Novel CREB3L3 Nonsense Mutation in a Family With Dominant Hypertriglyceridemia

Angelo B. Cefalù,* Rossella Spina,* Davide Noto,* Vincenza Valenti, Valeria Ingrassia, Antonina Giammanco, Maria D. Panno, Antonina Ganci, Carlo M. Barbagallo, Maurizio R. Averna

Objective—Cyclic AMP responsive element–binding protein 3–like 3 (CREB3L3) is a novel candidate gene for dominant hypertriglyceridemia. To date, only 4 kindred with dominant hypertriglyceridemia have been found to be carriers of 2 nonsense mutations in CREB3L3 gene (245fs and W46X). We investigated a family in which hypertriglyceridemia displayed an autosomal dominant pattern of inheritance.

Approach and Results—The proband was a 49-year-old woman with high plasma triglycerides (≤1300 mg/dL; 14.68 mmol/L). Her father had a history of moderate hypertriglyceridemia, and her 51-year-old brother had triglycerides levels as high as 1600 mg/dL (18.06 mmol/L). To identify the causal mutation in this family, we analyzed the candidate genes of recessive and dominant forms of primary hypertriglyceridemia by direct sequencing. The sequencing of CREB3L3 gene led to the discovery of a novel minute frame shift mutation in exon 3 of CREB3L3 gene, predicted to result in the formation of a truncated protein devoid of function (c.359delG–p.K120fsX20). Heterozygosity for the c.359delG mutation resulted in a severe phenotype occurring later in life in the proband and her brother and a good response to diet and a hypnotriglyceridemic treatment. The same mutation was detected in a 13-year-old daughter who to date is normotriglyceridemic.

Conclusions—We have identified a novel pathogenic mutation in CREB3L3 gene in a family with dominant hypertriglyceridemia with a variable pattern of penetrance. (Arterioscler Thromb Vasc Biol. 2015;35:2694-2699. DOI: 10.1161/ATVBAHA.115.306170.)

Key Words: codon, nonsense ▼ heterozygote ▼ hypertriglyceridemia ▼ mutation ▼ triglycerides

High plasma triglyceride concentration is a biomarker of a variety of familial and sporadic metabolic disorders. According to a recent proposed simplified definition1 of the hypertriglyceridemic states, fasting triglyceride plasma levels <2 mmol/L (175 mg/dL) can be considered normal while triglyceride levels between 2 to 10 mmol/L (175–885 mg/dL) and >10 mmol/L (885 mg/dL) identify subjects with mild to moderate and severe hypertriglyceridemia, respectively. Many common conditions, such as obesity, metabolic syndrome, and pregnancy, alimentary habits, including high alcohol intake, diseases as type 2 diabetes mellitus, hypothyroidism, renal diseases, paraproteinemias, and systemic lupus erythematosus, and some drugs are responsible of mild-to-moderate secondary forms of hypertriglyceridemia.2 Primary or genetic forms of hypertriglyceridemia include mild-to-moderate and severe forms; mild-moderate hypertriglyceridemia is more frequent than severe hypertriglyceridemia, and recent studies have indicated that this condition may have a polygenic cause with a complex heritability because of the effect of several rare, heterozygous loss-of-function gene variants.3 The rare severe hypertriglyceridemias are thought to be monogenic autosomal recessive and caused by homozygous or compound heterozygous loss-of-function mutations of few known genes pathophysiologically involved in the intravascular lipolysis of the triglyceride-rich lipoproteins, namely lipoprotein lipase (LPL), apolipoprotein CII (APOCII), apolipoprotein AV (APOAV), glycosylphosphatidylinositol (GPI)-anchored high-density lipoprotein–binding protein 1 (GPIHBP1), lipase maturation factor 1 (LMF1), and GPD1.4-6 Dominant familial hypertriglyceridemia recently renamed simple primary hypertriglyceridemia is a very common disorder with an estimated frequency of 1:20.2 The molecular basis of this form seems to be polygenic.1,3 The transcription factor cyclic AMP-responsive element–binding protein H (CREB-H, encoded by CRE-binding protein 3–like 3 [CREB3L3]) has been genetically associated with hypertriglyceridemia in humans,7 and...
to date, 4 kindred with dominant hypertriglyceridemia associated with CREB3L3 heterozygous mutations (245fs and W46X) have been described. Hypertriglyceridemia because of CREB3L3 pathogenic variants might represent a rare example of monogenic dominant hypertriglyceridemia. Here, we report a family harboring a novel mutation of CREB3L3 gene, which shows a dominant pattern of inheritance and a variable expression of the hypertriglyceridemia trait (Table 1).

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

Results
Phenotype
DNA samples were available from 6 subjects across 2 generations. Lipid profiles of the family members are presented in Figure 1. The proband (subject II-1) and her brother (II-3) showed moderate hypertriglyceridemia, whereas her sister (II-4) and her daughters (III-1 and III-2) showed a normal density lipoprotein cholesterol measurement, the levels of which show a dominant pattern of inheritance and a variable expression of the hypertriglyceridemia trait (Table 1).

Table 1. Primers Used for Sequencing the CREB3L3 Gene

<table>
<thead>
<tr>
<th>Exon</th>
<th>Primer</th>
<th>Sequence</th>
<th>Temp, °C</th>
<th>Size, bp</th>
</tr>
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<td></td>
<td>R</td>
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<td></td>
<td>R</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

F indicates forward; and R, reverse.

Table 1. Primers Used for Sequencing the CREB3L3 Gene

CREB3L3 Mutation in Hypertriglyceridemia

The proband’s response to treatment, including diet and various regimens of lipid-lowering medication, during the period between 2009 and 2015 is shown in Figure 2.

During this period, plasma triglyceride levels showed large fluctuations ranging from 150 to 1300 mg/dL (1.69–14.67 mmol/L). These differences were mainly attributed to the poor compliance to low-fat diet and hypolipidemic treatment with ω-3 fatty acids (EPA+DHA, 3 g/d) and fenofibrate (200 mg/d). On the occasion of our first evaluation, triglyceride plasma levels were 500 mg/dL (5.64 mmol/L); because of the reluctance of the patient to be treated with fibrates, a treatment with 4 g of ω-3 fatty acids supplementation was prescribed. Under ω-3 fatty acids therapy, she showed an improved lipid profile with triglyceride plasma levels as low as 213 mg/dL after 4 months of treatment (2.40 mmol/L, 43% reduction). For personal reason between February and July 2014, she discontinued the ω-3 fatty acids supplementation with a subsequent relapse of hypertriglyceridemia (plasma triglyceride, 537 mg/dL–6.06 mmol/L). She restarted the treatment and the triglyceride plasma levels decreased to 151 mg/dL (1.7 mmol/L; Figure 2).

ω-3 fatty acids therapy was also prescribed to subject II-3 (proband’s brother), but no follow-up data are available.

Genetic Analysis

There were 6 individuals in the family who had both plasma lipids phenotype and DNA available for genotyping. Of these 6 individuals, the proband (II-1) and her brother (II-3) were affected on clinical grounds (triglyceride >175 mg/dL). No functionally relevant mutations in the LPL, APOC2, APOA5, GPHBP1, and LMF1 genes were detected.

These results and the clinical suspicion of a dominant form of hypertriglyceridemia prompted us to analyze by direct sequencing the whole CREB3L3 gene.

The proband was found to be heterozygous for a novel frameshift mutation in exon 3 of CREB3L3 gene, predicted to result in the formation of a truncated protein devoid of function (c.359delG–p.K120fsX20). The presence of the mutation in exon 3 (Figure 3) was confirmed in 3 independent polymerase chain reaction amplifications and direct sequencing. The proband’s hypertriglyceridemic brother (II-3) and one of her normotriglyceridemic daughter (III-2) were found to be carriers of the same mutation in the heterozygous state (Figure 1).

Even if the c.359delG is predicted to generate a premature stop codon and it is expected to be a rare and deleterious variant, we screened this mutation by direct sequencing in 30 unrelated healthy Italian normolipidemic individuals; the mutation was not found in any of these subjects. Because this analysis was performed in the same ethnic background population of the proband, we cannot draw any certain conclusion about the frequency of the mutation in other ethnic groups.

More, the variant was not reported in the Exome Variant Server repository of the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/), the Exome Aggregation Consortium Website (ExAC, http://exac.broadinstitute.org), and the 1000 Genomes web catalog (http://www.1000genomes.org), and we did not detect any variant of the hypertriglyceridemia trait (Table 1).
by the analysis of whole CREB3L3 gene in other 7 unrelated patients with moderate hypertriglyceridemia, suggesting that the c.359delG is a rare mutation.

In order to try to explain the different lipid phenotype in mutation carriers, we investigated the possible genetic modulating effect on triglyceride levels by APOE genotypes and APOC3 variants. All investigated subjects were carriers of the E2/E3 genotype, but the proband who was E2/E4; direct sequencing of the whole APOC3 gene did not reveal any variant.

**Discussion**

In this study, we report a novel mutation of CREB3L3 gene, the c.359delG, in 3 related members (mother, daughter, and...
mother’s brother) of a family with a different and variable pattern of penetrance. Heterozygosity for the c.359delG mutation resulted in a severe hypertriglyceridemia phenotype in the proband and her brother with a late in life expression and a good response to diet and hypotriglyceridemic treatments based on fenofibrate and ω-3 fatty acids. The same mutation was detected in the daughter, 13 years old, who to date is normotriglyceridemic.

CREB-H was first identified by Abel et al. CREB-H is a liver-specific bZIP transcription factor that is selectively expressed in the liver and in the small intestine. CREB-H binds to the CRE consensus (TGACGTCA) and box-B–like elements present in the promoters of liver-expressed genes. CREB-H regulated genes are involved in acute phase response, iron absorption, and gluconeogenesis.

Recent data have suggested a prominent role of CREB-H in the hepatic lipid metabolism, notably in lipogenesis and triglyceride metabolism. The first evidence came from primary mouse hepatocytes incubated with fatty acids. In this experimental setting, fatty acids induced CREB-H mRNA hepatic expression, and this effect was attributed to the involvement of peroxisome proliferator–activated receptor α. Moreover CREB3L3−/− knockout mice show a 3-fold higher plasma triglyceride levels compared with wild-type mice suggesting a direct involvement of CREB-H in triglyceride metabolism. Additional experiments in CREB3L3−/− mice demonstrated that CREB-H does not affect hepatic very low-density lipoprotein secretion rate and that LPL activity was reduced indicating that hypertriglyceridemia in these mice is a consequence of an impaired triglyceride clearance from plasma. The infusion of wild-type animals plasma into CREB-H-deficient mice contributed to ameliorate the hypertriglyceridemia phenotype, suggesting that CREB-H effects on triglyceride clearance were mediated by circulating cofactors for LPL.

In fact CREB-H controls the expression of genes involved in triglyceride metabolism, including APOC2, APOA4, and APOA5, and the reduced expression of these known cofactors for LPL could give reasons for the hypertriglyceridemia phenotype. Moreover, CREB-H via peroxisome proliferator–activated receptor α induces FGF21, a liver expressed hormone, which increases insulin sensitivity and reduces plasma triglyceride levels. In early large genome-wide association studies CREB3L3 gene did

### Table 2. TG Levels in Carriers of CREB3L3 Mutations

<table>
<thead>
<tr>
<th>Pedigree Generation</th>
<th>Sex (M/F)</th>
<th>Age, y</th>
<th>TG Levels, mg/dL–mmol/L</th>
<th>BMI, kg/m²</th>
<th>Mutation</th>
<th>Reference</th>
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<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>62</td>
<td>4513–51</td>
<td>30.3</td>
<td>245fs</td>
<td>7</td>
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<tr>
<td></td>
<td>F</td>
<td>67</td>
<td>189–2.1</td>
<td>27.5</td>
<td>245fs</td>
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<td></td>
<td>M</td>
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<td>280–3.2</td>
<td>25.1</td>
<td>245fs</td>
<td>7</td>
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<td>F</td>
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<td>245fs</td>
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<tr>
<td></td>
<td>M</td>
<td>43</td>
<td>583–6.6</td>
<td>29.7</td>
<td>W46X</td>
<td>7</td>
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<tr>
<td></td>
<td>M</td>
<td>91</td>
<td>131–1.5</td>
<td>21.8</td>
<td>W46X</td>
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<td>5</td>
<td>F</td>
<td>49</td>
<td>537–6.1</td>
<td>22.5</td>
<td>K120fsX20</td>
<td>This report</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>51</td>
<td>553–6.2</td>
<td>25</td>
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<tr>
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<td>F</td>
<td>13</td>
<td>74–0.8</td>
<td>20</td>
<td>K120fsX20</td>
<td>This report</td>
</tr>
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</table>

F indicates female; M, male; and TG, triglyceride.
not emerge as a locus associated with the triglyceride trait.\textsuperscript{18} When this gene was resequenced in a cohort of 449 unrelated hypertriglyceridemia subjects, 12 individuals were identified as heterozygous carriers of nonsynonymous or insertional \textit{CREB3L3} gene mutations. The analysis of the families of the 4 unrelated nonsense mutation carriers allowed the identification of 7 additional carriers; the triglyceride mean plasma levels of the 11 carriers was significantly higher than those of the noncarriers family members (9.67 versus 1.66 mmol/L, 856 versus 147 mg/dL).\textsuperscript{7} Interestingly among the 11 heterozygotes for \textit{CREB3L3} nonsense mutations, 3 carriers were normotriglyceridemic, 4 had a mild-to-moderate hypertriglyceridemia, and only 2 had a severe hypertriglyceridemia (Table 2). These latter observations might suggest that other factors could contribute to the penetrance of the \textit{CREB3L3} genetic defect.

Recently, Johansen et al\textsuperscript{3} resequenced \textit{CREB3L3} in 413 hypertriglyceridemia subjects and 324 normal lipidemic controls and found an enrichment of \textit{CREB3L3} rare noncoding, synonymous, and nonsynonymous variants. Three of them (W46X, E240K, and K245Efs374X) were definitely damaging. Unfortunately, it was not reported the severity degree of the hypertriglyceridemia-associated phenotype. The family we described represents the fifth kindred in which a heterozygous frame shift mutation (c.359delG–p.K120fsX20) of \textit{CREB3L3} is associated with a severe hypertriglyceridemia phenotype.

Overall 7 rare variants have been reported in \textit{CREB3L3} gene so far.\textsuperscript{5,7} Among these, only 2 lead to premature stop codon (W46X and 245fs) and they both have been shown to alter the CREB-H protein function (Table 3) and to segregate in pedigrees with hypertriglyceridemia in families.\textsuperscript{7} The nonsense mutation reported here is expected to lead to a CREB-H protein with an intermediate size compared with the 2 previously described mutations and although we did not perform functional studies it can be speculated a deleterious effect on function.

Our proband was healthy, and the only relevant clinical feature was the presence of fatty liver detected by ultrasonography. This finding is interesting because of the absence of overweight and insulin resistance. In \textit{CREB3L3} knockout mice fed an atherogenic diet, Zhang et al\textsuperscript{19} demonstrated the development of hepatic steatosis underlining the role of CREB-H in the regulation of hepatic lipogenesis, lipolysis, and fatty acid oxidation. The proband’s daughter (subject III-2) is carrier of the mutation but her triglyceride levels were normal. The analysis of the 5 families (including the present) raises the issue of the penetrance of the heterozygous loss-of-function mutations of \textit{CREB3L3} gene. Because the majority of the heterozygotes have a mild-to-moderate hypertriglyceridemia, why do only few heterozygotes develop a severe hypertriglyceridemia form? To answer this question, we can rely only on the few published data. The 2 severe hypertriglyceridemia subjects belonging to 2 unrelated kindred harboring the \textit{CREB3L3} 245fs mutation were both obese and diabetics; in the \textit{CREB3L3} W46X kindred, the proband was overweight but the hypertriglyceridemia form was mild-to-moderate. Among the other 8 sporadic heterozygotes, carriers of \textit{CREB3L3} missense mutations, 5 showed a mild-to-moderate form of hypertriglyceridemia (n.2 G105R, n.1 P166L, n.1 D182N, and n.1 E240K) and 3 (n.1 P166L, n.1 V180M, n.1 E240K) a severe form of hypertriglyceridemia. It is worth to note that only 1 P166L heterozygote was diabetic. Overall, based on these sparse clinical information, it is difficult to draw any conclusion about the role as precipitating factors of overweight, obesity, and diabetes mellitus. An accurate follow-up of the identified heterozygotes would significantly contribute to a better understanding of the mechanisms that underlie the clinical expression of the \textit{CREB3L3} genetic defect.

The proband of our kindred presented a severe form of hypertriglyceridemia. The response to treatment with fenofibrate (200 mg/d) and ω-3 fatty acids (3 g/d) was excellent, and interestingly when fenofibrate was stopped a mild-to-moderate hypertriglyceridemia developed. This response to the treatment suggests that fenofibrate, as previously demonstrated in vitro, could stimulate the expression of CREB-H via the peroxisome proliferator–activated receptor α pathway in humans.\textsuperscript{14}

In conclusion, we identified a novel nonsense mutation of \textit{CREB3L3} gene, the c.359delG–p.K120fsX20, which was associated with a severe hypertriglyceridemia phenotype and showed a variable penetrance. No functional variants were identified in the other major known hypertriglyceridemia-associated genes. Moreover, we investigated the possible modulating action of \textit{APOE} common genotypes and \textit{APOC3} gene variants on triglyceride plasma levels. No \textit{APOC3} gene variants were identified in all family members; thus, we ruled out an effect on triglyceride levels in mutation carriers. Subjects carriers of the \textit{APOE2} allele show higher triglyceride
levels compared with the more common APOE3 allele carriers.20 The 3 mutation carriers (subjects II-1, II-3, and III-2) of this family are carriers of the APOE2 allele and in addition 2 of them (II-3 and II-2) share the same genotype (E2/E3), indicating that the variability of triglyceride plasma levels is not related to APOE genotype.

Further studies are warranted to identify putative modulators of the CREB-H partial deficiency. Based on our results and on the other already published observations, searching for CREB3L3 gene mutations should be part of the primary hypertriglyceridemia genetic work-up.

Acknowledgments

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Sources of Funding

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Disclosures

None.

References


Significance

Dominant familial hypertriglyceridemia also known as simple primary hypertriglyceridemia is a common disorder with an estimated frequency of 1:20. The molecular basis of this form seems to be polygenic. Recently, the transcription factor cyclic AMP-responsive element- binding protein H (CREB-H, encoded by CREB3L3) has been genetically associated with hypertriglyceridemia in humans. Only four kindred with dominant hypertriglyceridemia associated with CREB3L3 heterozygous mutations have been described. We describe a kindred in which a novel mutation of CREB3L3 gene (p.K120fsX20), cosegregates with hypertriglyceridemia in an autosomal dominant pattern. Two carriers of the mutation show a severe hypertriglyceridemia phenotype with a late in life expression and a good response to diet and a hypotriglycerideemic treatment. A 13-year-old girl belonging to this kindred, who is carrier of the mutation, was normotriglyceridemic suggesting a variable pattern of penetrance. Searching for CREB3L3 gene mutations should be part of the primary hypertriglyceridemia genetic work-up.
Novel CREB3L3 Nonsense Mutation in a Family With Dominant Hypertriglyceridemia

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Material and methods

Case description and pedigree
The proband is a 49-year-old woman. Her parents are apparently unrelated. Her father died at the age of 48 because of lung cancer and he had a history of moderate hypertriglyceridemia (referred up to 500 mg/dl). The mother and one older sister are reported to be nomolipidemic. Her 51 years old brother has been suffered of sporadic episodes of abdominal pain and discomfort and in one occasion his triglycerides levels were as high as 1600 mg/dl. The clinical history of the two 24 an 13 years old daughters is reported as unremarkable but no data on lipid profile were known.

Severe hypertriglyceridemia (about 1000 mg/dl) was first noted in the occasion of a routine laboratory check up. A diet restriction to about 15% of fat intake (<7% of saturated fat) and treatment with fibrates and ω-3 fatty acids were prescribed but the adherence to prescriptions was poor resulting in fluctuating plasma triglycerides levels (referred from 150 mg/dl and 1300 mg/dl).

On December 2013 the patient was referred for the first time to our Lipid Clinic. The physical examination was unremarkable. Mild fatty liver was detected upon ultrasound examination.

Routine laboratory tests, with the exception of plasma lipids, were within the reference range. Plasma triglyceride (TG), total cholesterol (TC) and HDL-C levels were respectively 500 mg/dl (5.64 mmol/l), 218 mg/dl (5.64 mmol/l), and 33 mg/dl (0.77 mmol/l). At the time of this clinical evaluation the adherence to low-fat diet was poor and she has discontinued the hypolipidemic drugs.

Based on this clinical history we decided to start a diagnostic genetic work out.

Informed consent was obtained from all subjects investigated.

Plasma lipid analysis
Blood samples from proband and her relatives (10 mL each) were collected into plain and containing EDTA (1 mg/mL) tubes to obtain serum, plasma and buffy coat by centrifugation at 3000 rpm for 15 min. Plasma TC, TG and HDL-C were measured using standard enzymatic–colorimetric procedures (Roche Diagnostics, Basel Switzerland) on a COBAS MIRA plus auto-analyzer (Roche Diagnostics, Basel Switzerland). LDL-C was calculated by the Friedewald formula.

Analysis of candidate genes
Genomic DNAs from studied subjects were extracted from whole blood using the Wizard DNA Purification System (Promega Italia, Italy). Candidate genes of recessive (LPL, APOC2, APOA5, GPIHBP1 and LMF1 genes) and dominant (CREB3L3) forms of hypertriglyceridemia were analyzed.

All exons, the promoter region the intron/exon boundaries of LPL, APOC2, APOA5, GPIHBP1 and LMF1 genes were amplified by PCR from genomic DNA using appropriate primers as previously described (1-2). Resequencing of the coding regions and consensus splice sites of APOC3 gene in all investigated subjects was performed using appropriate primers as previously described (3).

The coding regions including the intron/exon boundaries (reference sequence NM_032607) of CREB3L3 gene were amplified using the primers listed in Table 1.

PCR fragments were purified with a commercial kit (Wizard PCR Prep–DNA Purification System; Promega Italia, Italy) then sequenced directly in both directions using BigDye Terminator Cycle sequencing kit 3.1 in a 3500 Genetic Analyzer (Life Technologies Italia, Italy) and the results were analyzed with the SeqScape software version 3 (Life Technologies Italia, Italy).
The proband and other available family members were also genotyped for ε2, ε3, and ε4 polymorphisms in APOE gene by a commercially available preformulated TaqMan assay (Life Technologies) in a ViiA™ 7 Real-Time PCR System (Life Technologies).

References

