

Next-generation sequencing verified by multiplex ligation-dependent probe amplification to detect a new copy number variations in a child with heterozygous familial hypercholesterolemia

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Familial hypercholesterolemia (FH) is a common autosomal dominant disorder of lipid metabolism. Mutations in the low density lipoprotein receptor gene (*LDLR*) represent the majority of FH cases. *LDLR* mutations are codominantly inherited. Commonly, the clinical phenotype associated with heterozygous mutations is relatively mild, whereas homozygous mutations, compound heterozygous mutations, and double gene mutations in *LDLR* lead to severe phenotypes. However, some heterozygous FH (HeFH) and homozygous FH (HoFH) patients have similar lipid levels. Because of the quite different prognosis and treatments for these patients, accurate diagnosis is especially important. Gene testing plays an essential role in the diagnosis of FH, but finding the appropriate mutations by genetic testing is not always simple.

A 31-month-old female child presented with plane xanthomas and tendinous xanthomas that had been present since the age of 18 months, mainly located on the fingers, wrists, elbows, buttocks, and knees. They tended to increase in number and size; the diameter of the xanthomas ranged from 0.3 to 1.5 cm. The patient was of normal height (95 cm) and weight (14 kg). She had a regular heartbeat and no heart murmurs. Other physical examinations were normal. The levels of plasma total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) were 23.88 mmol/L (normal: <5.18 mmol/L) and 17.94 mmol/L (normal: <3.37 mmol/L), respectively, and the abdominal ultrasound was normal. Her father's plasma TC and LDL-C levels were 7.17 and 4.1 mmol/L, respectively, and her mother's levels were 8.15 and 3.69 mmol/L, respectively. Both parents were free of xanthomas and their physical examinations were normal. With informed consent from the patient's parents, next-generation sequencing (NGS) analysis for the core

family trio was performed by MyGenostics Inc. (Beijing, China). NGS detected a likely pathogenic mutation in the *LDLR* gene in the child and her father: chr19-11216187-11216188 (NM_000527) exon 4, c.606delC (p. H203Tfs*3). Through analyzing the depth of coverage (DOC), the report also showed suspected heterozygous deletions in exons 13 and 14 of the *LDLR* gene, considered a copy number variation (CNV) in this sample. Sanger sequencing was used to verify the point mutation. Multiplex ligation-dependent probe amplification (MLPA) was used to confirm the deletions in exons 13 and 14, which were shown to be present in the patient and her mother (Figure 1).

Although HeFH and HoFH are reported to result in similar lipid levels in some patients, HoFH patients often have higher cholesterol levels than HeFH patients. HeFH patients have an increased incidence of premature coronary heart disease, and initiation of drug treatment is recommended at 10 years of age, in addition to dietary intervention. However, HoFH can cause sudden death in adolescence and even childhood, and treatment is recommended as soon as the diagnosis is made. Therefore, genetic diagnosis is important for risk stratification and early precise treatment, followed by genetic consultation.

Traditionally, NGS is considered suboptimal for the genetic diagnosis of FH because it has low sensitivity for detecting CNVs. CNVs are large-scale mutations, and they account for approximately 10% of pathogenic *LDLR* variants.^[1] Therefore, routine CNV detection is recommended and necessary in the genetic diagnosis of FH. Methods for detection of CNV have progressed from Southern blotting in the 1980s to MLPA in the 2000s.^[2] MLPA is an ideal method for CNV detection in many diseases including FH.^[3-5] It is suited for analysis of *LDLR*

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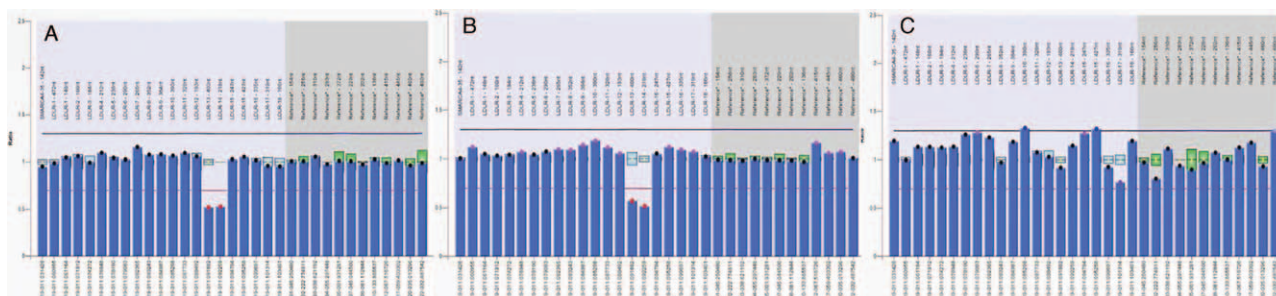


Figure 1: MLPA method output of the core family. (A) Heterozygous deletion in *LDLR* exons 13–14 in this child. (B) Heterozygous deletion in *LDLR* exons 13–14 in the child's mother. (C) The father's result is normal. *LDLR*: Low density lipoprotein receptor gene; MLPA: Multiplex ligation-dependent probe amplification.

because of its dedicated exon-by-exon level resolution. It is not rare that some patients undergoing only NGS have false-negative results because of missed pathogenic CNV variants. Fortunately, the results in our case report indicate that noting the DOC in NGS data can resolve this problem. DOC is a key measure that guarantees sequencing quality. When there is only one instead of 2 genomic templates (indicating absence of exons from one parent), the DOC reflected in the number of synthesized fragments will decrease by half. A previous report indicated 100% concordance in *LDLR* CNV detection between MLPA and NGS.^[5]

The core family trio underwent NGS. A novel deletion mutation in *LDLR*, chr19-11216187-11216188, c.606delC (p.H203Tfs*3), which led to early termination of transcription, was found in the child and her father. This mutation was in exon 4 of *LDLR*, the region with the largest number of known variants. This mutation supported the diagnosis of HeFH. However, xanthomas are unusual in patients with HeFH, and the patient's plasma lipid level was unusually high for a patient with HeFH. The patient's mother also had a high LDL level. Thus, the clinical picture was not consistent with a single deletion. The testing institution found an abnormal DOC when analyzing the raw data and informed us of suspected CNV of deletions in exons 13 and 14 in *LDLR*. We then conducted MLPA on the 18 exons of *LDLR*. Eventually, a large-scale deletion involving exon 13 to exon 14 of *LDLR* was confirmed in the patient and her mother. The patient's parents were diagnosed with HeFH, and the child was diagnosed with HoFH with compound heterozygous defects in *LDLR*.

In conclusion, we describe a patient with a paternally inherited point mutation and a maternally inherited CNV in *LDLR*. This unusual case suggests that CNV should be considered when a “heterogeneous mutation” is inconsistent with the high level of LDL-C. Furthermore, we recommend that core families undergo NGS, with close attention paid to possible abnormal DOC results, as the most rational choice for the genetic diagnosis of FH.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient's guardians have given their consent for their images and other clinical information to be reported in the article. The patient's guardians understand that their names and initials will not be published and due efforts will be made to conceal the identity of the patient, although anonymity cannot be guaranteed.

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Conflicts of interest

None.

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