS. J Lipid Res. 2006; 47:310–317. [PubMed: 16317172]

11. Muntoni S, Wiebusch H, Jansen-Rust M, Rust S, Schulte H, Berger K, Pisciotta L, Bertolini S, Funke H, Seedorf U, Assmann G. Heterozygosity for lysosomal acid lipase E8SJM mutation and serum lipid concentrations. Nutr Metab Cardiovasc Dis. 2013; 23:732–736. [PubMed: 22795295]
12. Abramov A, Schorr S, Wolman M. Generalized xanthomatosis with calcified adrenals. A.M.A. Journal of Diseases of Children. 1956; 91:282–286. [PubMed: 13301142]
13. Patrick AD, Lake BD. Deficiency of an acid lipase in Wolman's disease. Nature. 1969; 222:1067–1068. [PubMed: 5787090]
14. Fredrickson DS, Sloan HR, Ferrans VJ, Demosky SJ Jr. Cholesteryl ester storage disease: A most unusual manifestation of deficiency of two lysosomal enzyme activities. Transactions of the Association of American Physicians. 1972; 85:109–119. [PubMed: 4660005]
15. Burke JA, Schubert WK. Deficient activity of hepatic acid lipase in cholesterol ester storage disease. Science. 1972; 176:309–310. [PubMed: 5019788]
16. Muntoni S, Wiebusch H, Funke H, Ros E, Seedorf U, Assmann G. Homozygosity for a splice junction mutation in exon 8 of the gene encoding lysosomal acid lipase in a spanish kindred with cholesterol ester storage disease (CESD). Hum Genet. 1995; 95:491–494. [PubMed: 7759067]
17. Bernstein DL, Hulkova H, Bialer MG, Desnick RJ. Cholesteryl ester storage disease: Review of the findings in 135 reported patients with an under-diagnosed disease. Journal of hepatology. 2013; 58:1230–1243. [PubMed: 23485521]
18. Thelwall PE, Smith FE, Leavitt MC, Canty D, Hu W, Hollingsworth KG, Thoma C, Trenell MI, Taylor R, Rutkowski JV, Blamire AM, Quinn AG. Hepatic cholesteryl ester accumulation in lysosomal acid lipase deficiency: Non-invasive identification and treatment monitoring by magnetic resonance. Journal of hepatology. 2013; 59:543–549. [PubMed: 23624251]
19. Balwani M, Breen C, Enns GM, et al. Clinical effect and safety profile of recombinant human lysosomal acid lipase in patients with cholesteryl ester storage disease. Hepatology. 2013; 58:950–957. [PubMed: 23348766]
20. Muntoni S, Wiebusch H, Jansen-Rust M, Rust S, Seedorf U, Schulte H, Berger K, Funke H, Assmann G. Prevalence of cholesteryl ester storage disease. Arterioscler Thromb Vasc Biol. 2007; 27:1866–1868. [PubMed: 17634524]
21. Scott SA, Liu B, Nazarenko I, Martis S, Kozlitina J, Yang Y, Ramirez C, Kasai Y, Hyatt T, Peter I, Desnick RJ. Frequency of the cholesteryl ester storage disease common *LIPA* E8SJM mutation (c. 894G>A) in various racial and ethnic groups. Hepatology. 2013; 58:958–965. [PubMed: 23424026]

## Significance

Autosomal recessive hypercholesterolemia is a rare inherited disorder previously linked to mutations in *LDLRAP1*. In this report, we use exome sequencing and clinical phenotyping to diagnose cholesterol ester storage disease (CESD) in a small family with apparent autosomal recessive hypercholesterolemia. CESD is caused by mutations in *LIPA* and typically presents with hepatic disease and mixed hyperlipidemia. This study reveals a broader phenotypic presentation for loss of function mutations in *LIPA* than previously appreciated and suggests that *LIPA* mutations may be considered in the clinical evaluation of autosomal recessive hypercholesterolemia.

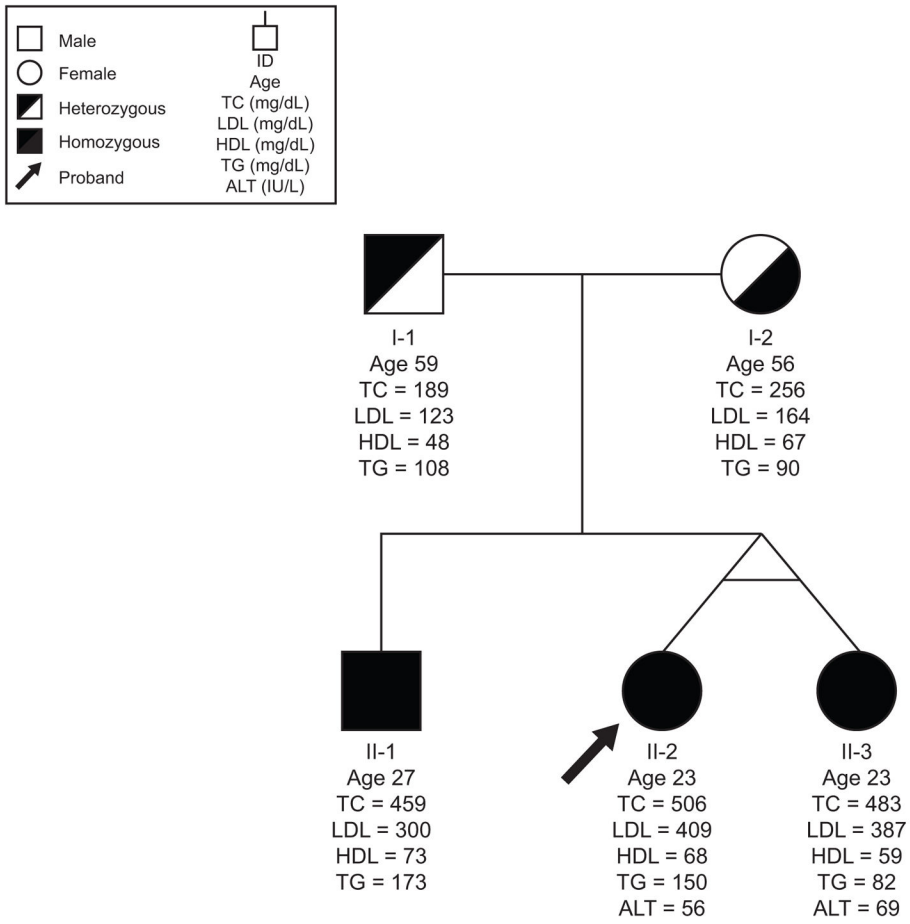


**Figure 1.**

Pedigree of the family demonstrating autosomal recessive hypercholesterolemia. Laboratory values are shown below each individual (TC = total cholesterol; LDL = low density lipoprotein cholesterol; HDL = high density lipoprotein cholesterol; TG = triglycerides; ALT = alanine aminotransferase). Individuals II-2 and II-3 are identical twins.

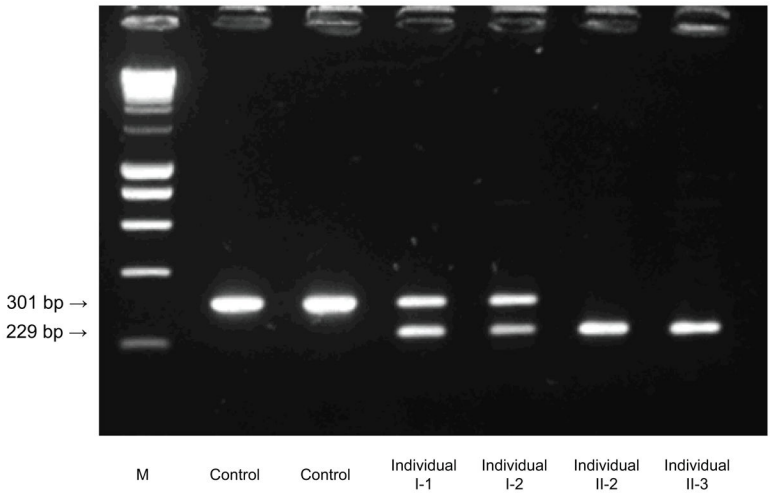


**Figure 2.**

RT-PCR of *LIPA* demonstrating skipping of exon 8 as a result of E8SJM. The upper and lower bands correspond to the expected products either containing (301 bp) or lacking (229 bp) exon 8, respectively. Control cDNA from individuals not carrying E8SJM demonstrates the expected product containing exon 8. Heterozygous carriers of E8SJM (Individuals I-1 and I-2) demonstrate the presence of one wild-type transcript and one transcript lacking exon 8, whereas homozygous E8SJM carriers (Individuals II-2 and II-3) demonstrate complete skipping of exon 8. M = molecular weight marker.

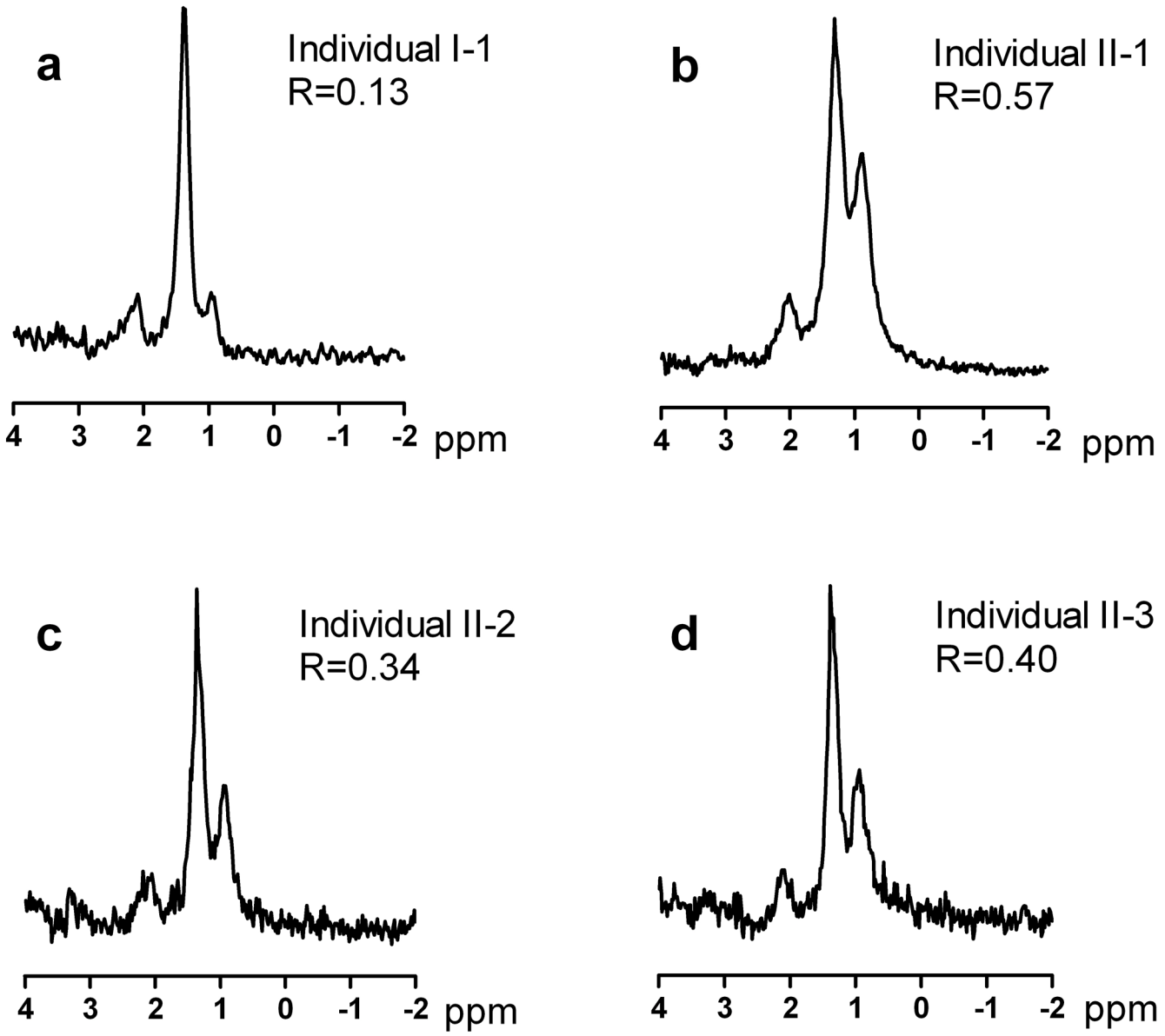


**Figure 3.**

Water suppressed MRS spectra demonstrating hepatic cholesterol deposition in homozygous carriers of *LIPA* E8SJM. R = ratio between peaks at 1.25 ppm and 0.9 ppm. Panel a: Individual I-1, the unaffected father of the proband, demonstrates a normal ratio. Panel b-d: Individuals II-2, II-3, and II-1, respectively demonstrate elevated ratios.

**Table 1**

Association of E8SJM and plasma lipid levels in the population

| Trait | N* | MAF† | Effect (mg/dL)‡ | 95% CI§ | p-value |
|---|---|---|---|---|---|
| LDL-C | 12, 581 | 0.16% | −0.059 | −13.3 – 13.2 | 0.9 |
| HDL-C | 12, 839 | 0.16% | 4.46 | −0.5 – 9.5 | 0.08 |
| TG | 13,443 | 0.16% | −0.16 | −0.3 –0.02 | 0.08 |

*N: number of individuals contributing to the analysis;

†MAF: minor allele frequency;

‡Effect: change in mg/dL for each copy of the minor allele;

§CI: confidence interval.

**Table 2**

Phenotypic consequences of homozygosity for *LIPA* E8SJM

| Study | ID* | TC† (mg/dL) | LDL-C‡ (mg/dL) | HDL-C§ (mg/dL) | TG‖ (mg/dL) | AST# (IU/L) | ALT** (IU/L) | Associated signs |
|---|---|---|---|---|---|---|---|---|
| Fasano et al[9] | | 298 | 221 | 35 | 216 | 58 | 110 | HSM†† |
| | | 323 | N/A‖‖ | N/A | 259 | 2–3 x ULN§§ | 2–3 x ULN | HSM |
| | | 189 | 130 | 40 | 93 | 56 | 110 | HM§§ |
| | | 263 | 197 | 31 | 178 | 75 | 102 | HM |
| Mutoni et al[16] | | 337 | 264 | 16 | 216 | N/A | N/A | HSM |
| Current study | II-2 | | | | | | | |
| | At presentation | 506 | 409 | 68 | 150 | N/A | 56 | None |
| | On statin therapy | 187 | 104 | 66 | 38 | 30 | 32 | |
| | II-3 | | | | | | | |
| | At presentation | 483 | 387 | 59 | 82 | N/A | 69 | None |
| | On statin therapy | 127 | 47 | 66 | 28 | 33 | 37 | |
| | II-1 | | | | | | | |
| | At presentation | 459 | 300 | 73 | 173 | N/A | N/A | None |
| | On statin therapy | 140 | 81 | 47 | 64 | 45 | 61 | |

*ID: Individual ID from family pedigree (Figure 1);

†TC: total cholesterol;

‡LDL-C: low-density lipoprotein cholesterol;

§HDL-C: high-density lipoprotein cholesterol;

‖TG: triglycerides;

#AST: aspartate aminotransferase (upper limit of normal in current study: 40 IU/L);

**ALT: alanine aminotransferase (upper limit of normal in current study: 34 IU/L);

††HSM: hepatosplenomegaly;

‡‡HM: hepatomegaly;

§§ULN: upper limit of normal;

|||N/A: not available