Genetic Analysis of Japanese Children Clinically Diagnosed with Familial Hypercholesterolemia

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Aim: This study aimed to elucidate the gene and lipid profiles of children clinically diagnosed with familial hypercholesterolemia (FH).

Methods: A total of 21 dyslipidemia-related Mendelian genes, including FH causative genes (LDLR, APOB, and PCSK9) and LDL-altering genes (APOE, LDLRAP1, and ABCG5/8), were sequenced in 33 Japanese children (mean age, 9.7 ± 4.2 years) with FH from 29 families.

Results: Fifteen children (45.5%) with pathogenic variants in LDLR (eight different heterozygous variants) and one child (3.0%) with the PCSK9 variant were found. Among 17 patients without FH causative gene variants, 3 children had variants in LDL-altering genes, an APOE variant and two ABCG8 variants. The mean serum total cholesterol (280 vs 246 mg/dL), LDL-cholesterol (LDL-C, 217 vs 177 mg/dL), and non-HDL cholesterol (228 vs 188 mg/dL) levels were significantly higher in the pathogenic variant-positive group than in the variant-negative group. In the variant-positive group, 81.3% of patients had LDL-C levels ≥ 180 mg/dL but 35.3% in the variant-negative group. The mean LDL-C level was significantly lower in children with missense variants, especially with the p.Leu568Val variant, than in children with other variants in LDLR, whereas the LDL-altering variants had similar effects on the increase in serum LDL-C to LDLR p.Leu568Val.

Conclusion: Approximately half of the children clinically diagnosed with FH had pathogenic variants in FH causative genes. The serum LDL-C levels tend to be high in FH children with pathogenic variations, and the levels are by the types of variants. Genetic analysis is useful; however, further study on FH without any variants is required.

Key words: FH, Gene, LDL-cholesterol, LDL receptor, PCSK9

Introduction

Familial hypercholesterolemia (FH, OMIM number #143890) is an autosomal dominant disorder characterized by hyper-low-density lipoprotein (LDL)-cholesterolemia, premature coronary artery disease (CAD), and tendon xanthomas. FH is caused commonly by pathogenic variants in genes encoding the LDL receptor (LDLR), apolipoprotein B (APOB), and proprotein convertase subtilisin/kexin type 9 (PCSK9). LDLR is the main causative gene for FH. In Japan, 54%–80% of adult patients with FH have pathogenic variants in LDLR or PCSK9.

Patients with FH have high serum LDL-cholesterol (LDL-C) levels from birth and are at risk of developing atherosclerosis at an earlier age than...
normal\textsuperscript{1, 4). The carotid intima-media thickness in children with a molecular diagnosis of heterozygous FH is significantly increased from the age of 12 years\textsuperscript{8). Therefore, it is important to diagnose FH at least up to 10 years of age and provide proper diet and exercise programs as soon as possible after the diagnosis. Untreated patients with FH have been reported to develop CAD at 35 years of age; however, children with FH who started low-dose statin therapy from 10 years of age developed CAD at 53 years of age, which is almost the same age as patients with dyslipidemia other than FH\textsuperscript{4). Thus, commencing statin therapy at 10 years of age delays the onset of CAD by 18 years\textsuperscript{9).}

In Japan, children with FH (<15 years old) are clinically diagnosed by 1) serum LDL-C levels ≥ 140 mg/dL, the 95th percentile value of Japanese children, and 2) a family history of FH or premature CAD, < 55 years of age in men and < 65 years of age in women, within their second-degree relatives\textsuperscript{9). However, diagnosing FH in childhood is not easy due to several reasons. First, children with FH, except homozygous FH, have no clinical findings, such as xanthomas and corneal rings. Second, children in general have few opportunities for receiving blood tests. Third, confirming their detailed family history of FH or premature CAD is difficult. This is because their parents have not been diagnosed with FH or have not reached the age to develop premature CAD. Fourth, the levels of serum LDL-C are physiologically variable during puberty\textsuperscript{10). Apart from the clinical diagnosis, FH is confirmed genetically when patients have pathogenic variants in \textit{LDLR}, \textit{APOB}, or \textit{PCSK9}. Thus, genetic analysis is a useful tool for children with FH when a definitive diagnosis is difficult.

To the best of our knowledge, studies on the genetic analysis of Japanese children with FH are scarce. Also, the genetic characteristics and identification rate of the pathogenic variants are unknown. Furthermore, the differences in lipid profiles between variant-positive and variant-negative children with FH are unknown. Variant-positive children are those with FH who have pathogenic variants in \textit{LDLR}, \textit{APOB}, or \textit{PCSK9}; conversely, variant-negative children have no pathogenic variants in these genes in this manuscript. We also aimed to understand the effects of the identified types of genetic variants on serum LDL-C levels.

**Methods**

**Subjects**

A total of 33 Japanese children from 29 families who visited the Clinic for FH Children at Showa University Hospital or Tokyo Metropolitan Children’s Medical Center between January 2016 and August 2019 were enrolled in the study (Table \textit{1}). They were clinically diagnosed with FH based on the criteria of the Japan Atherosclerosis Society (LDL-C ≥ 140 mg/dL and family history of FH or premature CAD)\textsuperscript{9).}

The height and weight of the children were measured, and then the height standard deviation (SD) score (Ht-SDS), percentage of overweight (POW), and body mass index (BMI)-for-age percentile (BMI%) were calculated using the data of the Annual Report of School Health Statistics 2000 from the Ministry of Education, Culture, Sports, Science and Technology, Japan. POW, which is commonly used for evaluating obese children in Japan, is the modified weight-for-height method. The diagnostic criterion for obesity is a POW ≥ 20% (≥ 120% of the standard weight)\textsuperscript{11). Xanthoma was checked via visual inspection and palpation. The thickness of the Achilles tendon was evaluated via X-ray in six cases.

A boy with Down syndrome, a girl with Graves’ disease, and a girl with type 1 diabetes mellitus were included in this study. The boy with Down syndrome was regularly examined for thyroid function and was found to be euthyroid. The girl with Graves’ disease was treated with thiamazole and was euthyroid. The girl with type 1 diabetes mellitus was treated with continuous subcutaneous insulin infusion, and her blood glucose levels were well controlled. The children had a family history of FH, and their mean serum LDL-C levels were 196, 203, and 156 mg/dL, respectively. Therefore, we considered that their high LDL-C levels were due to FH and not their primary diseases.

**Biochemical Analysis**

The serum levels of total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were measured using an automated analyzer. Non-HDL-C was calculated as the TC value minus the HDL-C value. During fasting, blood samples were not collected. A total of 94% (31/33) of the data were obtained before the initiation of lipid-lowering therapy. The remaining data from two
Involvement of the LDL-altering Variants in APOE, LDLRAP1, and ABCG5/8 for FH

A causative variant in the LDL-altering genes (APOE, LDLRAP1, and ABCG5/8) of the FH phenotype was defined if it fulfilled any of the following criteria: a) variants known to be disease causing for abnormalities of LDL-C in the HGMD were designated as “Pathogenic”; b) rare (allele frequency <1% among the East Asian population) protein truncating variants (premature stop, indel, or splice-site alteration) at LDLR; c) rare missense variants at LDLR, defined as those predicted as damaging by all four in silico software programs (M-CAP, Polyphen-2, SIFT, and MutationTaster); d) ClinVar-registered pathogenic or likely pathogenic variants causing FH in LDLR, APOB, or PCSK9; and e) PCSK9 p.Glu32Lys variant previously reported to cause FH in Japanese people.

Determination of the Pathogenic Variants for FH in LDLR, APOB, and PCSK9

A pathogenic variant for FH was defined if it fulfilled any of the following criteria: a) variants known to be disease causing for abnormalities of LDL-C in the HGMD were designated as “Pathogenic”; b) rare (allele frequency <1% among the East Asian population) protein truncating variants (premature stop, indel, or splice-site alteration) at LDLR; c) rare missense variants at LDLR, defined as those predicted as damaging by all four in silico software programs (M-CAP, Polyphen-2, SIFT, and MutationTaster); d) ClinVar-registered pathogenic or likely pathogenic variants causing FH in LDLR, APOB, or PCSK9; and e) PCSK9 p.Glu32Lys variant previously reported to cause FH in Japanese people.

Determination of Pathogenic Variants in Other Genes

A causative variant for the hypo- and hyper-
HDL-cholesterolemia phenotype in ATP-binding cassette transporter A1 gene \((ABC\text{A}1)\) and cholesteryl ester transfer protein gene \((CETP)\) was defined if it fulfilled any of the above criteria.

**Ethical Considerations**

The present study was approved by the Showa University Ethics Committee (No. 268), the Tokyo Metropolitan Children’s Medical Center (H28b-179), and Kanazawa University (313–6). All procedures were conducted in accordance with the ethical standards of the responsible institutional and national committees on human experimentation and the 1964 Declaration of Helsinki, as revised in 2013. Informed consent for genetic analysis was obtained from the parents of the patients.

**Statistical Analysis**

We used GraphPad PRISM version 7 (GraphPad Software Inc.; La, Jolla, CA, USA) for data analysis. \(P<0.05\) was considered statistically significant. Categorical variables were reported as the number of subjects and percentages. The chi-squared test was employed to compare the frequencies among different groups. Continuous variables were reported as mean ± SD or median (95% confidence interval [CI]). The differences between the two groups were evaluated using the non-parametric Mann–Whitney \(U\) test. Receiver operating characteristic (ROC) curve analysis was conducted using the JMP 15 software (SAS Institute Inc., Cary, NC, USA).

**Results**

**Patients**

The clinical characteristics and lipid profiles of the patients in the present study are presented in **Table 1**. Of the 33 patients, 10 were boys and 23 were girls (mean age: 9.7 ± 4.2 years). Their mean Ht-SDS, POW, and BMI% were within the normal ranges. One obese child was found in the variant-positive group, and two were found in the variant-negative group. The effect of obese children on the present study was negligible. All patients had hyper-LDL-cholesterolemia and a family history of FH. The mean serum LDL-C level was 196 mg/dL (median and 95% CI: 196 mg/dL, 181–212 mg/dL). A family history of premature CAD was observed in three patients. Tendon xanthomas and corneal rings were not found in any patient.

**Characteristics and Lipid Profiles in the Group with Pathogenic Variants**

**Table 1** presents the clinical characteristics and lipid profiles of the patients with or without pathogenic variants in the FH causative genes. Pathogenic variants in \(LDLR\) and \(PCSK9\) were found in 45.5\% (\(n=15; 13\) families, 44.8\%) and 3.0\% (\(n=1; \)one family, 3.4\%) of the patients. All the identified pathogenic variants were heterozygous. No variants were found in any of the other genes among the targeted exome sequencing. The remaining 51.5\% of patients (\(n=17; 15\) families 51.7\%) had no pathogenic variants in the FH causative genes.

No significant differences were observed in age, sex, Ht-SDS, POW, and BMI% between the variant-positive and variant-negative groups. The serum HDL-C and TG levels, prevalence of tendon xanthomas, and family history of premature CAD were similar between the groups. The mean serum TC (280 vs 246 mg/dL), LDL-C (217 vs 177 mg/dL), and non-HDL-C (228 vs 188 mg/dL) levels were significantly higher in the variant-positive group than in the variant-negative group. The median and 95% CI in the variant-positive and variant-negative groups were as follows: TC: 264 mg/dL (260–300) vs 229 mg/dL (225–267), LDL-C: 206 mg/dL (196–237) vs 164 mg/dL (155–200), and non-HDL-C: 216 mg/dL (206–249) vs 175 mg/dL (165–211).

**Fig.1** presents the distribution of individual LDL-C levels in children with FH. In the variant-positive group, 81.3% of the patients (13/16) had LDL-C levels \(\geq 180\) mg/dL, which is the recommended value for statin therapy in pediatric patients with FH (≥ 10 years of age) based on the guidelines of the Japan Atherosclerosis Society\(^9\)). Contrarily, only 35.3% of the patients (6/17) had serum LDL-C levels higher than 180 mg/dL in the variant-negative group (\(p<0.01\)).

ROC curve analysis was conducted to predict pathogenic variant-positive children. Although the number of our subjects was small, the highest LDL-C point of sensitivity plus specificity was 175 mg/dL. When 160, 180, or 200 mg/dL was set as the cutoff LDL-C values to determine the variant-positive children, the sensitivity was 93.8\% (15/16), 81.3\% (13/16), and 62.5\% (10/16), with a specificity of 47.1\% (8/17), 64.7\% (11/17), and 76.5\% (13/17), respectively.

**The Low-LDL-C FH Child with Variant and the High-LDL-C FH Child without Variant**

The pathogenic missense variant in \(LDLR\) (c.1702C>G, p.Leu568Val) was identified in a boy with FH, but his LDL-C level was 141 mg/dL (**Fig.1**). The boy was 8 years old. His father and paternal grandfather had been diagnosed with FH and were on statin therapy. A family history of premature
Pathogenic Variants in LDLR and PCSK9

Of the 16 pathogenic variants in LDLR or PCSK9 in total, 8 were different variants in LDLR, and 1 was a variant in PCSK9 (Table 2). Of these, 7 LDLR variants (c.2431A>T, p.Lys811*; c.682G>A, p.Glu228Lys; c.1067A>T, p.Asp356Val; c.1339T>C, p.Ser447Pro; c.1702C>G, p.Leu568Val; c.313+1G>T; c.1845+2T>C; and c.1245_1249dupCCGGA, p.Ser417Thrfs*12) and one PCSK9 variant (c.94G>A, p.Glu32Lys) were FH causative genes or the LDL-altering genes.

Cad was not noted in this family.

One child had abnormally high LDL-C level (309 mg/dL) in the variant-negative group (Fig.1). The child was a 2-year-old boy whose older sister (6 years old) also had high serum LDL-C level (229 mg/dL). His mother, maternal grandfather, and maternal great-grandfather also had hyper-LDL-cholesterolemia. The mother was on statin therapy; however, the treatment of the maternal grandfather was unknown. He was diagnosed with FH, even though he and his family did not have variants in the FH causative genes or the LDL-altering genes.

Table 2. Identified pathogenic variants in LDLR and PCSK9

| Variant names       | Predicted effect at the protein level | Number Patients/Families | Reference |
|---------------------|--------------------------------------|--------------------------|-----------|
| LDLR nonsense variant |                                       |                          |           |
| c.2431A>T           | p.Lys811*                             | n/a                      | Disease causing 3/2 (21) |
| LDLR missense variant |                                       |                          |           |
| c.682G>A            | p.Glu228Lys                           | 0.853                    | Disease causing 1/1 (22) |
| c.1067A>T           | p.Asp356Val                           | 0.891                    | Disease causing 1/1 Novel (27) |
| c.1339T>C           | p.Ser447Pro                           | 0.740                    | Disease causing 2/1 (23) |
| c.1702C>G           | p.Leu568Val                           | 0.751                    | Disease causing 5/5 (24) |
| LDLR splice-site variant |                                       |                          |           |
| c.313+1G>T          | -                                    | n/a                      | n/a       |
| c.1845+2T>C         | -                                    | n/a                      | n/a       |
| LDLR frameshift variant |                                       |                          |           |
| c.1245_1249dupCCGGA | p.Ser417Thrfs*12                      | n/a                      | Disease causing 1/1 (26) |
| PCSK9 missense variant |                                       |                          |           |
| c.94G>A             | p.Glu32Lys                            | -                        | n/a       |

n/a: not available. LDLR, low-density lipoprotein receptor; PCSK9, proprotein convertase subtilisin/kexin type 9.
The pathogenicity thresholds for each pathogenicity prediction software are as follows: M-CAP >0.025, PolyPhen-2 >0.8, and SIFT <0.05.

Fig. 1. Frequency histogram of the distribution of low-density lipoprotein cholesterol (LDL-C) values in our 33 patients
Closed bar: variant-positive children (n=16), open bar: variant-negative children (n=17).
Identified Variants in the LDL-Altering Genes

Of the patients without pathogenic variants in the FH causative genes, one patient (3.0% of patients, 3.4% of families) had compound heterozygous variants in \( APOE \) (c.784G>A, p.Glu262Lys, and c.787G>A, p.Glu263Lys), and two patients from different families (6.1% of patients, 6.9% of families) had heterozygous variants in \( ABCG8 \) (c.55G>C, p.Asp19His; c.1256T>A, p.Ile419Asn) (Table 3). These variants were reported to be associated with hyper-LDL-cholesterolemia.28

Identified Variants in \( ABCA1 \) and \( CETP \)

Of the patients without pathogenic variants in the FH causative genes, one patient (3.0% of patients, 3.4% of families) had a heterozygous variant in \( ABCA1 \) (c.3121C>G, p.Leu1041Val), and one patient with type 1 diabetes mellitus (3.0% of patients, 3.4% of families) had a heterozygous variant in \( CETP \) (c.1376A>G, p.Asp459Gly) (Table 3). Most \( ABCA1 \) variants are related to decreased HDL-C levels.29 However, the p.Leu1041Val variant in \( ABCA1 \) with CETP variant (p.Asp459Gly) was reported to be associated with high HDL-C.30 Moreover, the \( CETP \) variant (p.Asp459Gly) was reported to be associated with high HDL-C levels.30 Therefore, these variants were not considered to be related to the elevation of serum LDL-C.

Cholesterol Profiles by Genetic Variants

Table 4 presents the LDL-C and non-HDL-C profiles of our FH children with different types of

Table 3. Identified pathogenic variants in genes other than FH causative genes

| Gene      | Variant names       | Predicted effect at the protein level | Reference |
|-----------|---------------------|--------------------------------------|-----------|
| APOE      | c.784G>A, p.Glu262Lys | 0.649, 0.973, 0.079, Disease causing (28) |
| APOE      | c.787G>A, p.Glu263Lys | 0.541, 1.000, 0.051, Disease causing |
| ABCG8     | c.55G>C, p.Asp19His   | n/a, 0.769, 0.045, Polymorphism (12) |
| ABCG8     | c.1256T>A, p.Ile419Asn| 0.097, 0.975, 0.084, Disease causing (12) |
| ABCA1     | c.3121C>G, p.Leu1041Val | 0.156, 1.000, 0.000, Disease causing (29) |
| CETP      | c.1376A>G, p.Asp459Gly | n/a, 0.584, 0.016, Disease causing (30) |

Table 4. Cholesterol Profiles by Genetic Variants

| LDL-C | Non-HDL-C |
|-------|-----------|
|       |           |

n/a: not available. APOE, apolipoprotein E; ABCG8, ATP-binding cassette sub-family G member 8; ABCA1, ATP-binding cassette transporter A1; CETP, cholesteryl ester transfer protein. The pathogenicity thresholds for each pathogenicity prediction software are as follows: M-CAP > 0.025, PolyPhen-2 > 0.8, and SIFT < 0.05.
variants. Among children with pathogenic variants in \( \text{LDLR} \), both the mean serum LDL-C and non-HDL-C levels in children with missense variants \((n=9)\) were lower than those in children with other variants, such as nonsense, splice-site, and frameshift variants \((n=6, \text{both } p<0.05)\). Among children with missense variants in \( \text{LDLR} \), the mean LDL-C level in children with the \( \text{LDLR} \) p.Leu568Val variant \((n=5)\) was lower than that in children with other missense variants \((n=4, \text{both } p<0.05)\) but not the mean non-HDL-C level \((p=0.18)\). The mean serum LDL-C and the mean non-HDL-C levels in children with the LDL-altering variants in \( \text{APOE} \) or \( \text{ABCG8} \) \((n=3)\) were lower than in children with pathogenic variants in FH causative genes \((n=16, \text{both } p<0.05)\). Moreover, the mean LDL-C and the mean non-HDL-C levels in children with the LDL-altering variants \((n=3)\) were lower than in children with missense variants in \( \text{LDLR} \) other than p.Leu568Val \((n=4, \text{both } p<0.05)\). No significant differences were observed in the mean serum LDL-C and the mean non-HDL-C levels between the LDL-altering variant group \((n=3)\) and the \( \text{LDLR} \) p.Leu568Val variant group \((n=5)\) as well as between the LDL-altering variant group \((n=3)\) and the group without any variants \((n=14)\).

**Discussion**

In the present study, we demonstrated that 48.5% of our patients had pathogenic heterozygous variants in the FH causative genes (\( \text{LDLR} \) variants: 45.5%, \( \text{PCSK9} \) variant: 3.0%). Previous studies in other countries have shown that pathogenic variants in the FH causative genes (\( \text{LDLR}, \text{APOE}, \text{and } \text{PCSK9} \)) were identified in 50%–57% of children clinically diagnosed with FH \cite{f31-f33}. The rates of pathogenic variants were 57% (155/272) in Slovenia \cite{f31}, 56%...
had pathogenic variants in the FH causative genes and some other FH-related genes (e.g., STAP1) associated with the FH phenotype. Recently, the frequency of non-HDL-C in the group without any variants.

The p.Leu568Val variant in LDLR was the most commonly identified missense variant in the FH causative genes was different from that of the previously reported Japanese cases. The p.Leu568Val variant in LDLR was the most frequently identified in 15.2% of our patients ($n=5$; five families 17.2%). However, this variant was found only in 0.38% of patients ($4/1054$) by Mabuchi et al.\(^5\) and 2.9% of patients ($19/650$) by Hori et al.\(^7\). Mabuchi et al.\(^5\) reported that the p.Lys811* variant in LDLR was the most frequently identified in 27.7% of patients ($292/1054$). Contrarily, this variant was found in only 9.1% of our patients ($n=3$; two families 6.9%). Hori et al.\(^7\) reported that this variant was identified in only 3.2% of the patients ($21/650$). The differences in the frequency of gene profiles in patients with FH are not exactly known as the number of subjects in the present study is very small compared with those in the previous reports. However, it is suggested that the frequency of gene profiles may vary depending on the number, age, and region of the subjects.

In the present study, the serum TC, LDL-C, and non-HDL-C levels were significantly higher in the polygenic FH group than in the variant-negative group. It is suggested that children with FH with high serum LDL-C levels tend to have polygenic FH. In our study, 81.3% of FH children with polygenic FH were found to have greater than 180 mg/dL of LDL-C, and 64.7% of children with FH without polygenic FH had LDL-C levels less than 180 mg/dL. According to ROC analysis, the best round cutoff, the LDL-C level of 180 mg/dL was a potential candidate value to determine FH children with polygenic FH, with a sensitivity of 81.3% and specificity of 64.7%.

The serum LDL-C levels were significantly lower in children with missense variants in LDLR, especially in the case with the LDLR p.Leu568Val variant, than in children with other LDLR variants. The LDL-C levels in FH adults with loss-of-function variants, such as nonsense, splice-site, and frameshift variants, were reported to be higher than those in FH adults with missense variants.\(^38, 39\) In addition, the serum LDL-C level in FH adults with the p.Leu568Val variant in LDLR was reported to be low among the three frequent missense variants in LDLR, p.Cys338Ser, p.Asp433His, and this variant.\(^7\) Our results were the same as those in previous reports. However, it has been shown that most FH children with a pathogenic variant, despite the relatively low LDL-C at diagnosis, sooner or later develop higher cholesterol levels and need statin therapy.\(^40\)

Although the patients with LDL-altering variants were only three in the present study, the LDL-altering variants in APOE or ABCG8 weakly affect the serum LDL-C and non-HDL-C levels, as much as the LDLR p.Leu568Val variant. The LDL-altering variant group was predicted to have higher serum LDL-C and non-HDL-C levels than the group without any variants, but no significant differences were observed between the two groups. This could be due to the inclusion of siblings with abnormally high levels of LDL-C and non-HDL-C in the group without any variants.
We predicted that children with FH with LDLR or PCSK9 variants would develop CAD earlier than children without pathogenic variants when untreated. Patients with hypercholesterolemia develop CAD when their cumulative LDL-C levels reach approximately 6,200 mg/dL (160 mmol)\(^4\). Based on the results of the present study, it was estimated that untreated patients with pathogenic variants would develop CAD at 30.1 years of age (6,200/206), and patients without pathogenic variants would develop CAD at 37.8 years of age (6,200/164). Patients with pathogenic variants were estimated to reach cumulative LDL-C levels to develop CAD earlier at 7.7 years than patients without pathogenic variants. Actually, the father of the 4-year-old boy with the LDLR p.Glu228Lys variant in the present study had an acute myocardial infarction at the age of 32. Therefore, patients with pathogenic variants were at a higher risk of developing CAD than those without pathogenic variants in these genes.

In the present study, no children had homozygous FH. Children with severe FH require earlier lipid-lowering treatments, including plasma apheresis\(^4\). It is difficult for FH patients with “true homozygotes”, who have two identical pathogenic variants in two alleles of the FH causative gene, to reduce their serum LDL-C levels; however, statins and anti-PCSK9 antibodies are effective in children with less severe homozygous FH, such as double and compound heterozygotes\(^9\). In cases of severe FH or suspected homozygous FH, genetic analysis is a useful tool for treatment.

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Integration

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