

# Liver transplantation in a subject with familial hypercholesterolemia carrying the homozygous p.W577R LDL-receptor gene mutation

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**Abstract:** Mutations within the low density lipoprotein (LDL)-receptor gene result in familial hypercholesterolemia, an autosomal dominant inherited disease. Clinical homozygous affected subjects die of premature coronary artery disease as early as in early childhood. We identified a girl at the age of five yr with clinical homozygous familial hypercholesterolemia presenting with achilles tendon xanthomas and arcus lipoides. Her total cholesterol reached up to 1050 mg/dL. Molecular characterization of the LDL-receptor gene revealed a homozygous p.W577R mutation. Despite intensive treatment interventions with the combination of diet, statins, colestipol, and LDL-apheresis, the patient developed symptomatic coronary artery disease at the age of 16 yr. Subsequently, orthotopic liver transplantation was performed to cure the defective LDL-receptor gene. Clinical follow-up for almost nine yr post-transplantation revealed excellent liver function, normal liver enzymes, normal LDL-cholesterol, and regression of both tendon xanthomas and symptomatic coronary artery disease. In conclusion, liver transplantation can effectively reduce LDL-cholesterol in a familial hypercholesterolemia recipient with subsequent regression of xanthomas and atherosclerosis. Timing is extremely important in these exceptional cases to exclude the demand for heart transplantation due to severe coronary artery disease. In addition, the identification of the LDL-receptor as etiology of clinical homozygous hypercholesterolemia is a prerequisite once liver transplantation is considered as therapeutic option.

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This article is dedicated to the outstanding contribution of Rudolf Pichlmayr<sup>†</sup> to the field of transplantation and surgery.

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Elevated plasma levels of low density lipoprotein (LDL) cholesterol and its major protein component apolipoprotein B (apoB) represent an established risk factor for cardiovascular diseases. The liver plays a central role in the metabolism of LDL particles by secreting LDL precursors and also catabolizing apoB containing lipoproteins (1). The LDL-receptor (LDLR) is critical in mediating the catabolism of the cholesterol-enriched LDL particle (2). Hepatocytes express up to 80% of all LDLR in man. Mutations in the gene encoding the LDLR cause the clinical picture of autosomal dominant familial hypercholesterolemia (FH) (3). Patients with heterozygous FH usually respond well to currently available treatment regimens. However, homozygous FH with one in a million affected patients still represents a major therapeutic challenge. Untreated, these patients die because of premature coronary artery disease as early as in early childhood, and even with extensive therapeutic interventions such as LDL apheresis it is often not possible to reduce plasma LDL levels to near normal values. Therefore, other therapeutic options need to be explored. Early reports on the effectiveness of liver transplantation in FH subjects as a means of somatic gene transfer have demonstrated that normalizing liver LDLR can provide a cure for FH (4–6). Clinical signs of peripheral lipid accumulation also as xanthelasms and xanthomas may regress or disappear (7, 8). Consequently, experimental and clinical protocols have been developed for liver-directed gene therapy in an attempt to cure homozygous FH (9–12). However, although these studies provided proof of principle these strategies are far from entering the clinics (12–14). Therefore, once cholesterol lowering treatment options fail to prevent the development of significant atherogenesis, liver transplantation should be included as treatment option to correct the genetic defect of FH.

We report here on a case with genetically and functionally characterized homozygous LDLR deficiency. Orthotopic liver transplantation was carried out after symptomatic coronary artery disease was already present, and resulted in an excellent clinically stable long-term course of the disease. This case therefore highlights the importance of both timing and genetic testing and further

stresses the importance of including liver transplantation as a therapeutic option in the treatment panel of FH.

## Methods

The genetic characterization of patients with hypercholesterolemia was approved by the local ethic committees of the Medizinische Hochschule Hannover and the Charité. The index patient and the studied family members gave their written consent. DNA was extracted from peripheral blood leukocytes using spin columns (Qiagen, Hilden, Germany). PCR-based direct sequencing was performed as previously described (15, 16). Automated sequencing was carried out by using an ABI 310 Genetic Analyzer (Applied Biosystems, Darmstadt, Germany) according to the manufacturer's instructions. The identified mutations were confirmed on a second DNA sample isolated from a duplicate tube of blood followed by sequencing in both forward and reverse directions.

The newly identified nucleotide change c.1729T>C (p.W577R) creates a novel *MspI* restriction site; therefore, the presence for p.W577R can easily be detected by PCR and restriction digestion. PCR of exon 12 may be performed with the following primers, sense-5'CCTCTCCAGGTGCTTTTCTG-3', and anti-sense 5'-CAGCCTCTTTTCATCCTCCA-3', with an annealing temperature of 60°C. Digestion of the 240 bp PCR product with *Msp I* results in fragments of 33, 49, and 158 bp for the wild-type sequence, and in fragments of 33, 49, 54, and 104 for the mutated sequence. Fragments were analyzed on 2% agarose gels.

## Results

We report on a female index patient with a Turkish ethnic background. Homozygous familial hypercholesterolemia was diagnosed based on the clinical presentation at the age of five yr. At that time her serum total cholesterol and LDL-cholesterol were 780 mg/dL (ref.: <140) and 738 mg/dL (ref.: <105), respectively; VLDL-cholesterol 16 mg/dL (ref.: <30), HDL-cholesterol 27 mg/dL (ref.: >35), and triglycerides 99 mg/dL (<100).

Apolipoprotein E genotype was 3/4 (ref.: 3/3). She presented with bilateral arcus lipoides and achilles tendon xanthomas, but no xanthelasms. Coronary angiography revealed no atherosclerotic lesions at that time. Skin fibroblasts analysis showed a complete absence of LDLR-mediated binding of radiolabeled LDL (<5%). Her family history revealed a consanguineous marriage with her mother being at the same time the aunt of her father (Fig. 1). Both parents were healthy, but demonstrated elevated LDL-cholesterol levels, suggesting the classical trait of familial hypercholesterolemia. The father had a total cholesterol of 396 mg/dL (ref.: <200), and LDL-cholesterol of 343 mg/dL (ref.: <165), VLDL-cholesterol 17 mg/dL (ref.: <35), HDL-cholesterol 35 mg/dL (ref.: >40), and triglycerides 113 mg/dL (ref.: <150). The lipoprotein analysis in the mother revealed a total cholesterol of 330 mg/dL (ref.: <200), LDL-cholesterol of 280 mg/dL (ref.: <165), VLDL-cholesterol 6 mg/dL (ref.: <35), HDL-cholesterol 41 mg/dL (ref.: >45), and triglycerides 57 mg/dL (ref.: <150). A four-month-old sister of the index patient died of unknown etiology, no autopsy was performed. Another sister died at the age of four yr of myocardial infarction caused by extensive coronary artery disease in the presence of hypercholesterolemia and both tendinous and cutaneous xanthomas. Autopsy revealed disseminated advanced arteriosclerosis of the carotid, renal and coronary arteries and of the total aorta. Postprandial lipoprotein profile revealed total cholesterol of 719 mg/dL, LDL-cholesterol 661 mg/dL, HDL-cholesterol 13 mg/dL, and triglycerides 225 mg/dL. Her four yr older brother had elevated total cholesterol (312 mg/dL) and LDL-cholesterol (256 mg/dL), but no clinical symptoms, suggesting heterozygosity.

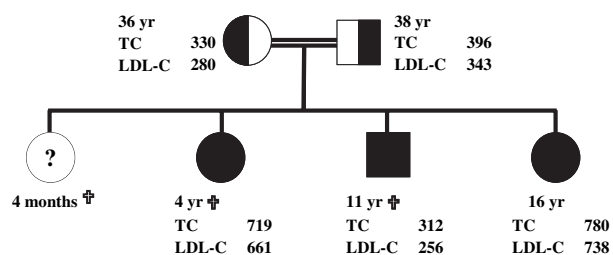


Fig. 1. Pedigree of reported family is illustrated. The index patient is now 26-yr old. Total cholesterol (TC) and LDL-Cholesterol (LDL-C) are depicted in mg/dL at time of diagnosis in the index patient 19 yr ago, off treatment. Lipid values in one of the brothers were obtained non-fasting at time of his death at age 4. Parents and her alive brother are heterozygous disease carriers. The four-month-old sibling died of sudden death without any more information available.

Lipid lowering drugs, initially nicotinic acid and colestipol, later statins in the addition to dietary cholesterol restriction failed to significantly lower serum cholesterol. Therefore, plasma apheresis was initiated at the age of six yr, later substituted by LDL-apheresis. A Cimino shunt was required and the histology of the radial artery revealed already signs of arteriosclerosis: intimal fibrosis protuberating into the arterial lumen, myxoid degeneration, and a destroyed elastic layer.

Eight yr later, at the age of 14, she developed characteristic pectangina caused by inferolateral ischemia assessed by technetium myocardial scintigraphy and exercise ECG test. Coronary angiography revealed a 90% stenosis of the right coronary artery, a stent was positioned, and subsequently nitrates and ticlopidin were administered. At the age of 16 yr a significant progression of coronary artery disease was revealed by angiography despite continued LDL-apheresis. At that time, we decided to perform liver transplantation, which was successfully realized five months later. There were no complications in the immediate and also long-term postoperative course. The patient was followed for nine yr after liver transplantation without any rejection episodes, treatment side effects or symptoms of angina pectoris. After liver transplantation plasma lipids normalized to normal (Fig. 2). The last plasma lipoprotein profile was obtained without any lipid lowering treatment: total cholesterol of 195 mg/dL, LDL-cholesterol 136 mg/dL, HDL-cholesterol 49 mg/dL, and triglycerides 50 mg/dL. To reduce LDL-cholesterol according the NTCP guidelines, we initiated a treatment with ezetimibe. Her additional current treatment consists of acetylsalicylic acid 100 mg/d, tacrolimus 2 × 1.5 mg/d (target level 4–5 ng/mL), and intermittent iron supplementation. Liver enzymes and hepatic synthesis parameters were all within the normal range. Coronary angiography was recently repeated, six and a half yr after liver transplantation, actually revealing regression of coronary artery disease.

Direct sequencing revealed a homozygosity for the p.W577R mutation within the LDL-receptor gene (LDLR) as underlying molecular defect. This mutation is located within exon 12 of the *LDLR*. This location translates into the EFG precursor homology domain of the receptor, which in turn consists of three EGF-like domains plus a  $\beta$ -propeller module (17, 18). This module contains six YWTD motifs. The total domain is responsible for lysosomal lipoprotein release and LDLR recycling. The mutation p.W577R affects tryptophane (W) within the fifth of the six YWTD motifs. Therefore, the functional properties of LDLR will



direct sequencing involving evaluation of the complete family before subjecting the patient to OLT. Notably, recently mutations in genes other than the LDLR have been identified that closely resemble the clinical picture of FH. These comprise mutations in (i) the binding site of apolipoprotein B to the LDLR, (ii) a novel gene termed ARH standing for autosomal recessive hypercholesterolemia that can already be suspected from pedigree analysis, as well as (iii) another recently identified gene encoding proprotein convertase subtilisin/kexin type 9 (PCSK9) and resulting in the clinical picture of autosomal dominant FH in the absence of mutations in the LDLR or apoB gene.

In summary, OLT is a safe and feasible therapeutic option to treat patients with FH with progression of atherosclerotic disease that can not be controlled by conservative therapy. A good timing of the procedure is mandatory to avoid atherosclerotic damage to the cardiovascular system resulting in complications that limit the success of OLT or will put the patient at an additional risk. Keeping these requirements in mind, OLT represents a means of somatic gene therapy to cure FH, an otherwise fatal liver-based metabolic disorder.

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