

Universal Screening for Familial Hypercholesterolemia in Children in Kagawa, Japan

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Aim: Familial hypercholesterolemia (FH) is an underdiagnosed autosomal dominant genetic disorder characterized by high levels of plasma low-density lipoprotein cholesterol (LDL-C) from birth. This study aimed to assess the genetic identification of FH in children with high LDL-C levels who are identified in a universal pediatric FH screening in Kagawa, Japan.

Method: In 2018 and 2019, 15,665 children aged 9 or 10 years underwent the universal lipid screening as part of the annual health checkups for the prevention of lifestyle-related diseases in the Kagawa prefecture. After excluding secondary hyper-LDL cholesterolemia at the local medical institutions, 67 children with LDL-C levels of ≥ 140 mg/dL underwent genetic testing to detect FH causative mutations at four designated hospitals.

Results: The LDL-C levels of 140 and 180 mg/dL in 15,665 children corresponded to the 96.3 and 99.7 percentile values, respectively. Among 67 children who underwent genetic testing, 41 had FH causative mutations (36 in the LDL-receptor, 4 in proprotein convertase subtilisin/kexin type 9, and 1 in apolipoprotein B). The area under the curve of receiver operating characteristic curve predicting the presence of FH causative mutation by LDL-C level was 0.705, and FH causative mutations were found in all children with LDL-C levels of ≥ 250 mg/dL.

Conclusion: FH causative mutations were confirmed in almost 60% of the referred children, who were identified through the combination of the lipid universal screening as a part of the health checkup system and the exclusion of secondary hyper-LDL cholesterolemia at the local medical institutions.

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Key words: Children, Familial hypercholesterolemia, Genetic testing, Low-density lipoprotein cholesterol

Introduction

Familial hypercholesterolemia (FH) is an autosomal dominant genetic disorder with an incidence of 1:200–500 in the general population¹⁻³.

Mutations in three genes, low-density lipoprotein receptor (LDLR), apolipoprotein B (APOB), and proprotein convertase subtilisin/kexin type 9 (PCSK9), have been detected in about 80% of FH patients⁴. Mutations in these genes lead to a high

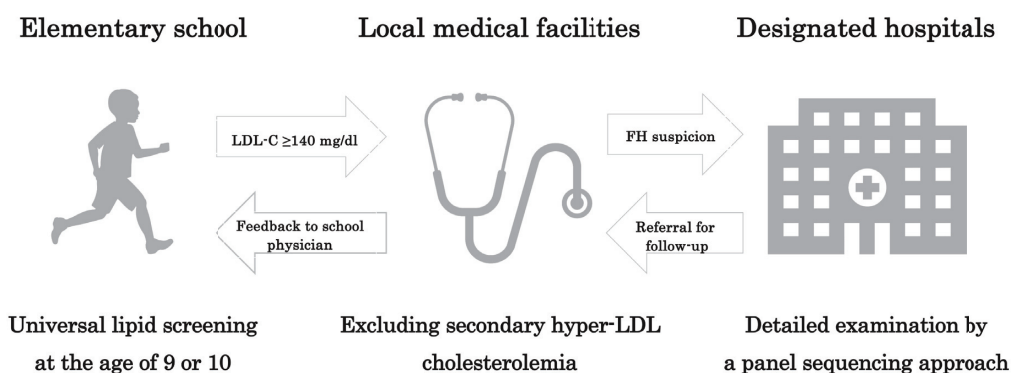


Fig. 1. Universal health checkups for the prevention of lifestyle-related diseases in children from Kagawa, Japan

Universal health checkups for the prevention of childhood lifestyle-related diseases include lipid screening and are annually performed in elementary schools in Kagawa, Japan. Children with LDL-C levels of ≥ 140 mg/dL are recommended to visit their local medical institution. After excluding secondary hyper-LDL cholesterolemia at the local medical institutions, the children were referred to the four designated hospitals. The treatment and follow-up were performed at the local medical institutions and/or four designated hospitals after the children were diagnosed with FH. FH, familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol.

level of plasma LDL-cholesterol (LDL-C), which is one of the most important causes of premature coronary artery disease (CAD) in FH patients^{2, 5}. Since early diagnosis and treatment are important to prevent CAD, screening is recommended in children aged 9–11 years⁶. However, the diagnosis of pediatric FH is still challenging^{7, 8}, as the clinical features in adult FH, such as xanthoma, and family history of premature CAD or FH are rarely confirmed in children and adolescents, and lipid blood tests are rarely administered.

From 2012, the Kagawa prefecture started the “Kagawa health checkups for the prevention of lifestyle-related diseases in children,” which include blood testing of LDL-C in addition to other metabolic parameters. More than 7,000 children aged 9 or 10 years have undergone universal lipid screening in primary schools in each year. Children with LDL-C levels of ≥ 140 mg/dL are recommended to visit their local medical institutions. When they are suspected of FH in these institutions after excluding secondary hypercholesterolemia, they are introduced to either of four designated hospitals for FH genetic testing. This study aimed to assess the genetic identification of FH in children with high LDL-C levels who are identified in a universal pediatric FH screening in the Kagawa prefecture.

Method

Study Population

All 17 local governments in the Kagawa prefecture annually perform health checkups for the prevention of lifestyle-related diseases in children. The procedure, which is performed in elementary schools, includes lipid screening. The Kagawa prefectural government collects and analyzes the medical data obtained from all local governments. A total of 15,665 (male/female=8,051/7,614) children aged 9 or 10 years voluntarily received health checkup in 2018 and 2019. The checkup rate in 2018 and 2019 was 92.2%. Children with LDL-C levels of ≥ 140 mg/dL received the recommendation letter to visit their local medical institutions. Children suspected of having FH at these institutions, after excluding secondary hyper-LDL cholesterolemia, were introduced to four designated hospitals for the diagnosis of FH, namely, Kagawa University Hospital, Shikoku Medical Center for Children and Adults, Mitoyo General Hospital, and Kagawa Prefectural Central Hospital (**Fig. 1**). Among the children assigned to the four hospitals, 67 with high LDL-C levels underwent genetic testing from April 2018 to August 2020.

Clinical Characteristics of FH in Children

In addition to the significant elevation of LDL-C levels (≥ 140 mg/dL) in children, we obtained medical

information on the family history of FH, family history of premature CAD, LDL-C level of a parent, and appearance of xanthoma in the second-degree relatives of the referred children. The weight and standing height were measured, and the percentage of overweight (POW) was determined as previously described⁹. Premature CAD was defined as CAD with an onset at <55 years of age for males and <65 years of age in females, respectively¹⁰. When the children who were referred from local medical institutes underwent genetic testing, the LDL-C levels were measured again, and concomitant endocrine and kidney disorders were excluded. Children were clinically diagnosed with FH according to the Guidance for Pediatric FH 2017¹⁰.

Basically, the doctors at the local medical institutions or four designated hospitals did not directly examine the LDL-C levels or Achilles tendon of the parents of the referred children from elementary school. The information on the parents, including their LDL-C levels and the family history of FH and premature CAD, was collected through interview or from their medical checkup data when parents brought them to the hospitals.

Genetic Testing

Among the children who were assigned to the four designated hospitals, 67 underwent genetic testing. Sequencing of the target genes was performed in Kanazawa University as previously reported¹¹. Briefly, the DNA extracted from peripheral white blood cells was pooled, selected for size, ligated to sequencing adapters, and amplified to enrich for target regions that were sequenced using the KAPA DNA Library Preparation. A custom NimbleGen in-solution DNA capture library (Roche NimbleGen Inc., Madison, WI) was designed to capture all coding exons in 21 dyslipidemia-related Mendelian genes, including three FH genes (*LDLR*, *PCSK9*, and *APOB*). The target coverage for each subject was more than 20-fold in more than 98% of all targeted exons.

Causative mutations for FH were determined in the *LDLR*, *PCSK9*, and *APOB* genes as previously reported¹¹. We defined a causative mutation for FH if it fulfilled any of the following criteria: a) rare protein-truncating mutations in the *LDLR* gene; b) rare damaging missense mutations in the *LDLR* gene, defined as those predicted as damaging by five *in silico* software programs (SIFT, Polyphen2-HDIV, Polyphen2-HVAR, MutationTaster-2, and LRT) as previously described¹¹; c) ClinVar-registered pathogenic or likely pathogenic mutations causing FH in *LDLR*, *PCSK9*, or *APOB*; or d) *PCSK9* p.Val4Ile and p.Glu32Lys mutations and *APOB* p.Arg3527Gln

mutation previously reported to cause FH in the Japanese population¹¹.

Statistical Analysis

Continuous variables, such as the patients' clinical characteristics, were compared using the Student's *t*-test, whereas categorical variables, such as sensitivity or specificity, were compared using the chi-squared test. The LDL-C levels measured at four designated hospitals were utilized for statistical analysis. Considering the results of the genetic testing as a reference, the sensitivity, specificity, and mutation detection rate were calculated. Further, 95% confidence intervals were based on binomial probabilities. The area under the curve (AUC) for predicting the presence of FH causative mutations was calculated by analyzing the receiver operating characteristic (ROC) curves. Descriptive statistics, such as means, standard deviation, and ranges, were analyzed for all variables. Statistical analyses were conducted using SPSS version 22.5 (SPSS Inc., Chicago, IL USA). A *P* value of <0.05 was considered statistically significant.

Ethical Considerations

The protocol was approved by the Ethics and Human Genome Committees at Kagawa University (Heisei 30-187), Shikoku Medical Center for Children and Adults, Mitoyo General Hospital, and Kagawa Prefectural Central Hospital. All procedures were performed in accordance with the ethical standards of the responsible committee on human experimentation and with the latest version of the Declaration of Helsinki. Written informed consent for the genetic testing of children was provided by at least one parent.

Results

Distribution of Pediatric LDL-C Levels in the Kagawa Prefecture, Japan

Fig. 2 presents the distribution of plasma LDL-C levels in 15,665 children who underwent lipid screening in 2018 and 2019. The 95 percentile value of the LDL-C level was 135 mg/dL, and the value of 140 mg/dL corresponded to the 96.3 percentile in this study. The LDL-C values of 160 and 180 mg/dL corresponded to the 99.0 and 99.7 percentile values, respectively.

Clinical and Biochemical Features of Children who Underwent Genetic Testing

Table 1 presents the clinical and biochemical features of 67 children who underwent genetic testing, along with their family history of FH and premature

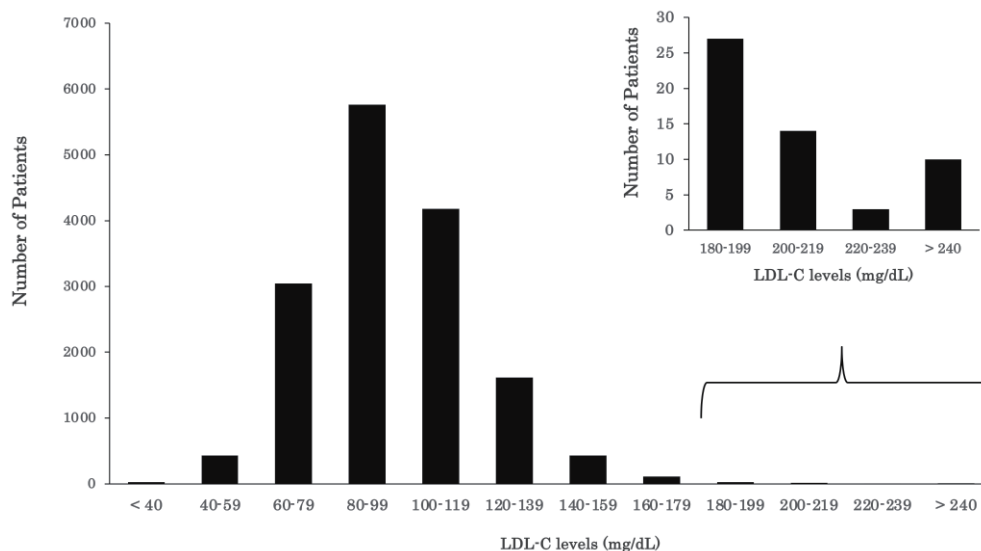


Fig. 2. Distribution of the LDL-C levels in children who underwent lipid screening in 2018 and 2019

The vertical axis in the small graph indicates the number of children who had LDL-C levels of ≥ 180 mg/dL. LDL-C, low-density lipoprotein cholesterol.

Table 1. Clinical and biochemical characteristics of children genetically tested

Characteristics	Patients <i>n</i> =67	mutant (+) <i>n</i> =41	mutant (-) <i>n</i> =26	<i>p</i> value mutant (+) vs. (-)
Age (IQR), y	10 (10-11)	10 (10-12)	10 (10-11)	0.810
Male sex, <i>n</i> (%)	33 (49)	21 (51)	12 (46)	0.686
Systolic BP (IQR), mmHg	96 (88-107)	97 (90-108)	95 (88-105)	0.562
Diastolic BP (IQR), mmHg	60 (55-66)	60 (55-66)	61 (50-66)	0.483
Height (IQR), cm	140.8 (133.1-149.0)	141.1 (133.0-151.0)	139.7 (135.0-147.5)	0.997
Weight (IQR), kg	33.3 (29.0-43.6)	32.0 (26.0-44.4)	36.2 (30.4-42.0)	0.498
POW (IQR), %	-2.2 (-11.1-5.7)	-5.2 (-12.2-2.1)	4.0 (-2.0-7.5)	0.032*
Cholesterol level (IQR), mg/dl				
LDL-cholesterol	178 (149-206)	184 (168-215)	152 (140-187)	0.005*
HDL-cholesterol	55 (50-65)	55 (50-65)	56 (51-63)	0.593
TG	74 (55-113)	74 (55-97)	77 (57-121)	0.895
Family history of FH, <i>n</i> (%)	10 (15)	8 (20)	2 (8)	0.294
Family history of premature CAD, <i>n</i> (%)	14 (21)	10 (24)	4 (15)	0.540
Parent's Xanthoma, <i>n</i> (%)	6 (9)	5 (12)	1 (4)	0.392
Parent's LDL-cholesterol ≥ 180 mg/dl, <i>n</i> (%)	36 (54)	26 (63)	10 (38)	0.078

CAD, coronary artery disease; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; POW: percentage of overweight; TG, Triglyceride; * $P < 0.05$ vs. mutant (-).

CAD and the prevalence of xanthoma and high parental LDL-C level (≥ 180 mg/dL). Among 67 children who underwent genetic testing, 41 had an FH causative mutation, and all of them had heterozygous one. In detail, 36 children had 19 mutations in *LDLR*, 4 children had 2 mutations in *PCSK9*, and 1 child had 1 mutation in *APOB* (**Supplemental Table 1**). The LDL-C levels of the children with an FH causative mutation were

significantly higher than those of children without it (184 versus 152 mg/dL, $P=0.005$). The POW was significantly lower in children with an FH causative mutation than in children without it (-5.2% versus 4.0%, $P=0.032$), although the POWs of both groups did not meet the criteria for obesity. No significant differences were observed in other clinical and biochemical features between children with and without a FH causative mutation (**Table 1**).

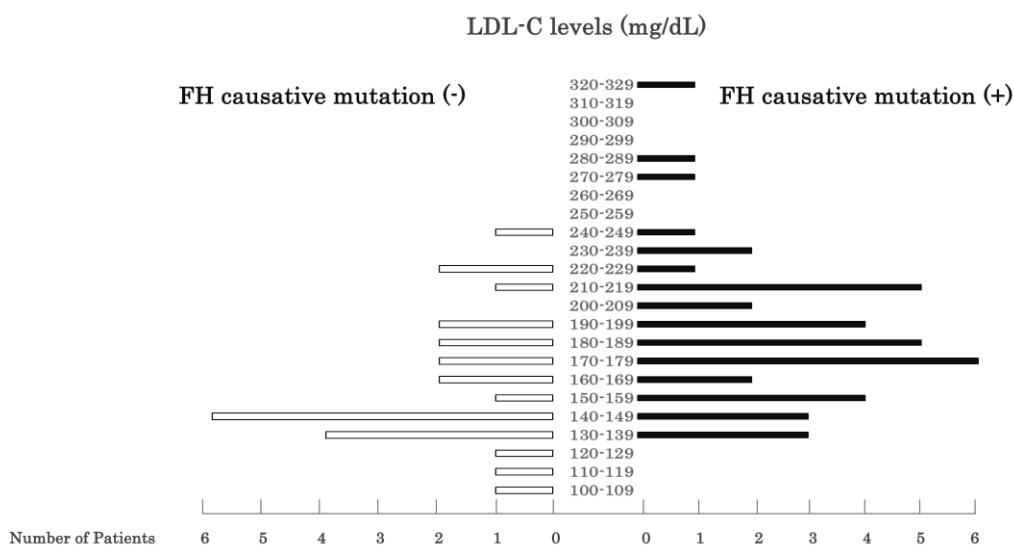


Fig. 3. Distribution of the plasma LDL-C levels in children with and without an FH mutation

Children with LDL-C levels of ≥ 140 mg/dL were divided into two groups according to the genetic testing result. The number of children is shown according to the LDL-C levels. Note that children without an FH mutation did not show LDL-C levels of ≥ 250 mg/dL. FH, familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol.

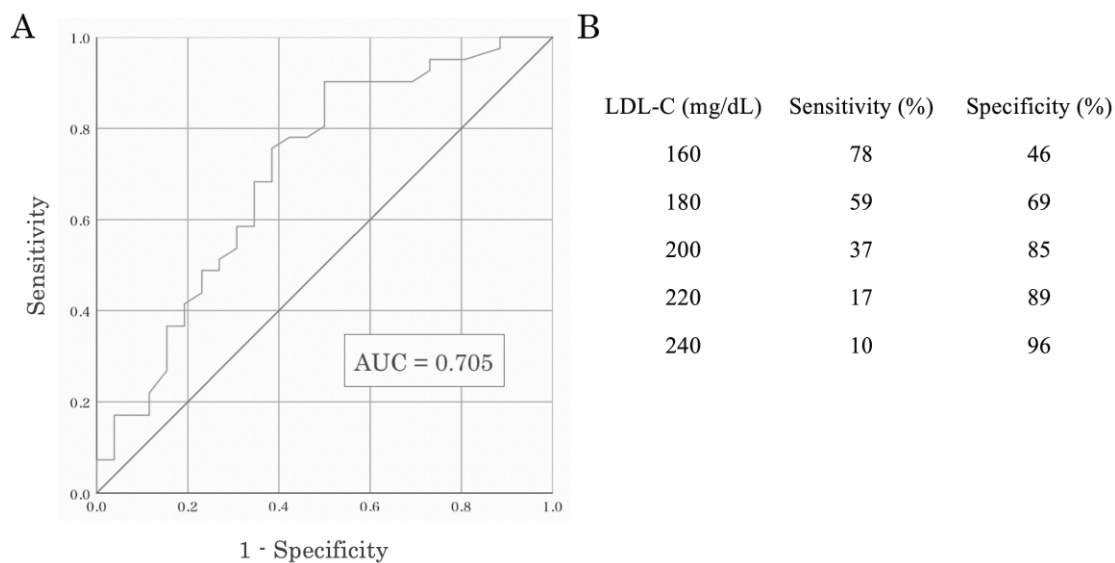


Fig. 4. ROC curve for plasma LDL-C levels for predicting the presence of FH mutation

A: ROC curve and AUC for LDL-C levels predicting the presence of causative FH mutations.

B: Sensitivities and specificities of representative LDL-C levels predicting the presence of causative FH mutation.

AUC: area under the curve; FH, familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol; ROC, receiver operating characteristic.

Fig. 3 presents the distribution of plasma LDL-C levels in 67 children who underwent genetic testing based on the presence and absence of an FH causative mutation. Although the children exhibited LDL-C levels of ≥ 140 mg/dL in Kagawa health checkups, ten demonstrated LDL-C levels of <140 mg/dL at the four designated hospitals. Notably, although the

number of children was small, all three children (male/female ratio: 1/2) with LDL-C levels of ≥ 250 mg/dL had FH causative mutations (**Fig. 3**).

Fig. 4A presents the ROC curve for plasma LDL-C levels for predicting the presence of an FH causative mutation. The AUC calculated by the ROC curve was 0.705 (0.572–0.838), indicating the low

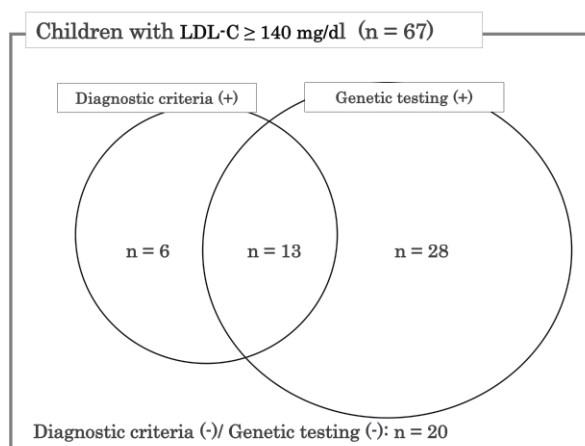


Fig. 5. Venn diagram presenting the number of children diagnosed with FH according to the Guidance for Pediatric FH 2017 and genetic testing

All the 67 children participating in this study were evaluated according to the clinical diagnostic criteria (Guidance for Pediatric FH 2017) and genetic testing. A total of 19 children were diagnosed with FH using the clinical diagnostic criteria, and 41 children had FH causative mutations. Among these children, 13 were positive in both the clinical diagnostic criteria and genetic testing. FH, familial hypercholesterolemia.

accuracy of using LDL-C levels for predicting FH causative mutations in children. The sensitivities and specificities of each LDL-C level are presented in [Fig. 4B](#).

Relation between the Current Clinical Diagnostic Criteria and FH Causative Mutations

[Fig. 5](#) presents a Venn diagram representing the number of children diagnosed with FH according to the clinical diagnostic criteria (Guidance for Pediatric FH 2017¹⁰) and genetic testing. Among the 67 children who demonstrated LDL-C levels of \geq 140 mg/dL and underwent the genetic testing, 19 met the clinical diagnostic criteria, 41 had FH causative mutations, and 13 fulfilled the clinical diagnostic criteria and had FH causative mutations ([Fig. 5](#)). Six children without FH causative mutations were clinically diagnosed with FH. Furthermore, 20 children with LDL-C levels of \geq 140 mg/dL did not meet the clinical diagnostic criteria and did not have FH causative mutations. According to the clinical diagnostic criteria (Guidance for Pediatric FH 2017), the sensitivity and specificity regarding FH causative mutations were 32% and 77%, respectively. Furthermore, we demonstrated the clinical and biochemical characteristics of 67 children who were divided into four groups based on the results of the clinical diagnostic criteria and genetic testing ([Supplemental Table 2](#)). No obvious clinical and biochemical characteristics to identify four groups could be observed. In a group negative for clinical diagnostic criteria and genetic testing, there was one

child who was diagnosed with sitosterolemia by examining the ABCG5/ABCG8 gene.

Discussion

This is the first study on the topic of universal screening for pediatric FH based on Kagawa health checkups for childhood lifestyle-related diseases prevention in Japan.

From 2012, Kagawa health checkups for the prevention of lifestyle-related diseases in children, including the measurement of LDL-C levels, is annually conducted in cooperation with the local government, elementary schools, and medical association in the Kagawa prefecture. In 2018, four designated hospitals, including Kagawa University Hospital, participated in this system focusing on the diagnosis and follow-up of FH. In addition to the performance of genetic testing at the four designated hospitals, we made and distributed the flyer about FH to the elementary schools and hosted a group study for elementary school nurses who play key roles in the introduction of children with high LDL-C levels to the local medical institutions/hospitals. Moreover, Kagawa pediatric association has recently made a comprehensive guidance program for doctors at the local medical institutions on how to follow-up the children who demonstrated abnormal values during the Kagawa health checkups. The tight cooperation among the local government, elementary schools, prefectural medical association, hospitals, and academia makes it possible to build up and polish the

systems to find and follow-up both the FH children and their parents/relatives.

To date, many universal lipid screening studies have been conducted to detect pediatric FH¹². The distribution of plasma LDL-C levels in pediatric health checkups in Kagawa is comparable to other universal lipid screening results^{13, 14} (Fig. 2). This indicates that our results are applicable to other regions in Japan and other countries. In this study, approximately 4% of children were found to have high plasma LDL-C levels (≥ 140 mg/dL) (Fig. 2). Among these children with high LDL-C levels, 67 underwent genetic testing for FH after excluding secondary hyper-LDL cholesterolemia. In addition, we found 41 patients who carried FH causative mutations (Fig. 5). Overall, 61% of the patients suspected with FH tested positive for FH causative mutations, a finding that is in agreement with those of other universal lipid screening studies^{15, 16}. The pediatric healthcare system for FH in Kagawa, Japan, is an effective universal lipid screening method.

In this study, we employed a panel sequencing approach focusing on genes associated with Mendelian lipid disorders. Although the definition of our causative mutation may not be perfect, most of the mutations found in this study have already been shown to be pathogenic in ClinVar. This study provides useful insights into the motivation for genetic testing of pediatric FH patients. First, genetic testing using panel sequencing can identify pediatric FH patients even when family history is not available. Second, patients with deleterious genetic variations other than the FH gene can be identified *via* panel sequencing, leading to their accurate diagnosis and risk stratification. Third, reverse cascade screening can be facilitated through the genetic testing of pediatric FH patients.

No significant difference was observed in the height and weight between the children with or without FH causative mutations. Although a significant difference was observed in the POW between children with and without an FH causative mutation, the POWs of these two groups were within the normal range (-20% – 20%) (Table 1)⁹. A high level of LDL-C in children with normal POW should be noted for FH diagnosis and treatment. Other clinical and biochemical characteristics did not differ between the children with and without an FH causative mutation, suggesting that it is difficult to detect pediatric FH using these characteristics.

Consistent with previous reports¹⁷, the LDL-C levels were high in children with FH causative mutations (Table 1), indicating that FH causative mutations can result in an increase in LDL-C levels.

Khera *et al.* reported an association between the presence of an FH mutation and a 50 mg/dL increase in LDL-C levels and a 3.8-fold (95% CI: 2.6 to 5.4) increase in the odds of CAD¹⁸. Therefore, early diagnosis and treatment are important to prevent premature CAD in children. Based on the results of genetic testing, we attempted to establish the threshold LDL-C level to diagnose pediatric FH. However, LDL-C level of 160 to 240 mg/dL did not demonstrate a precise correlation with FH causative mutations (Fig. 4). Among the 67 patients of this study, a remarkably high LDL-C level (≥ 250 mg/dL) was observed only in children with an FH causative mutation (Fig. 3). Therefore, it is likely that children with LDL-C levels of ≥ 250 mg/dL have FH causative mutations after excluding secondary hyper-LDL cholesterolemia.

Supplemental Table 2 presents the clinical and biochemical characteristics after 67 children were divided into four groups positive or negative in the clinical diagnostic criteria and genetic testing. However, other than the high LDL-C levels in children positive in the genetic testing and the high prevalence of family history and parent xanthoma in children positive in the clinical diagnostic criteria, we could not find any other differences in the clinical and biochemical characteristics among children in the four groups. Caution must be required when we see clinical characteristics of patients in Supplemental Table 2, because the number of children in this study is small and patient selection bias in the introduction to four designated hospitals from local medical institutes is highly possible.

In a study by Hori, the *LDLR* and *PCSK9* causative mutations were found in 46% and 7.8% of patients diagnosed with FH, respectively¹⁹. Compared with these ratios, our data provide the impression that the proportion of *LDLR* mutations is higher compared with the previous report. Although *LDLR*, *PCSK9*, and *APOB* have been proposed as the main FH causative genes, FH due to *LDLR* mutations may be associated with more typical FH symptoms in childhood. Importantly, we found the second Japanese case of a causative *APOB* gene mutation, namely, pGln3533AlafsTer16. The mutation is located outside the LDL-binding region and close to the first Japanese case^{11, 20}.

In addition, the present study suggests that even if the LDL-C level and diagnostic criteria are used, it may not be easy to detect FH causative mutations (Figs. 4, 5). The main reason may be attributable to the absence of a family history of FH, as the parents of pediatric FH patients are relatively young to suffer from CAD. Because genetic testing *via* panel

sequencing can identify pediatric FH patients, genetic testing may be a powerful method for diagnosing FH²¹⁾ in children whenever family history is not available.

When the children were diagnosed with FH, the pediatric doctors at the four designated hospitals started to treat them according to the Guidance for Pediatric FH 2017. The children treated with statins were mainly followed up at the four designated hospitals, and those who did not need statins but do need exercise or diet therapy were mainly followed up at local medical institutes. In addition, when the parents were diagnosed with FH *via* reverse cascade screening, the cardiologists corrected their LDL-C levels by intensive medical treatment and actively examined whether they have CAD. Indeed, we were able to diagnose many parents with FH who could not be diagnosed by the diagnostic criteria for adult FH and thus started to treat them. In the near future, we need to establish the systems to follow-up the children and their parents with the cooperation of pediatricians and cardiologists in order to efficiently strengthen the motivation of patients with FH to fight.

Limitations

In this study, although the primary health checkup rate was relatively high (90%–95%), the follow-up system was not well established. The second medical examination rate at the local medical facilities or at a designated hospital was only about 50%–70%, indicating that about half of the children with lifestyle-related diseases or propensities did not receive medical consultation and treatment. Indeed, according to the results of the Kagawa health checkups for the prevention of lifestyle-related diseases in children in 2018 and 2019, approximately 580 children with LDL-C levels of ≥ 140 mg/dL received the recommendation letter from elementary schools to visit the local medical institutes. However, no official data are available on the exact number of children who visited the local medical institutes, who were excluded due to secondary hypercholesterolemia, or who did not agree with further examinations, including genetic testing, at local medical institutes. With the cooperation of the local government and medical association in the Kagawa prefecture, we are now working on establishing the follow-up systems to obtain the exact number of children with LDL-C levels of ≥ 140 mg/dL who actually visit the local medical institutes and four designated hospitals.

Further improvement of the pediatric healthcare system in Kagawa requires the cooperation of all 17

local governments in the Kagawa prefecture, the Kagawa Medical Association, the Kagawa Pediatric Association, and the four designated hospitals in Kagawa.

Future Perspective

This healthcare system of Kagawa identified 41 children with FH causative mutations and six children clinically diagnosed with FH without FH causative mutations over a 2-year period. It was reported that for every 1000 children of 1 to 2 years of age who undergo a child–parent screening, 8 (4 parents along with 4 children) are positive for FH¹³⁾. Further improvement of the pediatric healthcare system in Kagawa would contribute to the reduction of CAD events in FH patients.

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Conflicts of Interest

Hayato Tada; Bayer Yakuin, Ltd, and Sanofi K.K.

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Supplemental Table 1. Causative mutations detected in 41 children among 67 children who received genetic testing

Gene	DNA	Amino acid	Number of children
LDLR	c. 301G>A	p. Glu101Lys	1
LDLR	c. 361T>C	p. Cys121Arg	1
LDLR	c. 682G>C	p. Glu228Gln	1
LDLR	c. 939C>A	p. Cys313Ter	1
LDLR	c. 967G>A	p. Gly323Ser	1
LDLR	c. 1056C>A	p. Cys352Ter	1
LDLR	c. 1201C>T	p. Leu401Phe	1
LDLR	c. 1207T>C	p. Phe403Leu	6
LDLR	c. 1252G>A	p. Glu418Lys	5
LDLR	c. 1502C>T	p. ALA501Val	1
LDLR	c. 1702C>G	p. Leu568Val	3
LDLR	c. 1705+1G>C		3
LDLR	c. 1706A>G	p. Asp569Gly	3
LDLR	c. 1747C>T	p. His583Tyr	1
LDLR	c. 1783C>T	p. Arg595Trp	3
LDLR	c. 1845+2T>C		1
LDLR	c. 2054C>T	p. Pro685Leu	1
LDLR	c. 2378T>C	p. Val763Ala	1
LDLR	c. 2579C>T	p. Ala860Val	1
APOB	c. 10596_10597insGCATT	p. Gln3533AlafsTer16	1
PCSK9	c. 94G>A	p. Glu32Lys	3
PCSK9	c. 161A>C	p. Glu54Ala	1

All of them were heterozygous.

Supplemental Table 2. Clinical and biochemical characteristics of 67 children who received genetic testing

Characteristics	Diagnostic criteria (-)/	Diagnostic criteria (+)/	Diagnostic criteria (-)/	Diagnostic criteria (+)/
	Genetic testing (-) <i>n</i> = 20	Genetic testing (-) <i>n</i> = 6	Genetic testing (+) <i>n</i> = 28	Genetic testing (+) <i>n</i> = 13
Age (IQR), y	10 (10-11)	11 (10-11)	10 (10-11)	12 (10-13)
Male sex, <i>n</i> (%)	8 (40)	4 (67)	12 (43)	9 (69)
Systolic BP (IQR), mmHg	92 (88-107)	96 (92-97)	96 (90-102)	105 (87-115)
Diastolic BP (IQR), mmHg	60 (50-66)	64 (53-66)	60 (55-66)	60 (56-69)
Height (IQR), cm	138.7 (135.0-148.0)	140.1 (137.7-145.8)	141.1 (135.4-145.8)	149.0 (130.0-155.3)
Weight (IQR), kg	36.5 (30.8-42.7)	33.3 (29.4-35.6)	31.5 (29.4-41.2)	32.6 (24.0-50.2)
POW (IQR), %	5.2 (-0.1-10.3)	-2.3 (-11.8-4.0)	-4.6 (-12.0-1.1)	-7.2 (-12.2-5.4)
Cholesterol level (IQR), mg/dl				
LDL-cholesterol	152 (141-189)	156 (120-176)	183 (163-215)	189 (171-214)
HDL-cholesterol	56 (52-64)	54 (49-56)	57 (50-67)	54 (48-57)
TG	77 (59-127)	74 (55-115)	75 (55-124)	67 (49-95)
Family history of FH, <i>n</i> (%)	0 (0)	2 (33)	0 (0)	8 (62)
Family history of premature CAD, <i>n</i> (%)	0 (0)	4 (67)	0 (0)	10 (77)
Parent's Xanthoma, <i>n</i> (%)	0 (0)	1 (17)	1 (4)	4 (31)
Parent's LDL-cholesterol ≥ 180 mg/dl, <i>n</i> (%)	6 (30)	4 (67)	16 (57)	10 (77)

Diagnostic criteria (+)/(-) and genetic testing (+)/(-) indicate positive or negative for clinical diagnostic criteria and genetic testing, respectively. IQR, interquartile range; POW: percentage of overweight; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, Triglyceride; CAD, coronary artery disease.