Whole Exome Sequencing Insufficient for a Definitive Diagnosis of a Patient with Compound Heterozygous Familial Hypercholesterolemia

Hirofumi Okada, Hayato Tada, Akihiro Nomura, Atsushi Nohara, Kazuyasu Okeie, Tsuyoshi Nozue, Ichiro Michishita, Masayuki Takamura, Hirofumi Takemura and Masa-aki Kawashiri

Abstract:
Homozygous familial hypercholesterolemia (HoFH) is a rare genetic disorder, and a genetic analysis is important to make a definitive diagnosis. A comprehensive genetic analysis using next generation sequencing (NGS) and whole exome sequencing (WES) is feasible. However, the application of NGS in the assessment of genomic structural variations is generally limited, and a substantial number of control samples are needed for such assessments. Thus, NGS alone is unlikely to detect genomic structural variations in a “singleton.” We present the case of a patient with compound HeFH (heterozygous FH), whose causative mutations in the LDLR gene could not be identified by WES, necessitating the application of the multiplex ligation-dependent probe amplification (MLPA) technique.

Key words: familial hypercholesterolemia, multiplex ligation-dependent probe amplification, whole exome sequencing


Introduction

Familial hypercholesterolemia (FH; OMIM no. 143890) is a relatively common monogenic hyper low-density lipoprotein (LDL) cholesterolemia, the frequency of which is estimated to be 1 in 250 to 300 general population. This condition is associated with premature death due to atherosclerotic cardiovascular disease (ASCVD) (1, 2). Homozygous FH (HoFH), including compound heterozygous FH (HeFH) and double HeFH, is a rare genetic disorder that is clinically characterized by xanthoma tuberosum in infancy, supravalvular aortic stenosis (SVAS), and premature ASCVD associated with extremely elevated levels of LDL cholesterol (LDL-C) (3-5). In addition, there are patients with oligogenic FH who present deleterious mutations in the FH gene coding for the LDL receptor (LDLR), the apolipoprotein B gene, the proprotein convertase subtilisin/kexin type 9 (PCSK9), as well as in some “accessory” genes, such as the ATP-binding cassette sub-family G member (ABCG) 5 and ABCG8 gene (6). Moreover, there are patients with “severe HeFH” who exhibit a more severe phenotype in comparison to those with typical HeFH (7). Accordingly, there are substantial overlaps between LDL-C and the phenotype of HoFH and HeFH, which result in different prognoses according to their genetic backgrounds. Furthermore, to achieve an accurate diagnosis, a comprehensive genetic analysis using next generation sequencing (NGS) has emerged as an efficient tool in cases of FH (8, 9). Among several comprehensive genetic analysis methods, whole exome sequencing (WES) is widely used as an established method. This technique has also been used to clarify the ge-

1Department of Cardiovascular Medicine, Kanazawa University Graduate School of Medical Sciences, Japan, 2Department of Genetics, Ishikawa Prefectural Central Hospital, Japan, 3Department of Cardiology, Koseiren Takaoka Hospital, Japan, 4Division of Cardiology, Department of Internal Medicine, Yokohama Sakae Kyosai Hospital, Japan and 5Department of Cardiovascular Surgery, Kanazawa University, Japan

Received for publication November 9, 2021; Accepted for publication December 16, 2021

Correspondence to Dr. Masa-aki Kawashiri, mk@med.kanazawa-u.ac.jp
Figure 1. A pedigree chart of the present case. Pin dots indicate affected members carrying the Cys302Gly/Arg303Trp mutations and black symbols indicate affected members carrying an exon 6 large deletion. Question marks indicate members with unknown genotype. “I,” “II,” “III,” and “IV” indicate the generation of this family. Squares indicate men, circles indicate women. Asterisks indicate members clinically diagnosed with hypercholesterolemia.

A 41-year-old woman with a history of chest pain and dyspnea on exertion for 1 month accompanied by extreme hyper LDL-cholesterolemia was referred to our hospital for further examination of her lipid and atherosclerotic cardiovascular diseases. She was born from a non-consanguineous marriage. She had a xanthoma tuberosum in her wrist joint at 3 years of age, which was resected. She received statins from 16 years of age for severe hypercholesterolemia (≥800 mg/dL); however, she discontinued the medications due to the risk of fetal malformation during her first pregnancy at 31 years of age. Moreover, she had a family history of hyperlipidemia as well as sudden cardiac death on the paternal and maternal sides of her family (Fig. 1, Table 1). A physical examination revealed xanthelasma and tendinous xanthoma on her finger extensor muscle and her Achilles’ tendons (Fig. 2). An ejection systolic grade IV/VI murmur was heard on the right second intercostal space radiating toward the jugular. The plasma lipid profile showed severe primary hypercholesterolemia, even under statin treatment (Table 2). Although the leaflets of her aortic valve were almost intact with preserved mobility (Fig. 3A, B, Supplementary material 1), severe left ventricular outflow tract stenosis was detected, with a peak gradient of 87 mmHg on transthoracic echocardiography (Fig. 3C). The patient had mild concentric left ventricular hypertrophy (10 mm) and a narrow annulus followed by SVAS (Fig. 3D). Cardiac computed tomography demonstrated severe calcification and a hypoplastic sinus of Valsalva due to SVAS (Fig. 4). Although coronary angiography showed slight irregularity of both the left main trunk and right coronary ostium, there was no significant stenotic lesion in her coronary arteries (Fig. 5A, B). Cardiac catheterization examination revealed SVAS with severe calcification and a pressure gradient of 75 mmHg between the left ventricle and aortic root (Fig. 5C, D).

Accordingly, her symptoms could be only be accounted for by SVAS, and aortic valve replacement surgery (Medtronic, ATS, 18 mm) with aortic root angioplasty was performed. Postoperative echocardiography showed significant hemodynamic improvement.

Her clinical diagnosis was tentatively estimated as HoFH because of her severe hyper LDL-cholesterolemia, which was refractory to statins, and her severe phenotype, which
Figure 2. Xanthelasma palpebrarum (A), xanthoma tendinosum on the finger’s extensor muscle (B), Achilles’ tendons (C), and radiograph of the left Achilles’ tendon (D).

Table 1. Genotype and Lipid Profile of the Proband and Her Family Members.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Genotype</th>
<th>Age</th>
<th>Sex</th>
<th>T-Chol</th>
<th>TG</th>
<th>HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband</td>
<td>c.904T&gt;G/c.907C&gt;T, exon 6 del, LDLR</td>
<td>41</td>
<td>female</td>
<td>523</td>
<td>122</td>
<td>43</td>
</tr>
<tr>
<td>II-1*</td>
<td>c.904T&gt;G/c.907C&gt;T, LDLR</td>
<td>75</td>
<td>male</td>
<td>205</td>
<td>140</td>
<td>49</td>
</tr>
<tr>
<td>II-14</td>
<td>exon 6 del, LDLR</td>
<td>68</td>
<td>female</td>
<td>226</td>
<td>137</td>
<td>91</td>
</tr>
<tr>
<td>III-4*</td>
<td>exon 6 del, LDLR</td>
<td>49</td>
<td>female</td>
<td>197</td>
<td>490</td>
<td>84</td>
</tr>
<tr>
<td>III-6</td>
<td>exon 6 del, LDLR</td>
<td>46</td>
<td>female</td>
<td>323</td>
<td>54</td>
<td>88</td>
</tr>
<tr>
<td>III-12</td>
<td>exon 6 del, LDLR</td>
<td>45</td>
<td>male</td>
<td>256</td>
<td>77</td>
<td>63</td>
</tr>
<tr>
<td>III-14</td>
<td></td>
<td>41</td>
<td>male</td>
<td>205</td>
<td>336</td>
<td>51</td>
</tr>
<tr>
<td>IV-5</td>
<td>c.904T&gt;G/c.907C&gt;T, LDLR</td>
<td>10</td>
<td>male</td>
<td>312</td>
<td>73</td>
<td>81</td>
</tr>
<tr>
<td>IV-6</td>
<td>exon 6 del, LDLR</td>
<td>7</td>
<td>female</td>
<td>254</td>
<td>42</td>
<td>51</td>
</tr>
</tbody>
</table>

* Asterisk indicates the patients under lipid lowering therapy.

T-Chol: total cholesterol, TG: triglycerides, HDL-C: High-density lipoprotein cholesterol

Table 2. Lipid and Apolipoprotein Profile of the Proband under Statin Therapy at the First Visit.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Apolipoprotein A-I</th>
<th>104 mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>423 mg/dL.</td>
<td>Apolipoprotein A-I</td>
<td>104 mg/dL</td>
</tr>
<tr>
<td>TG</td>
<td>52 mg/dL.</td>
<td>Apolipoprotein A-II</td>
<td>19.1 mg/dL</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>46 mg/dL.</td>
<td>Apolipoprotein B</td>
<td>222 mg/dL</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>354 mg/dL.</td>
<td>Apolipoprotein C-II</td>
<td>1.1 mg/dL</td>
</tr>
<tr>
<td>F-cholesterol</td>
<td>132 mg/dL.</td>
<td>Apolipoprotein C-III</td>
<td>5.7 mg/dL</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>313 mg/dL.</td>
<td>Apolipoprotein E</td>
<td>5.3 mg/dL</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>0.07 mEq/L.</td>
<td>Lipoprotein (a)</td>
<td>55.1 mg/dL</td>
</tr>
<tr>
<td>PG</td>
<td>105 mg/dL.</td>
<td>Free triiodothyronine</td>
<td>2.49 pg/mL</td>
</tr>
<tr>
<td>HbA1c</td>
<td>5.4 %</td>
<td>Free thyroxine</td>
<td>1.15 ng/dL</td>
</tr>
<tr>
<td>Total protein</td>
<td>7.2 g/dL.</td>
<td>Thyroid stimulating hormone</td>
<td>1 ng/dL</td>
</tr>
<tr>
<td>Albumin</td>
<td>3.9 g/dL.</td>
<td>Urine albumin</td>
<td>(-)</td>
</tr>
</tbody>
</table>

TG: triglycerides, HDL: high-density lipoprotein, LDL: low-density lipoprotein, PG: plasma glucose, Hb: hemoglobin
Figure 3. The transesophageal echocardiogram shows that the leaflets of the patient’s aortic valve had preserved mobility in the parasternal long axis view (A; end systolic) and in the parasternal short axis view (B; end systolic). Severe left ventricular outflow tract stenosis with a peak gradient of 87 mmHg is revealed by transthoracic echocardiography using continuous wave Doppler (C). Transesophageal echocardiography shows left ventricular outflow tract stenosis in the parasternal long axis view (D; end diastolic, AA=19 mm, STJ=23 mm, Ao=27 mm). LA: left atrium, LV: left ventricle, Ao: aorta, RA: right atrium, AA: aortic annulus, STJ: sinotubular junction

Figure 4. Computed tomographic findings of the patient’s aortic valve and ascending aorta. Arrowheads indicate the site of supravalvular aortic stenosis. AV: aortic valve, LV: left ventricle, RA: right atrium, RV: right ventricle

Exome Sequencing and Bioinformatic Analysis

At first, we performed WES only for the proband, and subsequently performed bioinformatic filtering as previously described (5). This study was approved by the Research Ethics Committee of Kanazawa University (Kanazawa, Japan), and written informed consent was obtained from the patient and her family members. All procedures were conducted in accordance with the Helsinki Declaration of 2008. Four independent filters were applied after standard variant quality control to successfully discover the causal variants. Variants were filtered out as follows: 1) benign variants predicted by SnpEff; 2) minor allele frequency >1% in the Asian population; 3) variants found in two healthy subjects; 4) unknown variants that could be causal mutations of FH.

As a result, we identified two point-mutations with amino acid substitutions in the \textit{LDLR} gene (NM_000527.4:c.904 T>G, c.907 C>T) on chromosome 19. We confirmed these two point-mutations in exon 7 using Sanger sequencing. Initially, we recognized that the two adjacent missense-mutations were derived from each parent. However, we later confirmed both mutations in her father and older brother, but not in her maternal relatives, including those exhibiting hyper LDL cholesterolemia. Consequently, these observa-

included xanthoma and SVAS. We attempted to determine her genetic background in order to explain the extreme disease manifestations.
Figure 5. Coronary angiography and aortography. Coronary angiography of the right coronary artery (A) and left coronary artery (B) demonstrates slight irregularity of both the left main trunk and right coronary ostium. Aortography of the aortic root shows supravalvular aortic stenosis with severe calcification (C; LAO: left anterior oblique, D; RAO: right anterior oblique). Arrowheads indicate the site of supravalvular aortic stenosis. LCA: left coronary artery, RCA: right coronary artery.

Discussion

We reported a rare case of compound HeFH with xanthoma tuberosum in infancy. SAVS is also recognized as a typical clinical manifestation of HoFH. A study using magnetic resonance imaging showed that 41% of patients with HoFH exhibiting SVAS (12) required aortic root enlargement and aortic valve replacement (mechanical prosthetic heart valve), even while under aggressive medical therapy (4).

HoFH exhibiting double deleterious mutations in the FH gene is a rare genetic condition, the prevalence of which is currently considered to be approximately 1 in 160,000-360,000 in many countries, including Japan (11, 13).

When we encounter a patient with severe hypercholesterolemia, it is important to differentiate HoFH from HeFH. Although most available cholesterol lowering medicines are effective for reducing LDL-C in HeFH (half of their LDLR are intact), they could not effectively reduce the LDL-C level in patients with HoFH without an intact LDLR (14). Thus, most patients with HoFH require LDL-apheresis therapy and/or microsomal triglyceride transfer protein inhibitor...
The clinical course of the patient. LDL: low-density lipoprotein

Figure 6. The clinical course of the patient. LDL: low-density lipoprotein

In the present case, we firstly attempted to determine the patient’s genetic background using WES. Two adjacent missense mutations in the LDLR gene could be revealed, however, these mutations originated from her father, and they were also found in some paternal relatives with the HeFH phenotype. However, no genetic mutations responsible for the elevation of LDL-C could be found among the patient’s maternal relatives by WES. Although WES can detect mutations across the exome, enabling us to establish a differential diagnosis, its application is not sufficient for the detection of structural genetic variations such as the large deletion reported in our patient. One possible way to identify such structural variations is through NGS data with consideration of the depth of coverage; however, this method typically requires hundreds of “controls” data-points. On the other hand, the MLPA technique could be used to detect structural variants with higher sensitivity (15, 16). It is noteworthy that up to approximately 10% of the genetic background of FH showed such structural genetic variations (15).

In conclusion, we suggest the use of the MLPA method along with WES for the detection of structural genetic variations to establish a quick diagnosis of FH, as significant numbers of patients with FH show structural genetic variations.

The authors state that they have no Conflict of Interest (COI).

Financial Support

A scientific research grant from the Ministry of Education, Science, and Culture of Japan (No. 19K17552), Ministry of Health, Labor and Welfare Sciences Research Grant for Research on Rare and Intractable Diseases, and Japanese Circulation Society (project for genome analysis in cardiovascular diseases).

References

7. Futema M, Plagnol V, Li K, et al. Whole exome sequencing of fa-

The Internal Medicine is an Open Access journal distributed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License. To view the details of this license, please visit (https://creativecommons.org/licenses/by-nc-nd/4.0/).

© 2022 The Japanese Society of Internal Medicine
Intern Med 61: 2883-2889, 2022