

SHORT COMMUNICATION

## NOVEL MUTATION IN THE *APOB* GENE (Apo B-15.56): A CASE REPORT

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### ABSTRACT

Familial hypobetalipoproteinemia (FHBL) is a rare co-dominant genetic disorder characterized by decrease of plasma low density lipoprotein-cholesterol (LDL-c) or apolipoprotein B (Apo-B) equal to or less than the 5<sup>th</sup> percentile for the population. We describe a 48-year-old male who presented with fatty liver disease (FLD), insulin resistance (IR), obesity and hypertension. Our patient thus met the latest diagnostic criteria of the metabolic syndrome (MS) proposed by the Adult Treatment Panel and the International Diabetes Federation. However, he had very low plasma concentration of LDL-c and Apo-B. DNA sequencing showed that he and two first-degree relatives affected by obesity and mild IR were heterozygous for a single nucleotide deletion on exon 15 of the *APOB* gene, which was predicted to form a truncated Apo-B designated Apo B-15.56.

**Keywords:** Apolipoprotein B (Apo-B), Genetic mutation, Familial hypobetalipoproteinemia (FHBL), Metabolic Syndrome (MS)

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### INTRODUCTION

Familial hypobetalipoproteinemia (FHBL) is a genetic disorder of lipid metabolism, transmitted as a co-dominant trait, whose frequency in the heterozygous form (as estimated by clinical criteria) is 1:500-1:1000 [1]. This condition is characterized by plasma levels of total cholesterol (TC), low density lipoprotein-cholesterol (LDL-c) and apolipoprotein-B (Apo-B) below the 5<sup>th</sup> percentile for the general population [2]. Most cases of FHBL are linked to nonsense/frameshift mutations of the *APOB* gene, located on chromosome 2, which prevent the complete translation of Apo-B mRNA and lead to the production of truncated forms of Apo-B [3]. More than 60 such mutations have been reported, and they are designated by reference to the size of the truncated form compared to the normal form Apo-B 100 [4].

Apo-B 100 is a  $\beta$  lipoprotein synthesized by the liver, consists of 4,536 amino acids and is present in very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low density lipoprotein (LDL) of plasma. Another product of the *APOB* gene is Apo-B 48, that consists of 2,152 amino acids and results from an edited form of Apo-B mRNA. Apo-B 48 is synthesized in the small intestine and is present in chylomicrons (5). The truncated forms of Apo-B may be secreted into the plasma along with plasma lipoproteins.

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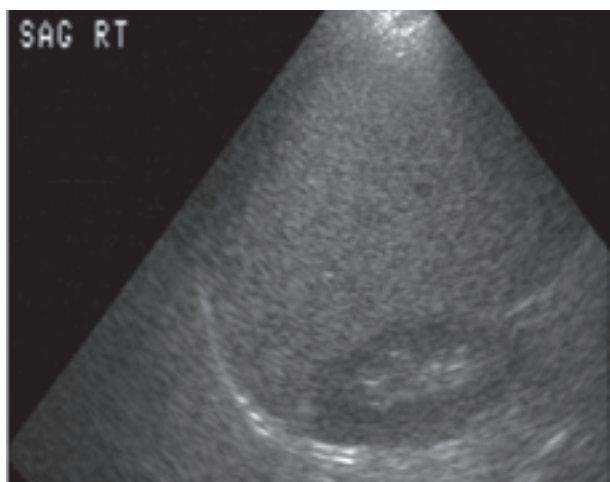
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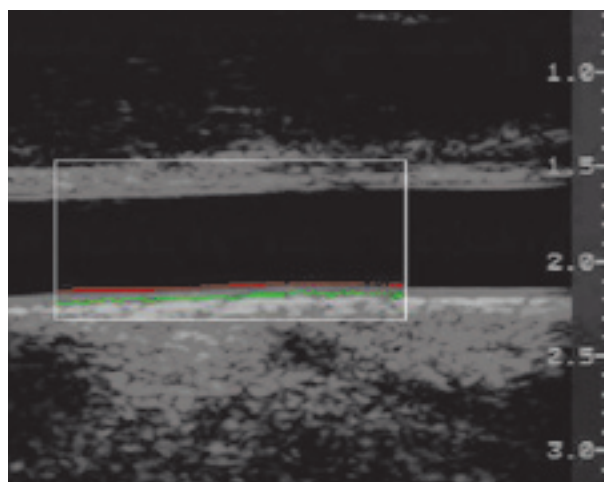
Only truncated Apo-B with a size above that of apoB-29/30 are secreted and can be detected by immunoblot with specific antibodies. Shorter truncations are less frequent, as they represent approximately one-third of all mutations, and cannot be detected in plasma [6,7]. Unfortunately, FHBL tend to be more severe in carriers of shorter truncations (2). In patients affected by FHBL, the mutant forms of Apo-B have a lower capacity for transporting triglycerides, while the production rate of the normal Apo-B 100 is reduced (usually to 25% of normal) [8]. This leads to a dysfunctional VLDL system that increases the susceptibility of the liver to the accumulation of lipids [1,9]. Fatty liver disease (FLD) and mild elevation of liver enzymes in serum are the main clinical features of FHBL, but oral fat intolerance and intestinal fat malabsorption have also been reported [1,2]. The increase of hepatic fat reported in FHBL subjects is not due to generalized or localized obesity, but rather to the Apo-B mutations *per se* [7]. In fact, intra-hepatic triglyceride content is not associated with IR in FHBL, suggesting that it represents a marker, not the cause, of metabolic dysfunction [10]. On the other hand, a significant decrease in arterial stiffness is observed in patients affected by FHBL compared with healthy controls, despite similar carotid intima-media thickness (IMT) [11]. This finding is consistent with a reduced lifelong exposure to Apo-B containing particles and suggests that FHBL could play a protective role towards cardiovascular disease (CVD). However, there is no systematic data about the prevalence

of CVD in FHBL, and convincing evidences of a protective effect of lifelong reduction of plasma LDL-c level toward atherosclerosis have only been emerging recently [12]. Here we report a novel mutation of the Apo-B 100 gene (Apo B-15.56), in a rural South Italian family, and describe the phenotypic manifestations.

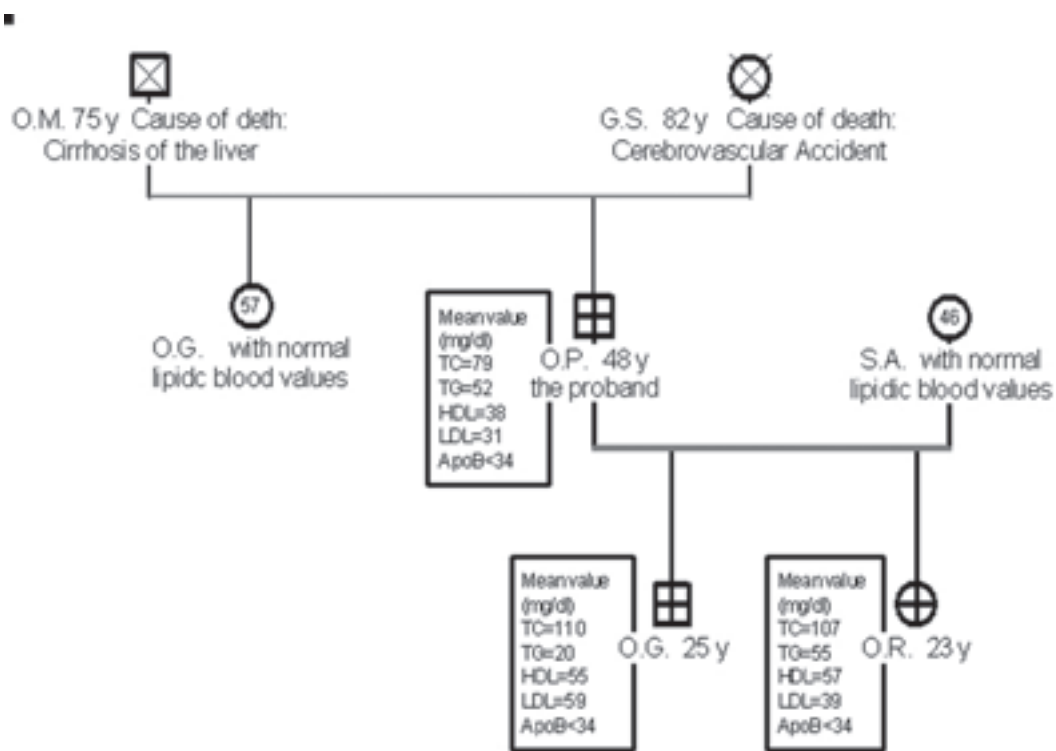
**Case Presentation.** O.P., a 48-year old man, was referred to our outpatients' clinic for a careful evaluation of his serum lipid levels which were apparently inconsistent with a diagnosis of MS. He had no history of smoking and his past medical history was significant only for hypertension, for which he was on antihypertensive medications. On physical examination the patient was obese (Body Mass Index = 32 kg/m<sup>2</sup>; waist circumference = 105 cm), and his blood pressure was 130/85 mm Hg. His liver was palpable 2 cm below the right costal margin. The remainder of the physical examination was unremarkable. A recent ultrasound imaging of the abdomen showed a severe hepatic steatosis (Figure 1). He had diffused atheromasic IMT in the following vascular regions: carotid arteries, abdominal aorta and iliac-femoral arteries, as documented by B-mode ultrasonographic examination. Maximum reported thickness was 1.3 mm bilaterally at the bifurcation of the common carotid artery (Figure 2). Laboratory testing showed plasma values of TC, LDL-c and Apo-B less than the 5<sup>th</sup> percentile, fasting plasma glucose 102 mg/dL and basal plasma insulin 24.2 microUI/mL. Family study revealed that his two children (O.G., male, 25 years old; and O.R., female, 23 years



**Figure 1.** Ultrasound examination of the abdomen, showing severe steatosis of the liver.



**Figure 2.** Ultrasound examination of the carotid artery district, showing diffuse IMT.



**Figure 3.** Pedigree of the family, including lipidic profiles (mg/dL) of FHBL subjects. TC: total cholesterol; TG: triglycerides; HDL: high density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol; ApoB: apolipoprotein B.

old) had a similar lipid phenotype (Figure 3) but moderate increase of insulin serum level (O.G.: 22.1 microUI/mL; O.R.: 19.5 microUI/mL) and waist circumference values (O.G. = 97 cm; O.R. = 83 cm).

A genetic study was then performed on the proband and his family. Genomic DNA was extracted from peripheral blood leukocytes by a standard procedure [13]. A single nucleotide deletion on exon 15 of the *APOB* gene was identified by polymerase chain reaction (PCR) amplification with adapted primers [14]. The amplification product was analyzed by 2% agarose gel electrophoresis and sequenced using Thermosequenase radiolabeled terminator cycle sequencing kit (Amersham Biosciences Europe GmbH, Freiburg, Germany) [15]. The identified mutation is predicted to form a truncated Apo-B species which we have designated Apo B-15.56. In this case we decided to treat O.P. with metphormin and acetylisalicylic acid in order to improve IR (marked by severe hepatic-steatosis), and to slow the progression of the atheromasic lesions, most

likely due to metabolic disorders and obesity. We prescribed to both children a program of aerobic exercise and balanced diet for gradual weight loss and reduction of waist circumference.

## DISCUSSION

It is well known that phenotypic expression of heterozygous forms of FHBL is variable according to environmental or genetic factors [7]. The FHBL heterozygotes may be asymptomatic, being usually identified after routine blood work or plasma cholesterol screening, or have clinical manifestations that require medical attention. Several authors considered these patients protected against atherosclerotic coronary heart disease (CHD), due to their reduced life-time exposure to atherogenic Apo-B containing lipoproteins [2]. However, we described a case of heterozygous form of FHBL, with phenotypic aspects characteristic of MS, caused by a novel gene mutation of Apo-B 100 (Apo B-15.56), which is probably related to a higher risk of cardiovascular events [16,17].

We also underline the probable relationship between FHBL and some pathological features that characterize MS, *i.e.*, obesity and IR [16]. Metabolic syndrome is a common disorder, characterized by visceral obesity, impaired glucose metabolism, raised blood pressure, atherogenic dyslipidemia, and the existence of a proinflammatory and prothrombotic state. Metabolic syndrome is a progressive condition, associated with a significantly increased risk of cardiovascular events and type 2 diabetes mellitus (T2DM) onset [17]. Metabolic syndrome is defined in various ways, but the latest diagnostic criteria proposed by the International Diabetes Federation are central obesity (waist circumference >94 cm in men and >80 cm in women), plus at least two of the following: fasting glucose serum level higher than 100 mg/dL, blood pressure values higher than 130/85 mmHg or antihypertensive treatment, fasting triglycerides serum level higher than 150 mg/dL, and HDL-c level lower than 40 mg/dL in men and 50 mg/dL in women [18]. This case illustrates the importance of a careful evaluation of lipid profiles, along with a genetic analysis, to get the correct diagnosis. Very low values of Apo-B and LDL-c are not included in the diagnostic criteria of MS [19], and are sometimes ignored by physicians, especially when patients are asymptomatic. However, FHBL is often associated with other disorders that could worsen the hepatic function and increase the risk of CVD development [17]. In this patient, the heterozygous form of FHBL was included in a clinical context of MS that could be the cause of a rapid development of atherosclerotic disease and metabolic dysfunctions.

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