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Objective—Mutations in *LPL* or *APOC2* genes are recognized causes of inherited forms of severe hypertriglyceridemia. However, some hypertrigliceridemic patients do not have mutations in either of these genes. Because inactivation or hyperexpression of *APOA5* gene, encoding apolipoprotein A-V (apoA-V), causes a marked increase or decrease of plasma triglycerides in mice, and because some common polymorphisms of this gene affect plasma triglycerides in humans, we have hypothesized that loss of function mutations in *APOA5* gene might cause hypertriglyceridemia.

Methods and Results—We sequenced *APOA5* gene in 10 hypertriglyceridemic patients in whom mutations in *LPL* and *APOC2* genes had been excluded. One of them was found to be homozygous for a mutation in *APOA5* gene (c.433 C>T, Q145X), predicted to generate a truncated apoA-V devoid of key functional domains. The plasma of this patient was found to activate LPL in vitro less efficiently than control plasma, thus suggesting that apoA-V might be an activator of LPL. Ten carriers of Q145X mutation were found in the patient's family; 5 of them had mild hypertriglyceridemia.

Conclusions—As predicted from animal studies, apoA-V deficiency is associated with severe hypertriglyceridemia in humans. This observation suggests that apoA-V regulates the secretion and/or catabolism of triglyceride-rich lipoproteins. **(*Arterioscler Thromb Vasc Biol*. 2005;25:411-417.)**

Key Words: apolipoprotein A-V deficiency ■ hypertriglyceridemia ■ hyperchylomicronemia ■ *APOA5* gene ■ nonsense mutation

Hypertriglyceridemia is conventionally defined as severe when the levels of fasting plasma triglycerides are >10 mmol/L;[1] it may be primary or secondary to other diseases. Severe hypertriglyceridemias are often referred to as hyperchylomicronemia syndromes, because of plasma accumulation of chylomicrons in the fasting state.[1,2]

Three inherited disorders have been described in which chylomicrons accumulate in plasma: familial lipoprotein lipase (LPL) deficiency (MIM 238600), familial apolipoprotein C-II deficiency (MIM 608083), and familial inhibitor of LPL (MIM 118830). Familial LPL deficiency is a rare autosomal-recessive disorder caused by a severe deficiency of LPL, the enzyme responsible for the hydrolysis of triglycerides in chylomicrons and very-low-density lipoprotein (VLDL).[2] LPL deficiency is usually diagnosed in childhood because of the presence of episodes of abdominal pain, recurrent pancreatitis, eruptive cutaneous xanthomatosis, hepatosplenomegaly, and failure to thrive.[2] The diagnosis is based on severely reduced/absent LPL activity in postheparin plasma and on demonstration of mutations in *LPL* gene in either homozygous or compound heterozygous state.

Familial apolipoprotein C-II (apoC-II) deficiency is an exceedingly rare autosomal recessive disorder caused by a severe defect of apoC-II, the physiological activator of LPL. The few mutations of *APOC2* gene reported so far cause a complete absence of apoC-II activity/protein in plasma, resulting in a functional LPL deficiency and in a block of the lipolytic cascade.[2] The clinical manifestations of apoC-II deficiency are similar to those of familial LPL deficiency.[2]

The third inherited disorder of the lipolytic cascade was reported in a single family in which very low levels of postheparin plasma LPL activity were thought to be caused by the presence of a circulating plasma inhibitor of LPL.[3]

During a systematic analysis of *LPL* and *APOC2* genes in children and adults with severe primary hypertriglyceridemia, we identified 10 patients who had no mutations in either of these 2 genes. These negative findings prompted us to investigate *APOA5* gene, another potential candidate gene in the pathogenesis of hypertriglyceridemia.[4] This recently discovered gene is located proximal to the well-characterized *APOA4-C3-A1* gene cluster on human chromosome 11 (11q23); it encodes the apolipoprotein A-V (apoA-V), a 363-amino acid protein (NP_443200) that bears strong similarity to other apolipoproteins, such as apoA-I and apoA-IV.[5,6] Mice with targeted inactivation of *apoa5* gene have a

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marked elevation (+400%) of plasma triglycerides with respect to controls, caused by an accumulation of VLDL.[5]However, mice overexpressing this gene have reduced plasma levels of triglycerides and VLDL (−70%).[5,7] Genetic studies in humans have indicated strong association between some common polymorphisms of *APOA5* gene and plasma triglyceride concentration.[5,8–14]

We found that 1 of our patients was homozygous for a single nucleotide substitution in exon 4 of *APOA5* gene, which is predicted to cause a premature termination of mRNA translation and the formation of a truncated apoA-V of 144 amino acids. This is the first observation that links apoA-V to a genetic defect of plasma lipoproteins in humans.

Methods

Proband

Proband K.Y. is a 9-year-old boy who was admitted to the hospital at age 5 years for recurrent episodes of abdominal pain and severe hypertriglyceridemia. His healthy parents are first cousins and came from Tunisia. Proband's brother was apparently healthy.

On physical examination, the proband was found to have planar xanthomas on the right elbow, several eruptive cutaneous xanthomas, and a mild hepatosplenomegaly. His body mass index (kg/m^2) was 14.

Routine laboratory tests, with the exception of plasma lipids, were within the reference range. Plasma triglyceride (TG) level was >50 mmol/L (10th to 90th percentiles: 0.48 to 1.30 mmol/L), total cholesterol (TC) was 7.76 mmol/L (10th to 90th percentiles: 3.57 to 5.50 mmol/L), and high-density lipoprotein (HDL) cholesterol was 0.61 mmol/L (10th to 90th percentiles: 1.03 to 1.80 mmol/L). Because he was suspected to have familial hyperchylomicronemia secondary to LPL or apoC-II deficiency, he was treated first with medium chain triglyceride (MCT)-containing diet and subsequently with ω-3 fatty acids (EPA+DHA 3.4 g/d), associated with a low-fat diet (<20 g/d).

Informed consent was obtained from all subjects investigated. The study protocol was approved by the institutional human investigation committee of each participating institution.

Plasma Lipids Analysis and LPL Assay

TC, HDL cholesterol, and TG levels were determined by standard methods. The assay of plasma apoC-II and apoC-III was conducted by immunonephelometry using polyclonal antibodies.[15]

Lipoprotein and hepatic lipase activities in postheparin plasma were assayed as previously reported.[16] In the first LPL assay, we used postheparin plasma that had been collected during the first clinical examination of the proband and had been stored at −80°C for some weeks. The second LPL assay was performed using freshly isolated postheparin plasma collected from the proband during ω-3 fatty acids treatment. LPL was also assayed in the parents and the brother of the proband. To test the capacity of apoA-V–deficient plasma to activate LPL activity, 10 μL of postheparin plasma from a control subject and proband K.Y. were incubated in the presence of 60 to 150 μL of control or proband's plasma (used as source of LPL activators).

Analysis of Candidate Genes

Genomic DNA was extracted from peripheral blood leukocytes by standard procedure. All exons and the promoter region of *LPL* and *APOC2* genes were amplified by polymerase chain reaction (PCR) from genomic DNA using appropriate primers.

The coding sequence of *APOA5* gene (exons 2 to 4) was amplified using the following primers: 2F (5′-GAG CCC CAA CAG CTC TGT GC-3′ and 2R (5′-GGC CCT CTG GCC AGC CAC CA-3′) for exon 2; 3F (5′-GGG AGG AGA GCC CAG GCC CT-3′) and 3R (5′-GAG GTT GAG GCA GCA GAG GC-3′) for exon 3; 4F (5′-CGG CCT GGA TAT CTG TCC CC-3′) and 4eR (5′-CCC AGT GCC TGC AAA GGC TC-3′) for the 5′ half of exon 4; and 4eF (5′-GGT GCT CTC CCG GAA GCT CA-3′) and 4R (5′-GCC TCT CCC TCC CTA CTC CC-3′) for the 3′ half of exon 4. Exon 4 was amplified in 2 overlapping fragments of 662 bp and 707 bp, respectively. The amplification conditions for exons 2 and 3 were 95°C for 40 seconds, 61°C for 40 seconds, and 68°C for 2 minutes for 29 cycles. The amplification conditions for the 2 regions of exon 4 were 95°C for 3 minutes (hot start), 95°C for 40 seconds, 61°C for 40 seconds, and 72°C for 7 minutes for 10 cycles, followed by 20 cycles at 95°C for 40 seconds, 61°C for 40 seconds, and 72°C for 7 minutes and 20 seconds. The mutation of *APOA5* gene found in the proband, which eliminates a *Pst*I restriction site, was confirmed by restriction fragment analysis of PCR-amplified 5′ region of exon 4 using the forward primer 5′-AGT TGG AGG AGG TGA AGG CTC G-3′ and the reverse primer 4eR (see previous). The amplification conditions were 94°C for 3 minutes, 94°C for 15 seconds, 60°C for 30 seconds, and 72°C for 1 minute for 30 cycles, followed by 72°C for 7 minutes. The amplification product (500 bp) was digested with *Pst*I and the fragments (148 and 78 bp for the wild-type and 226 bp for the mutant allele) were separated by agarose gel electrophoresis. The nucleotide substitutions were designated according to the recommendations of the Nomenclature Working Group for human gene mutations.[17]

The proband and family members were also genotyped for the following polymorphisms: −1131 T/C in *APOA5* gene; −482 C/T in *APOC3* gene; ϵ2, ϵ3, and ϵ4 in *APOE* gene; and D9N and N291S of *LPL* gene, using previously described methods.[5,18–20] The c.47 C/G (S16W) polymorphism located in exon 3 of *APOA5* gene was determined by direct sequencing.

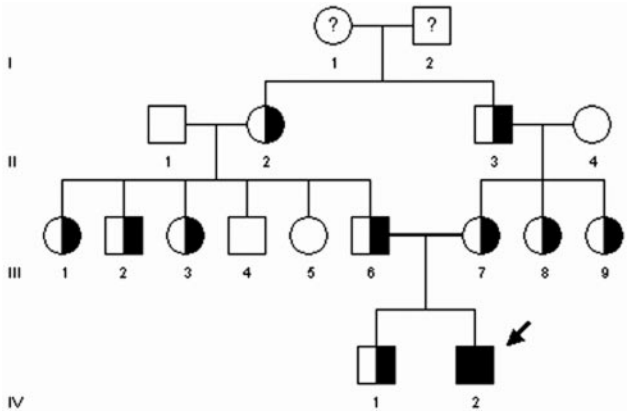

Figure 1. Pedigree of proband K.Y. The proband is indicated by an arrow. All subjects indicated in the Figure (apart from subjects I.1 and I.2) were genotyped for *APOA5* gene mutation c.433 C>T (Q145X). Half-shaded symbols indicate heterozygotes for this mutation.

Results

Analysis of Candidate Genes

Proband's pedigree is shown in Figure 1. Because most members of this large pedigree were living in Tunisia, we were unable to examine them directly. With the exception of the proband (IV.2), his parents (III.6 and III.7), and his brother (IV.1), who have been living in Sicily for several years, all lipid parameters of the other family members (Table 1) were assessed in the country of origin. Table 1 shows that the proband had severe hypertriglyceridemia and hypercholesterolemia; plasma TG levels of his parents were moderately elevated. Maternal grandfather (II.3) had moderate mixed hyperlipidemia; paternal grandfather (II.1) and 2 siblings of proband's father (III.2 and III.5) had mild hypertriglyceridemia.

TABLE 1. Plasma Lipid Concentration in Members of the K.Y. Family

Subject	ApoA-V Q145X	Age, y	Sex	BMI, kg/m^2	TC, mmol/L	TG, mmol/L	Glucose, mmol/L	ApoA-V −1131T/C	ApoA-V S16W	Apo C-III −482 C/T	ApoE Genotype
II.1	QQ	76	M	24.7	5.56	3.53	6.10	TT	SS	CC	ε3ε3
II.2	QX	66	F	25.7	5.82	2.12	6.22	TT	SW	CT	ε3ε3
II.3	QX	61	M	25.8	8.02	4.45	5.99	TT	SW	CT	ε3ε3
II.4	QQ	55	F	26.3	6.44	1.01	5.88	TT	SS	CC	ε3ε3
III.1	QX	46	F	29.4	4.42	1.58	5.60	TT	SW	CT	ε3ε3
III.2	QX	44	M	31.9	5.82	2.50	6.99	TT	SW	CT	ε3ε3
III.3	QX	40	F	33.5	2.95	0.67	7.10	TT	SW	CT	ε3ε3
III.4	QQ	33	M	27.4	4.34	0.99	6.10	TT	SS	CC	ε3ε3
III.5	QQ	31	F	28.9	5.30	2.70	5.44	TT	SS	CC	ε3ε3
III.6	QX	36	M	25.2	4.94	4.65	5.27	TT	SW	CT	ε3ε3
III.7	QX	32	F	26.3	3.75	3.24	5.44	TT	SW	CT	ε3ε3
III.8	QX	29	F	25.7	4.00	0.52	4.88	TT	SW	CT	ε3ε3
III.9	QX	17	F	19.0	4.47	0.93	4.88	TT	SW	CT	ε3ε3
IV.1	QX	10	M	17.8	5.09	0.75	4.16	TT	SW	CT	ε3ε3
IV.2	XX	5	M	13.5	7.76	50.16	4.60	TT	WW	TT	ε3ε3

Apo indicates apolipoprotein; BMI, body mass index, F, female; M, male; TC, total cholesterol; TG, triglyceride.

The pretreatment level of plasma postheparin LPL activity of the proband (5.08±0.39 μmol free fatty acid [FFA]/mL per hour, mean±SD of triplicate determinations), assayed in vitro in the presence of control plasma, was below the lower limit of our reference range (8 to 15 μmol FFA/mL per hour);[16] hepatic lipase activity was 40.4±2.3 μmol FFA/mL per hour (reference range 35 to 55 μmol FFA/mL per hour). A subsequent LPL assay performed with fresh postheparin plasma resulted in a value of 6.10±0.23 μmol FFA/ mL per hour (Table 2). LPL activity in proband's father and mother (obligate heterozygotes) and proband's brother were 8.1±0.40, 10.2±0.25, and 11.2±0.42 μmol FFA/mL per hour, respectively (mean±SD of triplicate assays).

The presence of consanguinity suggested that the proband had a rare recessive disorder of TG metabolism. The sequence of *LPL* gene revealed no pathogenic mutations but only a silent mutation in heterozygous state (c.1083 C>A, T361T). No mutations were found in *APOC2* gene.

In view of these negative results, we decided to sequence the *APOA5* gene on the assumption that mutations of this gene abolishing the production/activity of apoA-V might result in hypertriglyceridemia, as observed in *apoa5* gene knockout mice.[5]

The proband was found to be homozygous for a nucleotide substitution in exon 4, which converts the glutamine codon CAG into a termination codon TAG (c.433 C>T, Q145X).

The presence of the nonsense mutation in exon 4 was confirmed on 3 independent PCR amplifications of the 5′ half of exon 4 and in 2 different laboratories. We confirmed the presence of the mutation by restriction fragment analysis of the candidate gene region, taking advantage of the fact that the mutation abolishes a *Pst*I restriction site (see Methods and Figure 2). Several family members, including proband's parents, were found to be heterozygous for the mutation (Table 1). The proband was also homozygous for the rare alleles of 2 common polymorphisms located in exon 3 (c.47 C>G, S16W and c.123 C>A, I41I), and for the more frequent allele (−1131 T/T) in the promoter region of *APOA5* gene. In addition, the proband was homozygous for the rare allele of the −482 C/T polymorphism of *APOC3* gene (−482 T/T).[21]

Ten members of the proband's family, including proband's parents, were Q145X carriers. All of them were also heterozygous for S16W polymorphism of *APOA5* gene and for −482 C/T polymorphism of *APOC3* gene. The apoE genotype of the proband and for all family members investigated was ε3/ε3 (Table 1). None of the family members was found to carry the 2 LPL variants D9N and N291S.

Plasma Lipids and ApoC-III Levels

Mean plasma TG level (unadjusted and adjusted values for age and sex) was higher in Q145X carriers (adjusted TG value: 2.27±1.13 mmol/L) than in noncarrier family members (adjusted TG value: 1.74±1.26 mmol/L); however, this difference did not reach the level of significance. Mean plasma TC level (unadjusted and adjusted values for age and sex) was similar in the 2 groups (adjusted TC values: 5.05±0.93 versus 5.09±1.25 mmol/L).

The plasma level of apoC-III in the proband, measured during treatment with ω-3 fatty acids (when his plasma TG level was 5.7 mmol/L), was 27.4 mg/dL. The plasma level of apoC-III in proband's father, mother, and brother were 15.2, 16.1, and 11.2 mg/dL, respectively (normal range, 7 to 10 mg/dL).

Activation of LPL Activity by ApoA-V–Deficient Plasma

To test the capacity of proband's plasma (apoA-V–deficient plasma) to activate LPL activity, we performed the assays illustrated in Table 2. LPL activity of a control subject was

TABLE 2. Activation of LPL by ApoA-V–Deficient Plasma

Postheparin Plasma, 10 μL	Activator Plasma, μL	LPL Activity, μmol/mL per hour	Kruskal–Wallis Test
Control plasma	Control plasma		
	60	9.20±0.48(a)	
	100	8.95±0.27(b)	$P<0.02$
	150	8.38±0.26(c)	
Control plasma	ApoA-V–deficient plasma		
	60	6.10±0.23(d)	
	100	5.13±0.32(e)	$P<0.005$
	150	4.20±0.26 (f)	
Control plasma	ApoC-II–deficient plasma*		
	60	<1.0	
	100	<1.0	
Apo A-V–deficient plasma	Control plasma		
	60	6.83±0.36(g)	
	100	6.16±0.30(h)	$P<0.005$
	150	5.68±0.34 (i)	
Apo A-V–deficient plasma	Apo A-V–deficient plasma		
	60	4.36±0.36 (j)	
	100	4.11±0.39(k)	$P<0.005$
	150	3.31±0.25 (l)	

*Plasma from the patient described in reference 15.

LPL activity values are expressed as the mean±stand deviation of triplicate assays of 2 independent experiments.

Plasma TG and apo C-III levels were 1.14 mmol/L and 10.1 mg/dL in control plasma, 5.70 mmol/L and 27.4 mg/dL in proband's plasma (apoA-V–deficient plasma), and 22.80 mmol/L and 43.8 mg/dL in apoC-II–deficient plasma.

All the differences between groups evaluated by Mann–Whitney test, ie, (a) vs (d), (b) vs (e), (c) vs (f), (g) vs (j), (h) vs (k), and (i) vs (l), were significant at $P<0.005$.

found to be reduced by ≈40% when the assay was performed in the presence of apoA-V–deficient plasma as enzyme activator. Similar results were obtained when the proband's LPL activity was assayed in the presence of apoA-V–deficient plasma. LPL activity of both control and proband's plasma decreased as the concentration of apoA-V–deficient plasma increased (from 60 to 150 μL) in the incubation medium. A similar decrease, although less pronounced, was observed when control plasma was used as LPL activator (Table 2).

Treatment of Hypertriglyceridemia

As an initial treatment trial, the patient was given a low-fat diet supplemented with medium chain TGs. Because no beneficial response was observed after a 2-month period, we decided to treat the patient with ω-3 fatty acids (3.4 g/d), because chronic ω-3 fatty acid supplementation significantly lowers postprandial TG concentration, regardless of the type of fat in the test meal,[22] and had been successfully used in some cases of severe primary hyperchylomicronemia.[23] In view of the substantial reduction in serum TG (−90%) and TC (−60%) observed after 2 months, this treatment was continued for 4 months, monitoring the lipid profile on a monthly basis. During this period, plasma TG remained <8 mmol/L and the patient had no episodes of abdominal pain. At the end of 8 months of treatment, eruptive cutaneous xantomas had completely disappeared. Treatment with low-fat diet and ω-3 fatty acids was continued over the next 36 months. During this period, plasma TG showed large fluctuations ranging from 1.96 to 9.12 mmol/L, with a mean value of 5.74 mmol/L. We attributed these fluctuations to the poor compliance to treatment and to a less stringent follow-up schedule.

Discussion

In the present report, we described a patient who presented the classical features of the familial chylomicronemia syndrome (severe hypertrigliceridemia, recurrent episodes of abdominal pain, and eruptive xanthomatosis) and who was found to be homozygous for a pathogenic mutation in the recently discovered *APOA5* gene. This mutation is predicted to result in a truncated apoA-V of 144 amino acids ≈40% the size of the wild-type protein. This truncated protein, if synthesized and secreted, is presumably devoid of any function, because it lacks the region between residues 171 and 245, which contains 3 very hydrophobic, strongly amphipathic α-helices, predicted to have high surface activity for interaction with lipids.[24,25] Because the analysis of amino acid variability among human, rat, and mouse apoA-V sequences has revealed that several domains of the carboxy-terminal

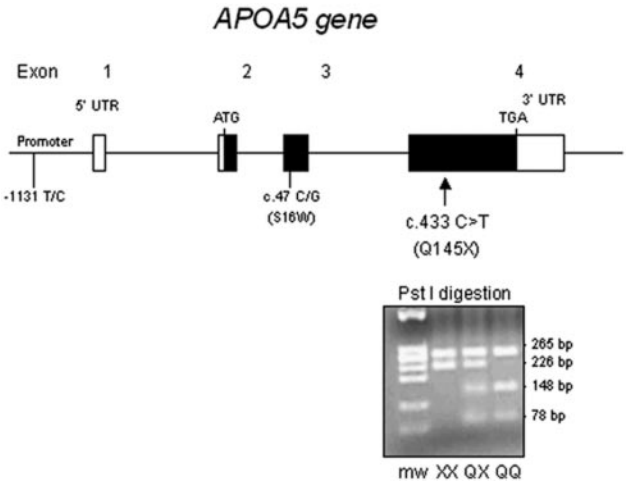

Figure 2. Upper panel, Localization of 2 common polymorphisms (−1131T/C and c.47C/G) and the pathogenic mutation (c.433C>T, Q145X) identified in *apoA-5* gene of proband K.Y. Lower panel, The result of the screening for the c.433C>T mutation based on PCR amplification and enzyme digestion with *Pst*I. Ln 1 shows proband; lane 2, proband's father; lane 3, unaffected family member; MW, molecular size marker.

half of the protein are highly conserved,[24] it is likely that these domains (all missing in the truncated protein) have a key functional role.

By extending the genetic analysis to all family members available for study, we discovered 10 carriers of the pathogenic mutation. Only 5 of them had plasma TG levels >1.70 mmol/L.[26] This result suggests that other genetic/environmental factors contribute to the elevation of plasma TG in heterozygotes for apoA-V deficiency as it occurs in heterozygotes for LPL deficiency in whom the level of fasting plasma TG may be in the normal range or slightly elevated, the latter in the case of subjects older than 50 or with some degree of adiposity.[16,27,28] In our kindred apoA-V mutation, carriers with plasma TG >1.70 mmol/L tended to be older (47.8±15.1 versus 28.4±15.1 years) and to have higher body mass index (+7.5%) and plasma glucose (+12%) than the mutation carriers with normal TG levels. The variability of plasma TG in apoA-V mutation carriers cannot be explained by some common polymorphisms of candidate genes (*APOA5*, *APOC3*, *LPL,* and *APOE*) affecting plasma TG levels (Table 1). However, postheparin LPL activity measured in proband's parents who had mild hyeprtrigliceridemia (subjects III.6 and III.7 in Table 1) was within the normal range, as was that found in proband's brother (subject IV.1 in Table 1) who had normal plasma TG.

To the best of our knowledge, this is the first pathogenic mutation of apoA-V found in humans. The plasma lipid phenotype observed in our proband resembles the one found in *apoa5* knockout mice, which have a marked elevation of serum TG and VLDL.[4,5]

The mechanism whereby apoA-V affects plasma TG and VLDL/chylomicron levels is a matter of intensive investigation. The apoA-V might act in the plasma by affecting the lipolytic cascade, in the hepatocytes/enterocytes by modulating the assembly/secretion of TG-rich lipoproteins, or in both these compartments. In human, rat, and mouse plasma, apoA-V is found mostly in HDL;[4–6] its concentration is very low (eg, 100 μg/dL in rat and 5 to 50 μg/dL in human plasma)[6,29] as compared with that of the other major HDL apolipoproteins (apoA-I and apoA-IV) and the LPL activator apoC-II. Fruchart-Najib et al[30] found that in mice overexpressing human *APOA5* gene, VLDL TG secretion by the liver was similar to that found in control mice, whereas plasma VLDL TG turnover was much faster and strongly correlated with increased LPL activity in postheparin plasma. They also showed that recombinant apoA-V interacted with LPL protein in vitro and increased its activity.[30] Schaap et al[31] using adenovirus-mediated transfer of murine *apoa5* gene into mice found that in vivo hyperexpression of apoA-V stimulated LPL-dependent clearance of TG-rich lipoproteins and in vitro stimulated LPL activity up to 2.3-fold only in the presence of apoC-II. These combined observations[30,31] strongly suggest that apoA-V acts as a stimulatory modifier of apoC-II induced LPL-mediated TG hydrolysis. Whether the minute amounts of apoA-V present in human plasma under physiological conditions are sufficient to promote this LPL activation remains to be firmly established. In this context, we tried to assess the capacity of proband's plasma (apoA-V–deficient plasma) to act as activator of LPL in vitro. We found that when apoA-V–deficient plasma was used as activator, LPL activities of a control subject and the proband were reduced as compared with those observed when control plasma was used as LPL activator. The finding that increasing the concentration of apoA-V–deficient plasma in the incubation mixture was associated to a further decrease of LPL activity strongly suggests that our apoA-V–deficient plasma contains an LPL inhibitor. Because the concentration of apoC-III in apoA-V–deficient plasma was 2.7-fold that of the control plasma used in the vitro assay (Table 2), it is conceivable to assume that the reduced LPL activation capacity of apoA-V–deficient plasma is the result of a combined effect of the absence of an activator (apoA-V) and the presence of an inhibitor (apoC-III).[31–33]

Shaap et al[31] also observed that *apoa5* hyperexpression in mice diminished VLDL TG production rate without affecting VLDL particle production, suggesting that apoA-V impaired lipidation of apoB. The hypothesis that apoA-V might act intracellularly by reducing VLDL TG secretion had been first proposed by Weinberg et al,[24] who, on the basis of the structural analysis and interfacial properties of apoA-V, suggested that this apolipoprotein might retard the assembly of TG-rich lipoproteins. They demonstrated that apoA-V transfected in COS-1 cells was poorly secreted, remained associated with the endoplasmic reticulum, and did not traffic to the Golgi.[24]

With regard to the treatment of hypetriglyceridemia in our patient, we first conducted a 2-month trial with a low-fat MCT-containing diet, which is one therapeutic option in hyperchylomicronemia syndrome, because MCTs are absorbed directly into the portal circulation and do not appear to contribute to the generation of chylomicron TG. The treatment with ω-3 fatty acids was found to have beneficial effects in terms of reduction of hypertriglyceridemia, episodes of abdominal pain, and eruptive cutaneous xanthomas.

Omega-3 fatty acids have not been frequently used in the treatment of genetic disorders of plasma lipolytic cascade (LPL and apoC-II deficiency), because in this group of patients the main defect is the inability to hydrolyze circulating plasma TG in VLDL and chylomicrons. Some cases, however, have been reported in which ω-3 treatment in combination with MCT-containing diet was found to have beneficial effects in these patients.[23]

The plasma TG-lowering effect of ω-3 fatty acids may be caused by several mechanisms. It has been known for a long time that ω-3 fatty acids inhibit the synthesis/secretion of VLDL TG by the liver.[22] Recent in vivo and in vitro studies[34] have demonstrated that this reduced VLDL secretion is related to an increased intracellular degradation of apoB-100 through a novel pathway designated postendoplasmic reticulum presecretory proteolysis.[35] This pathway is activated by the increased lipid peroxydation and oxidative stress related to the high hepatic content of ω-3 polyunsaturated fatty acids.[34] There is also recent evidence in normolipidemic humans that ω-3 fatty acids reduce postprandial TG and apoB-48 levels, chylomicron TG half-life, and chylomicron size, and that they increase preheparin LPL activity during the fed state.[36] These data indicate that ω-3 fatty acid supplementation accelerates TG clearance by increasing LPL activity,[36] thus providing a rationale for this treatment in genetic disorders of the lipolytic cascade.

In conclusion, we identified a pathogenic mutation in *APOA5* gene, predicted to cause apoA-V deficiency, in a patient with severe hypertriglyceridemia. Heterozygotes for this mutation had normal or moderately elevated plasma TGs. Supplementation of a low-fat diet with MCT had no effect on this type of hypertriglyceridemia, whereas treatment with ω-3 fatty acids was beneficial.

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