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A novel homozygous mutation causing lecithin–cholesterol acyltransferase deficiency in a proband of Romanian origin with a record of extreme gestational hyperlipidemia

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KEYWORDS:
Inherited HDL deficiency;
Molecular diagnosis;
Kidney disease;
Lipoprotein X;
LCAT deficiency;
Genetic analysis

Abstract: A patient from Romania with extraordinarily high total cholesterol levels and clinical and biochemical features consistent with familial lecithin–cholesterol acyltransferase deficiency is reported. The genetic analysis performed on our proband showed a novel homozygous mutation on codon 119 of lecithin–cholesterol acyltransferase gene that causes the substitution of glycine by aspartate. The same mutation, also in homozygosis, was observed in her older sister, whereas his brother presented it in heterozygosis.

Introduction

Extremely low levels of high-density lipoprotein cholesterol (HDL-C) can result in different clinical presentations depending on the etiology. Apolipoprotein A-I deficiency, Tangier disease, and lecithin–cholesterol acyltransferase (LCAT) deficiency are the main inherited monogenic disorders that cause severe HDL deficiency. LCAT is an enzyme responsible for cholesteryl ester (CE) formation in HDL. CE is accumulated in HDL particles, leading to maturation from small, discoidal particles to large, spherical HDL particles.[1] Two forms of LCAT deficiency have been described: a complete lack of LCAT activity, known as familial LCAT deficiency (FLD), and a partial deficiency, called fish eye disease (FED).[2] We report here a patient from Romania who presents with clinical and biochemical features consistent with FLD and who carries a novel homozygous mutation on the *LCAT* gene. This patient, however, also presented a record of extreme

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gestational hyperlipidemia with episodes consistent with acute, transient cholestatic events that complicated the interpretation of the case.

Case report

A 33-year-old Romanian woman was referred to our Lipid Unit for drug-resistant dyslipidemia. She presented with very high total cholesterol (TC) levels (834 mg/dL), extremely low HDL-C levels (12 mg/dL), and mild anemia. The patient was referred from the obstetric department after giving birth to a healthy child (February 2011). During pregnancy, she developed gestational diabetes managed with insulin therapy. Three years before this pregnancy, she had a spontaneous abortion after 20 weeks of gestation. Prior ultrasound kidney examinations, because of repetitive cystitis, showed no morphologic abnormalities. She also presented with normal renal blood tests. Five years before our assessment, the patient was referred to an endocrinologist because of dyslipidemia (TC: 297 mg/dL, triglycerides: 305 mg/dL). Increases in aspartate aminotransferase (160 U/L) and alanine aminotransferase (119 U/L) were also noted at that time. An abdominal ultrasound was performed. There were no hepatic abnormalities in shape or size, but she had a 14 cm long spleen. Dietary and lifestyle interventions were initiated at that time, as well as treatment with gemfibrozil and fenofibrate, for 2 years. Later controls showed persistent dyslipidemia (TC: 335 mg/dL, HDL-C: 6 mg/dL, triglycerides: 282; low-density lipoprotein cholesterol [LDL-C]: 273). Treatment was replaced by 80 mg atorvastatin, without improvement in TC (406 mg/dL) or triglycerides (590 mg/dL). The patient had no other remarkable past medical history. Her parents had no consanguinity. Both died over 75 years of age in Romania. She also has an older sister (44 years of age) with a history of dyslipidemia and 2 more siblings (a 42-year-old man and a 50-year-old woman) with no known chronic diseases. Like her, all siblings emigrated to Spain from Romania. Our proband had no known allergies and was not taking medications at the time of our first assessment. A complete blood count showed mild normochromic anemia (hemoglobin: 10.7 g/dL, hematocrit: 30.9%, mean red cell volume: 89.2) with some of the erythrocytes having the morphology of target cells. Liver blood tests revealed a progressive increase in transaminases (aspartate aminotransferase: 415 U/L and alanine aminotransferase: 200 U/L), gamma-glutamyltransferase (268 U/L), alkaline phosphatase (369 U/L), and bilirubin (1.5 mg/dL) at 10 weeks postpartum. These values normalized in subsequent controls. Tests during pregnancy and early puerperium showed largely normal liver laboratory tests. Creatinine was within normal limits, but 24-hour urine analysis revealed a daily protein excretion of 0.27 g/24 h. The autoantibody screen was negative. Immunoglobulin levels were normal. Further testing revealed TC and HDL-C levels of 703 and 10 mg/dL, respectively. The calculated LDL-C level by Friedewald formula was 666 mg/dL. Plasma triglycerides (136 mg/dL) and apoB concentrations (112 mg/dL) were within normal limits. On examination, the proband reported no symptoms. Her eyes revealed intense corneal opacity and a pale-yellow halo around the iris (Fig. 1).[3,4] These findings had been present for as long as she could remember. She reported no visual deficit. Splenomegaly was noted on palpation. Wrinkled, not-pruritic, yellowish, cutaneous lesions appeared on the trunk and upper extremities transiently, but disappeared shortly thereafter (Fig. 2). Blood pressure and electrocardiogram were within normal values. Chest and abdominal X-rays revealed essentially normal findings, but the abdominal computed tomography showed again a prominent spleen (16 cm). A liver manometry indicated normal pressure in the portal vein and superior vena cava. A liver biopsy performed 5 months after delivery showed small groups of cells containing lipid droplets consistent with microvascular steatosis. Autoimmune hepatitis and primary biliary cirrhosis were ruled out. Histologic analysis of the skin-lesion biopsy revealed abundant cells with a lipid-load macrophage content.[5] The context of the clinical characteristics and the biochemical and histologic analyses performed suggested LCAT deficiency. After informed consent, peripheral blood samples were taken from the proband to perform a more detailed biochemical study as well as a genetic analysis. The fetal calf (FC) serum concentration was 192 mg/dL, which was 89% of TC (reference value: <40%). HDL was separated by ultracentrifugation. The FC concentration in HDL was 11.2 mg/dL, which was 83% of HDL-C (reference value: <34%). The cholesterol esterification rate in serum, as a measure of endogenous LCAT activity, was null (reference value: >3.5% per hour).[6,7] The *LCAT* gene was amplified by polymerase chain reaction, and exons and exon-intron boundaries were sequenced.[6,7] This analysis revealed a novel homozygous mutation, c.356G>A (Fig. 3), which, to the best of our knowledge, has not previously described, and which is predicted to cause substitution of glycine by aspartate on position 119 of LCAT. These findings were independently confirmed by an analysis of the restriction enzyme MvaI

Figure 1 (1.5 column): The eyes. Both corneas present intense opacity. Iris is surrounded by a pale-yellow halo.

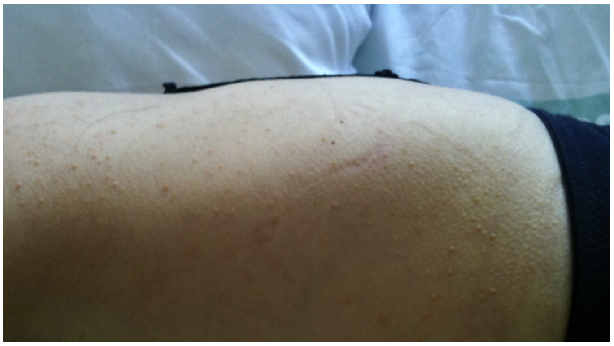

Figure 2 (1.5 column): Xanthomas. Wrinkled, not-pruritic, yellowish cutaneous lesions in trunk and upper extremities.

(Supplementary Fig. 1). Although the pathogenicity of the mutation has not been directly demonstrated, this result would be highly consistent with our clinical and biochemical findings. Furthermore, all in silico analyses performed with the PolyPhen2, Shift, Provean, i-Mutant 3.0, and PANTHER programs concluded that this mutation was a disease-causing mutation. We have recently attended the 3 siblings. One sister (aged 50 years) had similar corneal opacities but without skin lesions. Biochemical analyses showed (Supplementary Table 1) a TC of 174 mg/dL, an LDL-C of 142 mg/dL and an HDL-C of 5 mg/dL but without renal (24-hour protein excretion was within reference values) or hepatic impairment. ApoAI and ApoB levels were 44 and 36 mg/dL, respectively. The brother showed marked dyslipidemia (TC: 467 mg/dL, triglycerides: 1762 mg/dL, HDL-C: 22 mg/dL, ApoAI: 106 mg/dL, ApoB: 185 mg/dL). The other sister (aged 56 years) only showed mild hypertriglyceridemia (TC: 210 mg/dL, triglycerides: 258 mg/dL, HDL-C: 51 mg/dL, ApoAI: 151 mg/dL, ApoB: 113 mg/dL). Neither had corneal opacities or other biochemical impairments. No siblings were on lipid-lowering therapy as the proband left statin therapy 1 year ago. DNA sequencing of the siblings (Supplementary Table 1) revealed the mutation in her 50-year-old sister (in homozygosis) and her brother (in heterozygosis) previously identified in the proband. The 56-year-old sister did not inherit the mutation. FC serum concentration was 82.5% of TC in the affected sister and 30.5% and 24% in the brother and the unaffected sister.

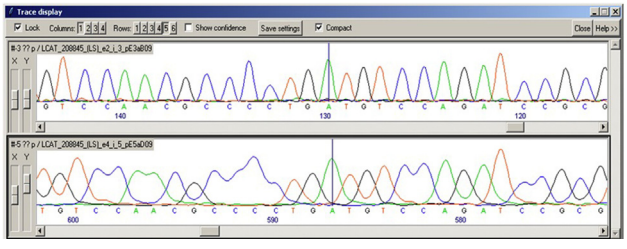

Figure 3 (Double column): *LCAT* sequencing. *LCAT* sequencing of both strands of amplicons containing exons 2 and 3 of the gen showed in both cases had an homozygous c.356G>A exchange (labeled as number 130), which corresponds to a p.(Gly119Asp) exchange.

The presence of Lp-X in the affected siblings was confirmed by a differential protein composition of the lipoprotein fraction with a density <1.063 g/mL but >1.016 g/mL as isolated by sequential ultracentrifugation (Fig. 4A), as well as by the unique co-existence of these 2 types of lipoprotein families (Fig. 4B). All these results are fully consistent with the known properties of Lp-X.[8] Therefore, both affected siblings presented Lp-X in addition to LDL in the analyzed density, whereas none of the siblings unaffected by LCAT deficiency (one without the mutation and the other heterozygous) presented with Lp-X.

Therefore, our diagnosis, first based on DNA sequencing, was fully supported by the biochemical findings obtained from the detailed serum analysis of the proband and the siblings.

Intermittent anemia was seen during follow-up with controls of the proband. A close follow-up of the renal function of the proband was also performed. Microalbuminuria was present (3.5 mg/dL; microalbuminuria/creatinuria ratio of 52.7 µg/mg), and the urine analysis was positive for red blood cells (++). To date, daily protein excretion has not increased, and renal blood tests are within normal values. Current tests show persistent hyperlipidemia.

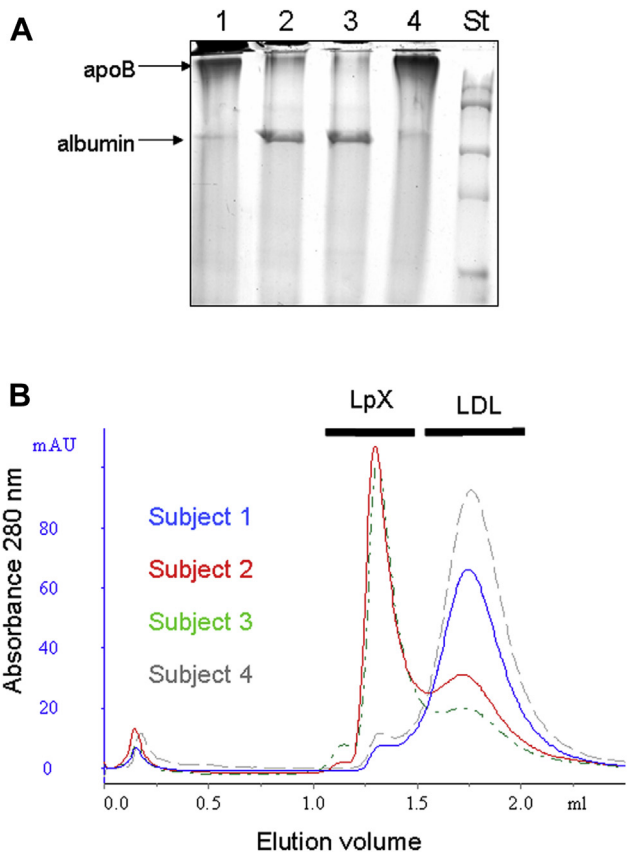

Figure 4 (Single column): Demonstration of Lp-X. (A) SDS polyacrylamide gel electrophoresis showing the main protein content of the lipoprotein fraction isolated by a density of <1.063 g/mL but >1.016 g/mL in patient 1 (unaffected sister), 2 (proband), 3 (affected sister), and 4 (unaffected brother). (B) Absorbance at 280 nm of the lipoprotein fractions isolated by a density of <1.063 g/mL but >1.016 g/mL and subjected to size exclusion chromatography. The numbers of the patients are the same as in panel (A).

FC percentages did not change. As expected, HDL-C levels remained low, and opacities on the eyes persisted. Both the proband and the affected sister were subjected to regular blood tests to control cardiovascular risk. The brother is on follow-up as well. For a more detailed biochemical follow-up of the proband over 10 years, see Supplementary Table 2.

Discussion

Clinical presentation of complete FLD includes corneal opacity and proteinuria that can lead to renal failure, splenomegaly, and moderate normochromic anemia. Therefore, it can very often be clinically distinguished from Tangier disease and apoA-I deficiency, the two other monogenic inherited HDL deficiencies. We report a case of a novel homozygous mutation on codon 119 that causes the substitution of glycine by aspartate. Our proband, despite being relatively young, presented with most clinical and biochemical characteristics of complete FLD. However, the proband also presented a record of extreme gestational hyperlipidemia with episodes consistent with acute, transient cholestasis that complicated the interpretation of the case. The role of LCAT deficiency in the pathogenesis of atherosclerosis has not yet been clearly established. However, control of risk factors, such as LDL-C, with statins and follow-up of proteinuria must be considered. This report is, to the best of our knowledge, the third report of LCAT deficiency in Spain[6,7] although the previous reports were in families of Spanish origin. We did not find reports of LCAT deficiency from Romania.

LCAT was first identified by Glomset et al. in 1962. It is a human enzyme of 67-kDa size encoded by a gene on region 16q22 of chromosome 16, comprising 6 exons and 5 introns. This protein is composed of 416 amino acids and mainly synthesized in the liver but also in the brain and testicles.[1,2] At present, 95 mutations of the *LCAT* gene are listed in the Human Gene Mutation Database, which was accessed on January 19, 2017. Of those, 75 were missense/nonsense mutations, 1 was a splice site mutation, 10 were small deletions, 1 was a gross deletion, 5 were small insertions, 2 were small indels, and 1 was a gross insertion/duplication.[9] LCAT esterifies the FC on lipoproteins, mainly HDL, by catalyzing the transfer of a fatty acid (usually linoleic acid) from the C-2 position of lecithin or phosphatidylcholine to the 3-hydroxyl group of free cholesterol to form CE. LCAT activity is also detected in apo-B-containing lipoproteins (VLDL and LDL) although in a smaller proportion.[1,2] Apo A-I content in HDL is the main LCAT activator.

Norum and Gjone first described FLD in 1967 in a 33-year-old woman.[10,11] LCAT deficiency is a rare autosomal recessive disease with a prevalence of 1/1,000,000 individuals. Inherited mutations in both alleles of the *LCAT* gene result in 2 forms of LCAT deficiency. Patients with total loss of activity of the enzyme are classified as FLD; those who maintain partial LCAT activity suffer FED. Patients with FED are relatively asymptomatic but present with corneal opacities and HDL-C deficiency without Lp-X, renal disease, or anemia. FLD patients present with biochemical abnormalities in their serum that include decreased HDL-C levels, mild-to-severe hypertriglyceridemia, increases in serum FC, and the presence of Lp-X, which are a class of large, multilamellar, and heterogeneous phospholipid vesicles also enriched in FC and triglycerides with very low CE and apoB content. Named Lp-X because of their abnormal electrophoretic mobility, these lipoproteins were present in the case reported here. LDL-C levels are often low because of increased catabolism of abnormal LDL particle.[12] Calculated LDL-C by Friedewald formula, however, could be increased in this case, at least in part, because of the presence of Lp-X as suggested by the fact that the apoB concentration was not increased. Corneal opacification and anemia are 2 of the most common findings in LCAT deficiency. Corneal opacification starts early in life, as young adults form a ring near the limbal area. Vision is not typically impaired. The corneas of these patients contained large depositions of free cholesterol and phospholipids.[13] The anemia in these patients was hemolytic, normochromic, and normocytic, with hemoglobin levels of approximately 10 g/dL. The anemia is thought to be caused by FC and phosphatidylcholine deposition in erythrocyte membranes, which would induce shortening of the erythrocyte lifespan and enhanced catabolism, probably secondary to hypersplenism.[14] Foam cells and sea-blue histiocytes were found on analysis of bone marrow samples. FLD prognosis depends on renal function because it is a major cause of morbidity and mortality. Proteinuria can be noted early, as well as microscopic hematuria, and might progress to nephrotic syndrome and end-stage renal disease, typically by the fourth or fifth decade of life. The renal disease is thought to be characterized by deposition of Lp-X particles and could be facilitated by pro-oxidative, proinflammatory mechanisms associated with HDL deficiency. Light microscopic examination of renal biopsies in the early stage of the disease reveal foam cells in the glomerular tufts and thickening of the glomerular basement membrane. Electron microscopy shows lipid deposition in the glomerular basement membrane, in the extracellular matrix, in Bowman's capsule, and in the vascular endothelium.[15] Close follow-up consultation and monitoring of blood pressure, blood glucose, and other disorders that can affect renal function is imperative. Kidney disease may progress to renal failure requiring dialysis or renal transplantation. However, disease can recur after this procedure.

It is well known that phenotypic expression of monogenic disorders shows interindividual variability. Unknown polygenic and environmental differences may thus be responsible for the FLD phenotype of the index case that contrasts with that of her sister, who, despite no renal involvement, shows clinical signs (intermittent anemia, anisocytosis, and corneal opacity) and biochemical findings (Lp-X, cholesterol esterification rate) also

characteristic of FLD. Likewise, the extreme hypertriglyceridemia observed in the proband and in her brother, but not in the other siblings, could indicate the presence of additional genetic or unknown environmental factors that impair triglyceride metabolism in some members of this family.[16]

To date, no premature coronary heart disease has been reported in patients with LCAT deficiency.[17] Several studies with animals and in patients have not shown a clear association between LCAT activity and atherosclerosis. For instance, Calabresi et al.[14] found no significant difference in carotid intima–media thickness between 40 carriers of *LCAT* gene mutations and controls. Other data, however, show that heterozygosity in *LCAT* gene defects is associated with increased intima–media thickness in mutation carriers vs controls, thus suggesting that LCAT protects against atherosclerosis.[18] In any case, the primary endpoint in these patients is to prevent renal insufficiency by controlling any added cardiovascular risk that may worsen renal function. The ongoing investigational use of recombinant human LCAT may constitute a powerful therapy in the future.

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The authors confirm that the article has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed.

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Appendix

Supplementary Table 1 Biochemical analyses and DNA sequencing of the proband, siblings and the son

	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)*	FC (mg/dL)	FC/TC (%)	Triglycerides (mg/dL)	TC/ HDL-c	Apo AI (mg/dL)	Apo B (mg/dL)	LCAT mutation
Index case (patient)	272.24	5.03	223.49	213.81	78.54	218.60	54.12	37	59	Homozygous
Affected sister	174.40	5.03	141.76	143.93	82.53	138.06	34.67	44	36	Homozygous
Unaffected sister	209.59	50.66	107.25	50.27	23.98	258.42	4.14	155	113	Absent
Brother	467.13	22.43	†	142.50	30.51	1762.04	20.83	106	185	Heterozygous
Son	258	24	83			80	5.1			N.D.

FC, fetal calf; LCAT, lecithin–cholesterol acyltransferase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

*Calculated LDL-C by Friedewald formula (LDL-C = TC – HDL-C − TG/5).

†Friedewald formula cannot be applied in hypertriglyceridemia.

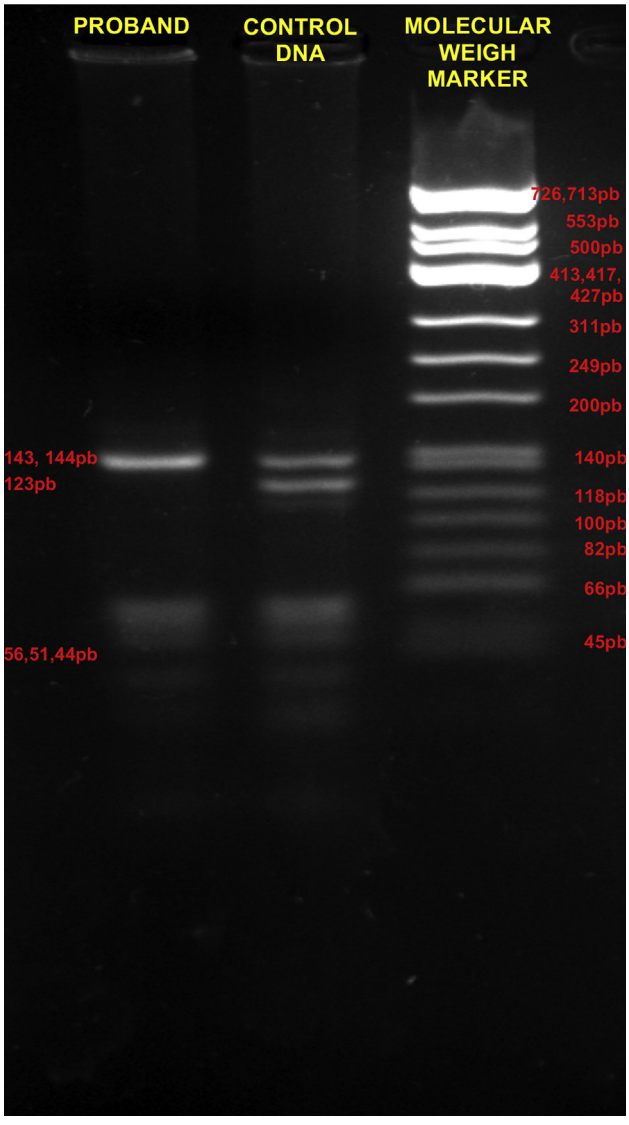
Supplementary Table 2 Biochemical follow-up of the proband over 10 years

	Feb 8, 2006	Feb, 2008	Feb 27, 2008	Aug 22, 2008	Sep 10, 2008	Oct 30, 2008	Dec 4, 2008	Apr 8, 2009	Jun 2, 2009	Jul 8, 2009	Feb 16, 2011	Feb 18, 2011	Feb 21, 2011	Mar 3, 2011	Mar 15, 2011	May 4, 2011	Jun 1, 2011	Jul 12, 2011	Jul 21, 2011	Sep 23, 2011	Sep 12, 2012	Dec, 2012	May 23, 2013	May 2, 2017
ALT (U/L)		Lifestyle intervention + fibrates		Abortus	160	Fibrates stopped, change to atorvastatin				88		Delivery (caesarian section)	23	41	71	415	323	72	Implementation of rosuvastatin			Interruption of statin by the patient	42	30
AST (U/L)					119					53				39	61	200	191	44					37	30
GGT (U/L)					198					30	47		39	78	135	268	249	96					35	47
AF (U/L)					310					112	150			251	298	369	311	167					93	107
Bil (mg/dL)					1					0.7	0.6				0.9	1.5	1.2	0.6					1.1	1.2
TC (mg/dL)	297		335		531		244						834			703	637	348		209	171		209	272
TG (mg/dL)	305		282		2553		125				3810		2329			136	174	606		175	144		167	219
LDL-C (mg/dL)*			273		†		216		†		†		†			666	594	†						223
HDL-C (mg/dL)			6				3	4	10				12			10	8	5		<5	<5		<5	5

AF, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Bil, bilirubin; GGT, gamma-glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

*Calculated LDL-C by Friedewald formula (LDL-C = TC − HDL-C − TG/5).

†Friedewald formula cannot be applied in hypertriglyceridemia.



Supplementary Figure 1 Restriction analysis of the proband. Digestion of LCAT exons 2 and 3 (475 bp) with Mval (BstNI) target: CC'W,GG. Fragments anticipated (bp) for control DNA: 5, 21, 32, 44, 51, 56, 123, 144.