Int J Clin Exp Pathol 2016;9(4):4462-4466
www.ijcep.com /ISSN:1936-2625/IJCEP0022587

Case Report

A newly-found homozygous mutation in the *LDLR* gene in a patient with homozygous familial hypercholesterolemia

Xiaoqian Zhang[1,2], Xi Wang[1], Anli Tong[1], Naishi Li[1], Chongmei Lu[2], Weibo Xia[1]

[1]*Department of Endocrinology, Key Laboratory of Endocrinology, Ministry of Health, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, P. R. China;* [2]*Department of Internal Medicine, Peking Union Medical College Hospital, Beijing, P. R. China*

Received December 23, 2015; Accepted March 7, 2016; Epub April 1, 2016; Published April 15, 2016

Abstract: Homozygous familial hypercholesterolemia (HoFH) is a rare, inherited disorder, characterized by multiple cutaneous and tendinous xanthomas, markedly elevated plasma low-density lipoprotein cholesterol (LDL-C) levels, systemic atherosclerosis, premature coronary heart disease and homozygous mutation of genes related to LDL-C elimination. This study reported an 8-year-old boy with HoFH, discussed its clinical manifestations, identified its genetic mutation locus and conducted a familial genetic analysis. This Chinese boy gradually developed multiple cutaneous and tendinous xanthomas, with elevated plasma level of low-density lipoprotein cholesterol (LDL-C), approximately 17.00 to 20.00 mmol/L at age of 5. Treatment with Statins and Ezetimibe made little effect on his LDL-C level. By means of genetic analysis, a homozygous mutation at exon 4 of *LDLR* gene, c.459delC (p.Gln154Serfs*52) was found in the patient. His parents, sister, uncle and grandmother had a heterozygous mutation in the same site, as well as hypercholesterolemia, with their LDL-C level ranging from 3.89 to 5.60 mmol/L. Clinical manifestations, family history and findings of genetic analysis of the patient satisfied the diagnostic criteria of HoFH. *LDLR* gene is the common mutational gene of FH. The homozygous mutation in exon 4 of *LDLR* gene, c.459delC, has not been reported previously.

Keywords: Homozygous familial hypercholesterolemia, *LDLR* gene, homozygous mutation

Introduction

Homozygous familial hypercholesterolemia (HoFH) is a rare autosomal co-dominant disease, characterized by multiple cutaneous and tendinous xanthomas, markedly elevated plasma low-density lipoprotein cholesterol (LDL-C) levels, systemic atherosclerosis and premature coronary heart disease. Despite its rarity, the condition is considered to be life-threatening and tends to have a poor prognosis. If left untreated, most patients do not survive for more than 30 years because of cardiac events. Therefore, early diagnosis and prompt initiation of lipid-lowering therapy are critical for HoFH patients. This study reported an 8-year-old boy with HoFH, discussed its clinical manifestations, identified its genetic mutation locus and conducted a familial genetic analysis.

Case report

An 8-year-old male Chinese patient was admitted in Mar. 2015 with a complaint of elevated plasma low-density lipoprotein cholesterol (LDL-C) levels and multiple xanthomas for 3 years.

In Mar. 2012, he developed white rice-shaped nodules on the proximal interphalangeal joints at age of 5. These nodules gradually grew larger. One year later, he visited a local hospital and was found to have elevated total cholesterol (TC) level (21.04 mmol/L). Then he was administered Simvastatin for 10 weeks and Atorvastatin for 4 weeks but his TC level remain unchanged. The patient gradually developed skin lesions on the extensor aspect of wrists, elbows, ankles, knees and buttocks, and the lesions were xanthous and flat, with

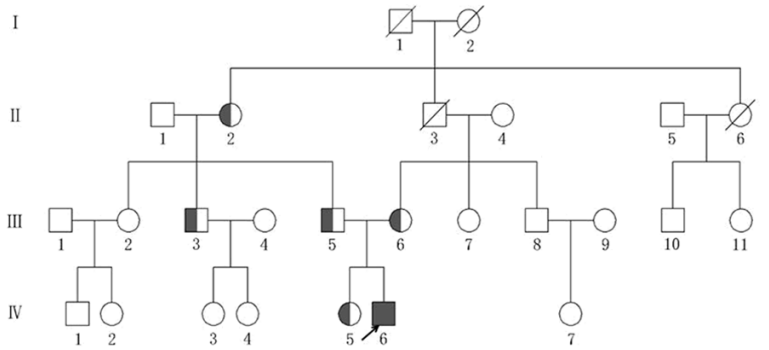

Figure 1. Genogram of the HoFH patient. IV6, HoFH patient (Proband); I1, I2, the cause of death was unknown; II3, died from cardiac event at 45; II6, died from cardiac infarction at 50; II2, III3, III5, III6 and IV5 were evidenced to be HeFH by gene analysis; TC level and gene analysis of other family members were unavailable.

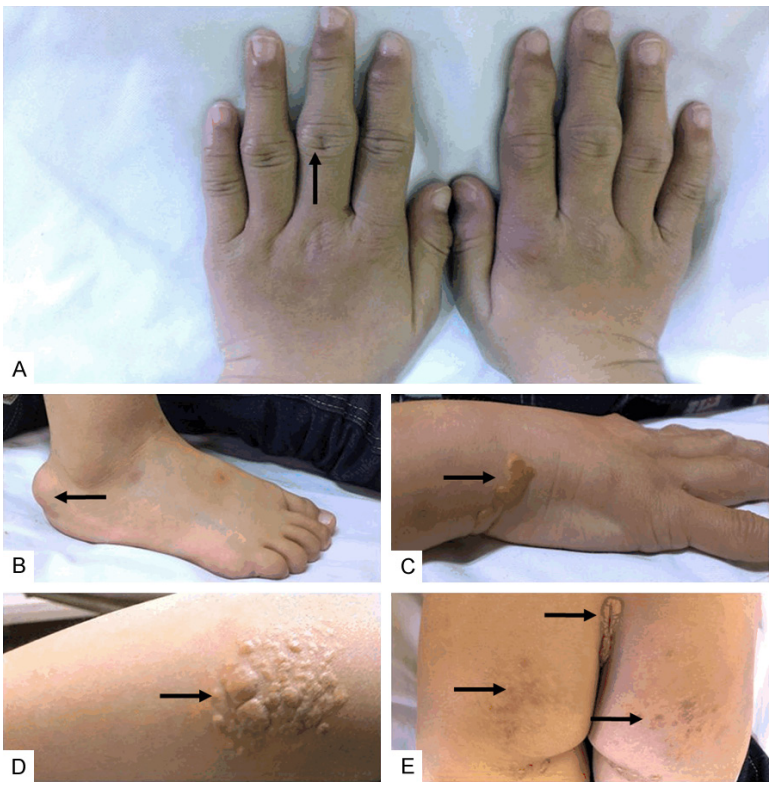

Figure 2. The cutaneous and tendinous xanthoma of the patient. (A and B) Tendinous xanthomas at the proximal interphalangeal joints (A) and ankles (B); (C-E) Cutaneous xanthomas at wrist (C), elbow (D), and buttocks (E).

irregular borders. At the same time, slow-growing, painless and regularly-bordered nodules appeared on his wrists and ankles (**Figure 1**). In Mar. 2015, he was referred to Department of Endocrinology of our hospital and received laboratory and imaging examinations. Total TC, total triglyceride (TG), low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C), apolipoprotein (Apo) A1, Apo(A), ApoB were 17.34 mmol/L, 0.85 mmol/L, 16.54 mmol/L, 0.62 mmol/L, 0.65 g/L, 454 g/L, and 4.1 g/L, respectively. Ultrasound and CT didn't reveal any abnormalities in coronary artery, carotid artery, vertebral artery and aorta-ventralis. His parents were closely related. His parents, sister, uncle and grandmother also had hypercholesterolemia, with their LDL-C level ranging from 3.89 to 5.60 mmol/L, but didn't had xanthomas. Two of the family members died from acute cardiac events. The pedigree and biochemical data are given in **Figure 2** and **Table 1**. This patient was clinically diagnosed as having HoFH, and was asked to be on low-cholesterol diet and to take Rosuvastatin and Ezetimibe. A test conducted 8 weeks later showed that his LDL-C level was not substantially reduced. Then he was advised to initiate LDL-C apheresis. His parents and other family members gave their informed consent for the research and publication. The study was approved by a local ethical committee.

Mutation identification

Genomic DNAs were extracted from peripheral blood leukocytes of the patient and his core family members by employing DNAzol (Life Technologies). Eighteen exons of low density lipoprotein receptor (LDLR) gene were amplified in a 50 μl final reaction volume containing 200 μM dNTP, 2 μM of each primer, 100 ng of genomic DNA, and 2 U Taq polymerase (Life Technologies). The thermal cycling profile for PCR consisted of an initial denaturation of 94°C for 5 minutes, annealing at 56°C and a final extension at 72°C for 5 minutes. The primers were determined as previ-

Table 1. Lipid levels of the patient and his family members

	Age (years)	TC (mmol/L)	TG (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)	ApoA1 (g/L)	ApoB (g/L)	Apo (A) (g/L)
Patient (IV6)	8	17.34	0.85	16.54	0.62	0.65	4.1	454
Father (III5)	40	7.11	0.95	4.43	0.97	1.04	1.63	46
Mother (III6)	39	8.92	1.28	5.60	1.45	1.11	1.83	567
Sister (IV5)	17	6.36	0.48	3.89	1.11	0.90	1.23	409
Uncle (III3)	45	8.51	1.12	5.37	1.29	1.09	1.86	455
Grandmother (II2)	65	12.81	1.71	_	1.70	_	_	_

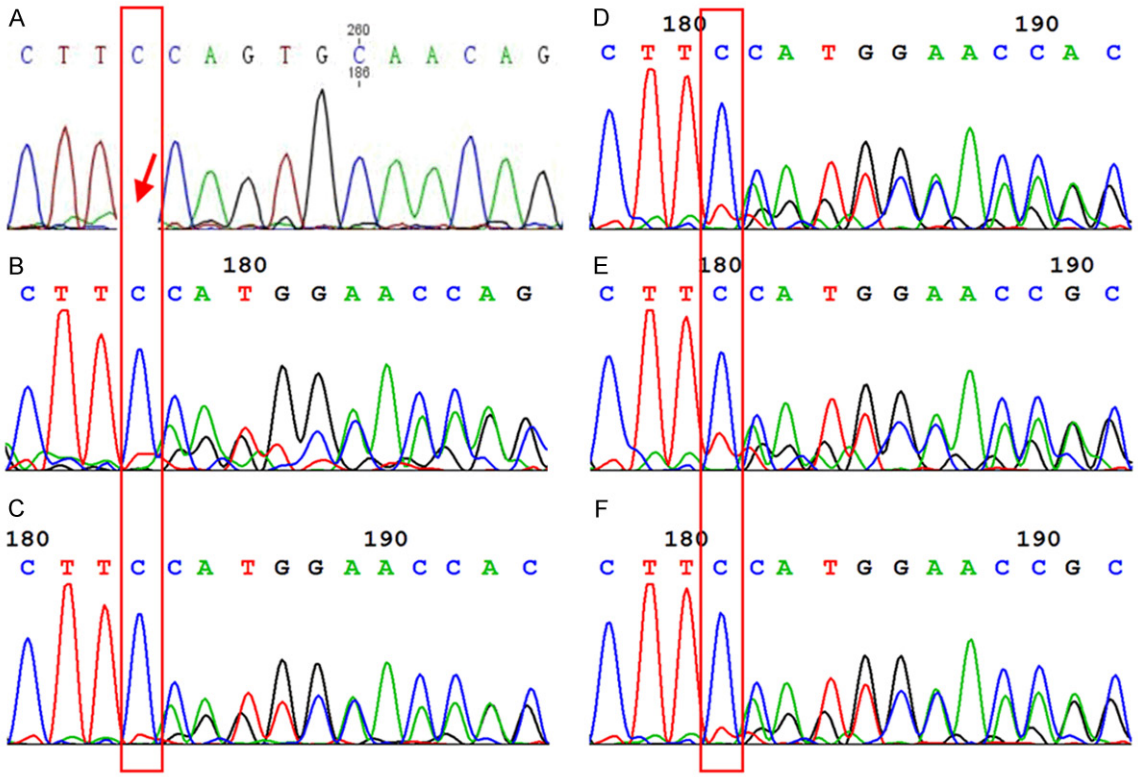

Figure 3. *LDLR* gene mutation of the HoFH patient and his family. A. Homozygous c.459delC mutation at exon 4 of *LDLR* gene of the patient (IV6); B-F. Heterozygous c.459delC mutation of *LDLR* gene of other family members of the patients. B. The patient's father (III5); C. The patient's mother (III6); D. The patient's sister (IV5); E. The patient's grandmother (II2); F. The patient's uncle (III3).

ously described [1]. The PCR products were purified and bidirectionally sequenced. A homozygous mutation at exon 4 of *LDLR* gene, c.459delC (p.Gln154Serfs*52), was found in the patient (**Figure 3A**). Heterozygous mutation in the same site was found in his parents, sister, uncle and grandmother (**Figure 3B-F**).

Discussion

HoFH is a rare hereditary metabolic disease with a global prevalence at 1-3/1,000,000 [2]. The condition has been proved to be of autosomal dominant inherence. The recently-identified genes included *LDLR*, *ApoB*, pro-protein convertase subtilisin/kexin 9 (*PCSK9*) and the LDLR adaptor protein 1 (LDLRAP1) gene [3] and *LDLR* gene is found to be associated with to virtually 65%~79% of FH cases [4]. LDLR is a glycoprotein on the surface of cell membrane and plays a critical role in LDL-C elimination. Its coding gene is on the short arm of chromosome 19 (19p13) [5]. Nearly 1700 mutations were catalogued in London university database (http://

www.ucl.ac.uk/fh). To date, tens of HoFH cases have been reported, and more than 20 homozygous mutations were identified [6-8]. In this study, we presented a new homozygous mutation in exon 4 of *LDLR* gene, c.459delC, which has not been reported previously. This type of homozygous mutation, as shown by computerized analysis, generates a frame-shift mutation, resulting in up to a 100% loss of receptor activity.

By the diagnostic criteria of the European Atherosclerosis Society, a definite diagnosis of HoFH patient should be based on: pre-treatment LDL-C level >13 mmol/L, plus one of followings: (1) cutaneous or tendinous xanthoma before age of 10, or (2) Two mutations at two alleles of the *LDLR, APOB, PCSK9, or LDLRAP1* gene locus, or (3) presence of heterogeneous FH in both parents [7]. Clinical manifestations, family history and findings of genetic analysis of the patient satisfied the diagnostic criteria of HoFH. For the treatment of the disease, the goal is to keep the LDL-C level under 3.5 mmol/L and, for the children with high risk factors, the LDL-C level should be controlled under 2.5 mmol/L. The treatment for the disease generally consists of, among others, lifestyle modification (including relinquishing smoking, balanced diet, exercises), blood pressure control and statins-based medication. However, apheresis (including LDL apheresis and plasmaphaeresis) may be needed when the aforementioned treatment is inadequate or when patients are intolerant of statin therapy [7, 9]. Nowadays, some newly developed drugs, such as lomitapide, mipomersen, PCSK9 inhibitor, as treatment alternative etc, are being under clinical trials as treatment alternatives for HoFH. For this patient, his LDL-C level had not been well controlled by rosuvastatin and ezetimibe for 8 weeks. Then he was advised to initiate LDL-C apheresis and so far imaging examinations didn't exhibit any atherosclerosis or cardiac ischemia. He and his family members are now under a long-term follow-up for further study.

Conclusion

This study reported a HoFH patient with homozygous mutation at exon 4 of *LDLR* gene, c.459delC, which has not been reported previously. His parents, sister, uncle and grandmother had both elevated LDL-C level and heterozygote mutations at the same sites. FH is closely linked to atherosclerosis and cardiovascular events and early screening and treatment are of great importance for FH patients and their family members.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Anli Tong, Department of Endocrinology, Key Laboratory of Endocrinology, Ministry of Health, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing 100730, P. R. China. E-mail: tonganli@hotmail.com

References

[1] Mollaki V, Progias P, Drogari E. Novel LDLR variants in patients with familial hypercholesterolemia: in silico analysis as a tool to predict pathogenic variants in children and their families. Ann Hum Genet 2013; 77: 426-34.

[2] Raal FJ, Santos RD. Homozygous familial hypercholesterolemia: current perspectives on diagnosis and treatment. Atherosclerosis 2012; 223: 262-8.

[3] Marques-Pinheiro A, Marduel M, Rabes JP, Devillers M, Villeger L, Allard D, Weissenbach J, Guerin M, Zair Y, Erlich D, Junien C, Munnich A, Krempf M, Abifadel M, Jaïs JP; French Research Network on ADH, Boileau C, Varret M. A fourth locus for autosomal dominant hypercholesterolemia maps at 16q22.1. Eur J Hum Genet 2010; 18: 1236-42.

[4] Do R, Stitziel NO, Won HH, Jørgensen AB, Duga S, Angelica Merlini P, Kiezun A, Farrall M, Goel A, Zuk O, Guella I, Asselta R, Lange LA, Peloso GM, Auer PL; NHLBI Exome Sequencing Project, Girelli D, Martinelli N, Farlow DN, DePristo MA, Roberts R, Stewart AF, Saleheen D, Danesh J, Epstein SE, Sivapalaratnam S, Hovingh GK, Kastelein JJ, Samani NJ, Schunkert H, Erdmann J, Shah SH, Kraus WE, Davies R, Nikpay M, Johansen CT, Wang J, Hegele RA, Hechter E, Marz W, Kleber ME, Huang J, Johnson AD, Li M, Burke GL, Gross M, Liu Y, Assimes TL, Heiss G, Lange EM, Folsom AR, Taylor HA, Olivieri O, Hamsten A, Clarke R, Reilly DF, Yin W, Rivas MA, Donnelly P, Rossouw JE, Psaty BM, Herrington DM, Wilson JG, Rich SS, Bamshad MJ, Tracy RP, Cupples LA, Rader DJ, Reilly MP, Spertus JA, Cresci S, Hartiala J, Tang WH, Hazen SL, Allayee H, Reiner AP, Carlson CS, Kooperberg C, Jackson RD, Boerwinkle E, Lander ES, Schwartz SM, Siscovick DS, McPherson R, Tybjaerg-Hansen

A, Abecasis GR, Watkins H, Nickerson DA, Ardissino D, Sunyaev SR, O'Donnell CJ, Altshuler D, Gabriel S, Kathiresan S. Exome sequencing identifies rare LDLR and APOA5 alleles conferring risk for myocardial infarction. Nature 2015; 518: 102-106.

[5] Cuchel M, Bruckert E, Ginsberg HN, Raal FJ, Santos RD, Hegele RA, Kuivenhoven JA, Nordestgaard BG, Descamps OS, Steinhagen-Thiessen E, Tybjærg-Hansen A, Watts GF, Averna M, Boileau C, Borén J, Catapano AL, Defesche JC, Hovingh GK, Humphries SE, Kovanen PT, Masana L, Pajukanta P, Parhofer KG, Ray KK, Stalenhoef AF, Stroes E, Taskinen MR, Wiegman A, Wiklund O, Chapman MJ; European Atherosclerosis Society Consensus Panel on Familial Hypercholesterolaemia. Homozygous familial hypercholesterolaemia: new insights and guidance for clinicians to improve detection and clinical management. A position paper from the Consensus Panel on Familial Hypercholesterolaemia of the European Atherosclerosis Society. Eur Heart J 2014; 35: 2146-57.

[6] Walzer S, Travers K, Rieder S. Homozygous familial hypercholesterolemia (HoFH) in Germany: an epidemiological survey. Clinicoecon Outcomes Res 2013; 5: 189-92.

[7] Bruckert E. Recommendations for the management of patients with homozygous familial hypercholesterolaemia: Overview of a new European Atherosclerosis Society consensus statement. Atheroscler Suppl 2014; 15: 26-32.

[8] Santos RD. What are we able to achieve today for our patients with homozygous familial hypercholesterolaemia, and what are the unmet needs? Atheroscler Suppl 2014; 15: 19-25.

[9] Raal FJ, Santos RD. Homozygous familial hypercholesterolemia: current perspectives on diagnosis and treatment. Atherosclerosis 2012; 223: 262-8.