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Subfraction analysis of circulating lipoproteins in a patient with Tangier disease due to a novel ABCA1 mutation

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ABSTRACT

Tangier disease, characterized by low or absent high-density lipoprotein (HDL), is a rare hereditary lipid storage disorder associated with frequent, but not obligatory, severe premature atherosclerosis due to disturbed reverse cholesterol transport from tissues. The reasons for the heterogeneity in atherogenicity in certain dyslipidemias have not been fully elucidated. Here, using high-performance liquid chromatography with a gel filtration column (HPLC-GFC), we have studied the lipoprotein profile of a 17-year old male patient with Tangier disease who to date has not developed manifest coronary atherosclerosis. The patient was shown to be homozygous for a novel mutation (Leu1097Pro) in the central cytoplasmic region of ATP-binding cassette transporter A1 (ABCA1). Serum total and HDL-cholesterol levels were 59 mg/dl and 2 mg/dl, respectively. Lipoprotein electrophoretic analyses on agarose and polyacrylamide gels showed the presence of massively abnormal lipoproteins. Further analysis by HPLC-GFC identified significant amounts of lipoproteins in low-density lipoprotein (LDL) subfractions. The lipoprotein particles found in the peak subfraction were smaller than normal LDL, were rich in triglycerides, but poor in cholesterol and phospholipids. These findings in an adolescent Tangier patient suggest that patients in whom these triglyceride-rich, cholesterol- and phospholipid-poor LDL-type particles accumulate over time, would experience an increased propensity for developing atherosclerosis.

1. Introduction

Tangier disease is a rare autosomal recessive disorder which results from mutations in the gene specifying ATP-binding cassette transporter 1 (ABCA1) [1]. Since the first identification of a causative mutation in the *ABCA1* gene [2–4], more than fifty mutations causing amino acid substitutions or other changes in the various regions of the ABCA1 protein have been reported [5–7]. Because of the physiological role of ABCA1 in cholesterol efflux from cells, particularly macrophages, to HDL, patients with functional defects show the typical clinical manifestations including ectopic lipids with foam cell development in tissues, including orange-yellow tonsils, hepato-splenomegaly, and the prognosis-determining complication, advanced atherosclerosis [8]. Carriers of a single *ABCA1* mutation (heterozygotes) have variable reductions in plasma HDL levels, with the decrease associated with variable reductions in ABCA1-mediated cellular cholesterol efflux; consequently, there is also a variably increased risk for developing coronary artery disease [9]. On the other hand, while HDL-cholesterol is virtually absent in patients homozygous for *ABCA1* mutations, this condition is accompanied by severe coronary atherosclerosis in only approximately 50% of them [10]. To date, the mechanism underlying the heterogeneity of risk for atherogenicity has not been fully elucidated.

One of the possible ameliorating factors that may attenuate the progression of atherosclerosis in Tangier disease is the decreased concentration of LDL in the circulation. On the other hand, a confounding factor is the accumulation of atherogenic lipoproteins. These are generated by the of disturbed lipid exchange via lipid transfer proteins between immature HDL and apolipoprotein (apo) B-containing lipoprotein particles [11]. In this context, using high-performance liquid chromatography with a gel filtration column (HPLC-GFC), we have recently identified abnormal lipoproteins highly associated with renal damage in familial lecithin:cholesterol acyltransferase (LCAT) deficiency, another severe hypo-alpha-lipoproteinemia due to the disturbance

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of HDL maturation [12]. The application of the recently established technology for the detection of small amounts of lipoproteins in cases with severe hypo-alpha-lipoproteinemia revealed that an absence of HDL likely causes the accumulation of abnormal apoB-containing lipoproteins, accompanied with ectopic lipid accumulations in kidneys and other tissues [12].

Here, we report a novel gene mutation leading to an amino acid substitution in the central cytoplasmic domain of ABCA1 in a patient diagnosed with Tangier disease. The patient had a severe deficiency in circulating HDL, yet his coronary arteries to date are intact. Therefore, we applied for the first time our recently established HPLC-GFC analysis protocol to investigate the serum lipoprotein particles in this patient, and to compare them with the abnormal lipoproteins identified by traditional gel electrophoretic analyses.

2. Materials and methods

2.1. Biochemical and genetic analyses

Blood samples were obtained from the patient and his parents, and serum was prepared and stored at −80 °C until use. Serum total cholesterol (TC) and triglyceride (TG) levels were measured using fully enzymatic methods with a JCA-BM1650 auto analyzer (JEOL JAPAN Ltd., Tokyo, Japan). HDL-cholesterol was measured by the selective inhibition method with the above analyzer. Apolipoproteins were measured by turbidimetric immunoassay. Free Cholesterol (FC) was quantitated by an enzymatic method. Cholesterol ester (CE) concentrations were calculated by subtracting FC values from TC values. LCAT activity was measured using dipalmitoyl lecithin as the substrate [13]. Genomic DNA was extracted from peripheral blood leukocytes by a standard procedure [13]. All the coding regions (50 exons) including exon–intron boundaries and the promoter regions of the ABCA1 gene were amplified using established primer sequences [14], and the sequencing reactions were performed as reported previously [13,15]. The Apo A-I and LCAT genes were sequenced using the previously reported primers [13,16]. The study was approved by the Ethics Committees of Toho University Sakura Medical Center, and informed consent was obtained from the patient and his parents.

2.2. Lipoprotein analysis

The serum lipoprotein profile was determined by polyacrylamide gel and agarose gel electrophoresis as previously described [13,15]. Subfractionation of serum lipoproteins was performed by HPLC-GFC as described [12]. The subfractioned samples were analyzed simultaneously by online enzymatic method to quantify TC, FC, TG, and phospholipid (PL) (Skylight Biotech, Akita, Japan). The resulting raw chromatograms (elution time versus lipid concentration) were further processed by computer program with modified Gaussian curve fitting for resolving the overlapping peaks by mathematical treatment. Finally, the system subdivided the lipoprotein particles of normal subjects according to size into the following 20 subclasses: chylomicrons (CM, >80 nm; fractions 1–2), very low-density lipoprotein (VLDL, 30–80 nm; fractions 3–7), low-density lipoprotein (LDL, 16–30 nm; fractions 8–13), and high density lipoprotein (HDL, 8–16 nm; fractions 14–20). In the fractionation analysis reported previously [12], standard particle diameters have been reported to be >90, 75, 64, 53.6, 44.5, 36.8, 31.3, 28.6, 25.5, 23.0, 20.7, 18.6, 16.7, 15.0, 13.5, 12.1, 10.9, 9.8, 8.8, and 7.6 nm for fraction numbers 1 through 20, respectively.

2.3. Statistical analysis

The results are expressed as means ± standard deviation (SD). Comparisons were assessed for significant differences by paired Student's *t*-test, where appropriate. Values of $p < 0.05$ were considered statistically significant. All statistical analyses were performed using the `Stat View 4.0′ statistical analysis package (Abacus Concept, Inc.).

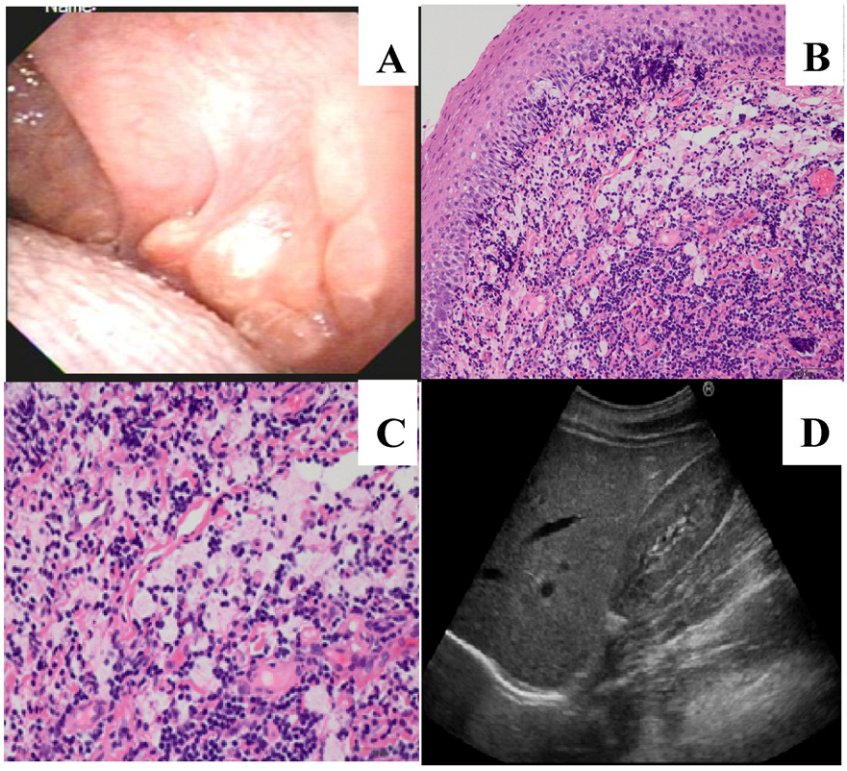

Fig. 1. Tonsillar and spleen pathology. A, Tonsil with orange-yellow appearance in the patient after tonsillectomy. B and C, H–E staining of a tonsillar biopsy specimen; magnification, 200× (B) and 400× (C). An obvious lipid accumulation with many infiltrated lipid-laden macrophages (foam cells) is seen in the tissue. D, Ultrasonographic image of spleen in the patient. Mild splenomegaly was observed.