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ORIGINAL ARTICLE
Dyslipidemia

Utility of Discrepancies Between Friedewald and Martin Equations in Predicting Pathogenic Variants of Familial Hypercholesterolemia in Children

Ryosuke Tani, MD; Keiji Matsunaga, MD, PhD; Tomoko Inoue, MD; Katsufumi Nishioka, MD; Kanako Irie, MD, PhD; Sonoko Kondo, MD, PhD; Takashi Iwase, MD, PhD; Hai Ying Fu, MD, PhD; Shigeru Ito, MD, PhD; Tsuyoshi Sasaki, MD, PhD; Sumiko Yoshida, MD, PhD; Ichiro Yokota, MD, PhD; Yoichi Hoshikawa, MD, PhD; Katsunori Yokoyama, MD, PhD; Takuji Fujisawa, MD, PhD; Hayato Tada, MD, PhD, FJCS; Masayuki Takamura, MD, PhD, FJCS; Takashi Kusaka, MD, PhD; Tetsuo Minamino, MD, PhD, FJCS

Background: The Friedewald equation, commonly used to calculate low-density lipoprotein cholesterol (LDL-C), occasionally yields inaccurate estimations for very-low-density lipoprotein cholesterol, suggesting the need for an alternative method such as the Martin equation. In this study, we aimed to evaluate the discrepancies between the Friedewald and Martin equations, particularly in the context of pathogenic variants associated with familial hypercholesterolemia (FH).

Methods and Results: We evaluated the discrepancies between LDL-C levels calculated using the Friedewald and Martin equations, and for the presence of pathogenic variants of FH in 53 children with hypercholesterolemia detected through universal lipid screening. Genetic testing revealed pathogenic variants of FH in 24 of the 53 children. Chi-squared tests indicated a significant difference in the presence of pathogenic variants of FH between the "Friedewald ≥ Martin" and "Friedewald < Martin" groups (X^2=11.348, P<0.001). Even in 37 children with LDL-C <180 mg/dL, discrepancies between the equations were still associated with the presence of pathogenic FH variants (Fisher's exact test, P=0.028).

Conclusions: Discrepancies in LDL-C levels measured by the Friedewald and Martin equations might serve as a useful predictive marker for identifying pathogenic variants of FH, especially in cases of LDL-C <180 mg/dL, which are often challenging to diagnose.

Key Words: Familial hypercholesterolemia; Friedewald equation; Genetic testing; Martin equation

Familial hypercholesterolemia (FH) is an autosomal hereditary disorder affecting 1 in 300 individuals. Pathogenic variants in 4 FH-associated genes (low-density lipoprotein receptor (*LDLR*), apolipoprotein B-100 (*APOB*), proprotein convertase subtilisin/kexin 9 (*PCSK9*)), and the LDLR adaptor protein 1 gene (*LDLRAP1*), have been identified in 60–80% of clinically diagnosed children with FH.[1–3] These variants are associated with a higher risk of cardiovascular events, even among individuals with moderately elevated levels of low-density lipoprotein cholesterol (LDL-C: 130–189 mg/dL).[4] Diagnosing FH using clinical criteria alone can be challenging in children with LDL-C <180 mg/dL; therefore, genetic testing is recommended for confirmation.[5,6] Although genetic testing is crucial for FH diagnosis and management, there are substantial barriers to its integration into standard clinical practice. Consequently, there is a strong demand for predictive markers that can identify pathogenic variants of FH.

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Department of Cardiorenal and Cerebrovascular Medicine (R.T., K.M., T. Inoue, T.M.), Department of Pediatrics (K.N., K.I., S.K., T. Iwase, T.K.), Faculty of Medicine, Kagawa University, Kagawa; Department of Biochemistry, Hyogo Medical University, Hyogo (H.Y.F.); Department of Pediatrics, Kagawa Prefectural Central Hospital, Kagawa (S.I.); Department of Pediatrics, Mitoyo General Hospital, Kagawa (T.S.); Department of Clinical Research, National Hospital Organization Shikoku Medical Center for Children and Adults, Kagawa (S.Y.); Department of Pediatrics, Division of Pediatric Endocrinology and Metabolism, National Hospital Organization Shikoku Medical Center for Children and Adults, Kagawa (I.Y.); Department of Health and Welfare, Kagawa Prefectural Government, Kagawa (Y.H., K.Y.); Fujisawa Pediatric Clinic, Kagawa (T.F.); and Department of Cardiovascular Medicine, Graduate School of Medical Sciences, Kanazawa University, Ishikawa (H.T., M.T.), Japan

Mailing address: Tetsuo Minamino, MD, PhD, FJCS, Department of Cardiorenal and Cerebrovascular Medicine, Faculty of Medicine, Kagawa University, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan. email: minamino.tetsuo.gk@kagawa-u.ac.jp

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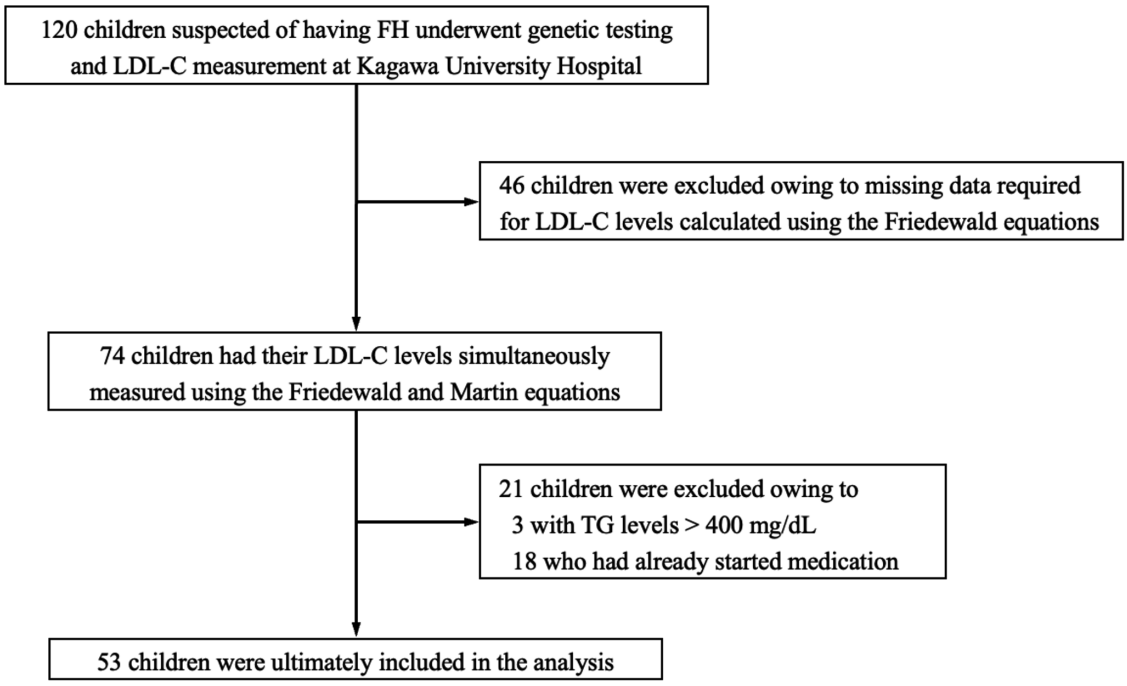

Figure. Flowchart of the inclusion and exclusion criteria for the study population.

The Friedewald equation utilizes a fixed factor of 5 for the ratio of triglyceride (TG) to very low-density lipoprotein cholesterol (VLDL-C).[7] However, because this ratio can vary across different TG and cholesterol levels, the Martin method has been proposed as an alternative.[8] VLDL-C levels are elevated in FH,[9] suggesting that LDL-C levels calculated using the Friedewald equation will be higher than those calculated using the Martin equation. Therefore, in this study we aimed to evaluate the discrepancies between the Friedewald and Martin equations, particularly in the context of pathogenic variants associated with FH.

Methods

Study Population

Children aged 9 or 10 years with LDL-C ≥140 mg/dL, identified during primary school health screenings, were advised to consult their primary care physicians or local medical facilities, following the guidelines of the "Kagawa health checkups for preventing lifestyle-related diseases in children" by the Kagawa Pediatric Association. Children with LDL-C ≥200 mg/dL were directly referred to 1 of 4 designated hospitals: Kagawa University Hospital, Shikoku Medical Centre for Children and Adults, Mitoyo General Hospital, and Kagawa Prefectural Central Hospital. For children with LDL-C levels between 140 and 199 mg/dL, further evaluation was conducted to detect potential secondary causes of lipid abnormalities, such as obesity, diabetes, thyroid disorders, nephrotic syndrome, and cholestatic liver disease. Additionally, family histories of FH and premature coronary artery disease (CAD) were examined. If no secondary cause was identified and FH was suspected, the child was also referred to 1 of the 4 designated hospitals. The screening program was later expanded to other prefectures, with Chutoen General Medical Centre as a collaborative research institution. This retrospective, single-center observational study enrolled 120 consecutive children from local medical facilities suspected of having FH who underwent both genetic testing and LDL-C measurement at Kagawa University Hospital between January 2018 and May 2024 (**Figure**). Of them, 46 were excluded because of missing data required for LDL-C levels calculated using the Friedewald equation; the other 74 had their LDL-C levels measured using both equations at the first follow-up. Of them, 3 had TG levels >400 mg/dL, and 18 had already started medication, resulting in their exclusion. Consequently, 53 children were ultimately included in the analysis.

Collection of Clinical Data

We collected clinical data, including age, sex, height, body weight, percentage of overweight, heart rate, and blood pressure. In addition, we collected their lipoprotein profiles, including total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol (HDL-C), TG, and non-HDL-C levels under non-fasting conditions during the first follow-up. Automated instrumentation (TBA-2000FR and TBA-FX8, Canon Medical Systems) was used to determine the serum concentrations of TC, TG, and HDL-C based on their respective assays. The Friedewald and Martin equations were used to calculate serum LDL-C concentrations [TC − HDL-C − TG/5 (Friedewald) and TC − HDL-C − TG/adjustable factor (Martin)].[7,8] Serum non-HDL-C concentrations were determined by subtracting the HDL-C level from the TC level.

Table 1. Characteristics of the Study Participants

	All (n=53)	PVs of FH (+) (n=24)	PVs of FH (–) (n=29)	P value
Age (years)	10 [10–11]	10 [10–11]	10 [10–11]	0.366
Male sex (%)	27 (51.0)	13 (54.2)	14 (48.3)	0.669
Height (cm)	141.4±8.8	142.2±11.8	141.1±7.0	0.364
Body weight (kg)	38.2±9.3	38.6±10.0	38.1±9.0	0.872
POW (%)	7.2 [–3.8 to 12.8]	0.6 [–5.7 to 11.7]	7.7 [–2.0 to 15.3]	0.473
Heart rate (beats/min)	86±14	86±19	85±12	0.816
Systolic BP (mmHg)	97 [90–106]	99 [95–106]	96 [90–105]	0.377
Diastolic BP (mmHg)	62 [54–67]	65 [58–68]	57 [53–64]	0.520
TC (mg/dL)	237 [210–257]	257 [237–333]	217 [206–245]	<0.001
Friedewald LDL-C (mg/dL)	153 [130–183]	187 [158–262]	139 [124–153]	<0.001
Martin LDL-C (mg/dL)	151 [132–184]	186 [156–259]	140 [126–159]	<0.001
HDL-C (mg/dL)	54 [49–65]	60 [52–67]	52 [45–65]	0.113
TG (mg/dL)	89 [55–129]	73 [48–89]	121 [79–175]	0.001
Non-HDL-C (mg/dL)	178 [158–202]	202 [174–279]	162 [150–180]	<0.001

Unless indicated otherwise, data are presented as the mean±standard deviation, median [interquartile range], or n (%). BP, blood pressure; FH, familial hypercholesterolemia; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Non-HDL-C, non-high-density lipoprotein cholesterol; POW, percentage of overweight; PVs, pathogenic variants; TC, total cholesterol; TG, triglycerides.

Genetic Analysis

Genetic analysis at Kanazawa University utilized a next-generation sequencing platform to examine the children's genotypes.[10] *LDLR* (NM_000527.5), *APOB* (NM_000384.3), *PCSK9* (NM_174936.4), and *LDLRAP1* (NM_015627.3) coding regions were sequenced, as documented previously. We also evaluated copy number variations at the *LDLR* locus using the eXome Hidden Markov Model.[11] Adhering to the American College of Medical Genetics and Genomics guidelines, the pathogenic variant of FH was determined during multidisciplinary meetings involving genetics specialists.

Ethical Considerations

The Ethics Committee of Kagawa University approved this study (H30-059). All procedures were conducted following the ethical standards of the Human Research Committee (institutional and national) and the Declaration of Helsinki (1975, revised in 2008). Written informed consent for the genetic testing of the children was given by at least 1 parent.

Statistical Analysis

Categorical data are presented as percentages. For normally distributed continuous variables, the mean±standard deviation is reported. Non-normally distributed continuous variables are expressed as medians with interquartile ranges (IQR). The mean levels of continuous variables were compared using Student's t-test, and median levels were compared using the Wilcoxon Mann-Whitney rank-sum test. We evaluated the presence of pathogenic variants of FH based on LDL-C levels and discrepancies in LDL-C levels calculated using the Friedewald and Martin equations, which were tested using the chi-squared test. Fisher's exact test was used when >20% of the expected cell frequencies were <5. In addition, sensitivity and specificity were evaluated simultaneously. The Wilcoxon matched-pairs signed-rank test was performed to assess paired differences in LDL-C levels calculated using the Friedewald and Martin equations. Statistical analyses were conducted using IBM SPSS Statistics version 28 (IBM Corp., Armonk, NY, USA), with significance set at $P<0.05$.

Results

Clinical Characteristics

The clinical characteristics of 53 children are shown in **Table 1**. The median [IQR] age was 10 [10–11] years, with 27 of 53 (51.0%) children being male. The median [IQR] LDL-C level using the Friedewald equation was 153 [130–183] mg/dL and with the Martin equation was 151 [132–184] mg/dL. The results of genetic testing for pathogenic variants of FH in the children are presented in **Table 2**. Pathogenic variants were identified in 24 of the 53 children, and when the children were subsequently categorized into 2 groups based on the presence of these variants, significant differences were observed in all serum lipid parameters except for HDL-C. Pathogenic variants in *LDLR* were identified in 22 individuals, of whom 3 exhibited a copy number variation at the *LDLR* locus.[12] Furthermore, 2 had pathogenic variants in *PCSK9*. All identified variants were heterozygous.

Association Between Pathogenic Variants of FH, LDL-C Levels, and Discrepancies in LDL-C Calculated With the Friedewald and Martin Equations

Using the 2 equations, 36 children had higher LDL-C levels with the Friedewald equation (Friedewald ≥ Martin), and 17 had higher levels with the Martin equation (Friedewald < Martin) (**Table 3**). Of the 36 children in the "Friedewald ≥ Martin" group, pathogenic variants of FH were identified in 22. Among the 17 children in the "Friedewald < Martin" group, 2 were identified with pathogenic variants of FH. Chi-squared tests indicated a significant difference in the presence of pathogenic variants of FH between the "Friedewald ≥ Martin" and "Friedewald < Martin" groups ($\chi^2=11.348$, $P<0.001$). In the "Friedewald ≥ Martin" group, children with LDL-C ≥180 mg/dL had a significantly higher prevalence of pathogenic variants of FH compared

Table 2. Pathogenic Variants of FH in Children Who Underwent Genetic Testing

Gene / Region	Variant type	No. of children	ACMG	Judgment
LDLR				
c.301G>A	Missense	1	PM1/PP2/PP3/PP4/PP5 Likely pathogenic	Pathogenic
c.401G>A	Missense	1	PM1/PM2/PM5/PP3 Likely pathogenic	Likely pathogenic
c.682G>C	Missense	1	PM1/PM2/PP1/PP3/PP5 Likely pathogenic	Pathogenic
c.967G>A	Missense	1	PM2/PM5/PP2/PP3 Likely pathogenic	Likely pathogenic
c.1187-10G>A	Splice-site	1	PP3/PP4/PS3 Likely pathogenic	Pathogenic
c.1207T>C	Missense	5	PM1/PM2/PM5/PP3 Likely pathogenic	Pathogenic
c.1702C>G	Missense	3	PM2/PP1/PP3/PP5 Likely pathogenic	Pathogenic
c.1705+1G>C	Splice-site	3	PVS1/PM2/PM4/PP4 Pathogenic	Pathogenic
c.1783C>T	Missense	1	PM1/PM2/PP3/PP4 Likely pathogenic	Pathogenic
c.1845+2T>C	Missense	1	PVS1/PM2/PS4/PP5 Pathogenic	Pathogenic
c.2579C>T	Missense	1	PVS1/PM2/PM4/PP4 Pathogenic	Pathogenic
exons 2–3	Deletion	1	Copy number variation	Pathogenic
exons 7–14	Deletion	2	Copy number variation	Pathogenic
PCSK9				
c.94G>A	Missense	2	PS1/PS3/PP4/PP5 Pathogenic	Pathogenic

All children were heterozygous variants. ACMG, American College of Medical Genetics and Genomics; FH, familial hypercholesterolemia; *LDLR*, low-density lipoprotein receptor; *PCSK9*, proprotein convertase subtilisin/kexin type 9.

with those with LDL-C <180 mg/dL (χ^2=9.715, P=0.002). However, in the "Friedewald < Martin" group, the prevalence of pathogenic variants did not differ significantly between children with LDL-C ≥180 mg/dL and those with LDL-C <180 mg/dL (Fisher's exact test, P=0.228).

Of the 53 children, 16 had LDL-C ≥180 mg/dL and 37 had LDL-C <180 mg/dL (**Table 4**). Chi-squared tests indicated a significant difference in the presence of pathogenic variants of FH between the LDL-C ≥180 mg/dL and LDL-C <180 mg/dL groups (χ^2=16.486, P<0.001). Among children with LDL-C ≥180 mg/dL, the presence of pathogenic variants was not significantly different between the "Friedewald ≥ Martin" and "Friedewald < Martin" groups (Fisher's exact test, P=0.242). However, among children with LDL-C <180 mg/dL, pathogenic variants were more prevalent in the "Friedewald ≥ Martin" group than in the "Friedewald < Martin" group, with a significant difference (Fisher's exact test, P=0.028). The Wilcoxon Mann-Whitney rank-sum test showed no significant differences in LDL-C levels calculated using the Friedewald and Martin equations (53 cases, P=0.975). Similarly, no significant differences were observed in those with pathogenic variants of FH (24 cases, P=0.726) or in those without (29 cases, P=0.780). When the Wilcoxon matched-pairs signed-rank test was used, a significant difference was observed between LDL-C levels calculated using both equations for the cases of children with pathogenic variants (P<0.001), but no significant difference was observed for children without pathogenic variants (P=0.316).

Discussion

In this study, we evaluated the discrepancies between levels of LDL-C measured by the Friedewald and Martin equations, as well as the presence of pathogenic variants of FH in 53 children with hypercholesterolemia detected through universal lipid screening. There were 2 main findings: (1) discrepancies in the LDL-C levels between the Friedewald and Martin equations could serve as a predictive marker for identifying the presence of pathogenic variants of FH and (2) even in children with LDL-C <180 mg/dL, discrepancies between the results with these equations could similarly identify the presence of pathogenic variants of FH, which are often challenging to diagnose.

The Friedewald equation utilizes a fixed factor of 5 for the TG to VLDL-C ratio,[7] but this ratio can vary across different TG and cholesterol levels. Tada H et al. revealed that the cholesterol levels in TG-rich lipoproteins, including VLDL-C, are significantly elevated in FH.[9] Consequently, the Friedewald equation tends to underestimate VLDL-C levels in FH, resulting in LDL-C levels calculated using this method being higher than the actual values. In con-

Table 3. Distribution of Discrepancies in LDL-C Levels Calculated With the Friedewald and Martin Equations, as Well as the Presence of Pathogenic Variants of FH

	PVs of FH (+)	PVs of FH (−)	Sensitivity (%)	Specificity (%)	χ^2 value	P value
All children	24	29				
Friedewald ≥ Martin	22	14	91.7	51.7	11.348	<0.001
Friedewald < Martin	2	15				
Friedewald ≥ Martin	22	14				
LDL-C ≥180 mg/dL	13	1	59.1	92.9	9.715	0.002
LDL-C <180 mg/dL	9	13				
Friedewald < Martin	2	15				
LDL-C ≥180 mg/dL	1	1	50.0	93.3		0.228
LDL-C <180 mg/dL	1	14				

Friedewald ≥ Martin: LDL-C levels calculated using the Friedewald equation were higher than those calculated using the Martin equation. Friedewald < Martin: LDL-C levels were lower using the Friedewald equation compared with the Martin equation. Abbreviations as in Table 1.

Table 4. Distribution of LDL-C Levels and the Presence of Pathogenic Variants of FH

	PVs of FH (+)	PVs of FH (−)	Sensitivity (%)	Specificity (%)	χ^2 value	P value
All children	24	29				
LDL-C ≥180 mg/dL	14	2	58.3	93.1	16.486	<0.001
LDL-C <180 mg/dL	10	27				
LDL-C ≥180 mg/dL	14	2				
Friedewald ≥ Martin	13	1	92.9	50.0		0.242
Friedewald < Martin	1	1				
LDL-C <180 mg/dL	10	27				
Friedewald ≥ Martin	9	13	90.0	51.9		0.028
Friedewald < Martin	1	14				

Friedewald ≥ Martin: LDL-C levels calculated using the Friedewald equation were higher than those calculated using the Martin equation. Friedewald < Martin: LDL-C levels were lower using the Friedewald equation compared with the Martin equation. Abbreviations as in Table 1.

trast, the Martin equation adjusts for VLDL-C based on TG levels and non-HDL-C, resulting in lower LDL-C estimates compared with the Friedewald equation.[13,14] In this study, of the 24 children with pathogenic variants of FH, 22 were in the "Friedewald ≥ Martin" group.

In adults, VLDL-C levels often increase because of conditions such as diabetes mellitus or obesity, making accurate assessment more challenging.[15,16] A similar challenge in VLDL-C estimation also arises when lipid-lowering medications are used.[17] In contrast, the benefits of using this calculation method were more apparent in the studied children, who typically have fewer complicating factors and do not take these medications.

We emphasize that this method can be performed using only the parameters available from standard biochemical assays. Furthermore, if the Friedewald equation has been calculated, the Martin equation can be applied without additional testing or extra cost. Having this alternative method of calculation is important for when the appropriateness of genetic testing for children is uncertain, and may facilitate early consultation with specialists. We believe it can be extremely effective for early diagnosis of hidden FH.[18]

Discrepancies in LDL-C Levels Calculated With the Friedewald and Martin Equations, When LDL-C Levels <180 mg/dL

Of the 37 children with LDL-C levels <180 mg/dL, 10 carried pathogenic variants. Notably, 9 of them were in the "Friedewald ≥ Martin" group. Even in children with LDL-C <180 mg/dL, discrepancies between the results of the equations might be useful predictive markers of the presence of pathogenic variants of FH.

In children with LDL-C levels <180 mg/dL, a family history of FH, parental LDL-C levels, and premature CAD are strong indicators for FH diagnosis;[19] however, a lack of family history does not rule out the possibility of FH in children. Furthermore, diagnosing FH in children in whom phenotypic expression is not yet evident is challenging; therefore, continuous monitoring is recommended for early diagnosis and treatment of FH.[20–22] Early diagnosis of FH in children can facilitate the detection of FH in parents before the onset of CAD. Therefore, to protect families from FH, genetic testing should not be delayed, as it enables early diagnosis of FH. The results from this study indicated that even in children with LDL-C levels <180 mg/dL, discrepancies between the results for the Friedewald and Martin equations can support the use of genetic testing and thus help identify FH in children.

Clinical Implications

In children with LDL-C levels ≥180 mg/dL and suspected or confirmed FH, genetic testing should be incorporated into risk stratification. In addition, even if LDL-C levels

are <180 mg/dL and FH is suspected, genetic testing should be considered. Genetic testing is particularly recommended in children who fall into the "Friedewald ≥ Martin" group.

In this study, 16 children had LDL-C levels ≥180 mg/dL, as calculated using the Friedewald equation; 14 of them had pathogenic variants of FH. LDL-C 180 mg/dL corresponds to the 99.7 percentile in children, reflecting the prevalence of FH and necessitating aggressive medication therapy.[5] In addition, considering the possibility of persistently high LDL-C levels from childhood in their parents, cumulative LDL-C levels should be calculated using appropriate methods. Coronary assessments, such as coronary computed tomography, which has a high negative predictive level and resolution, should be considered more aggressively.[23–26] For children with LDL-C levels <180 mg/dL, consistent and consecutive follow-up or genetic testing should be conducted as necessary to diagnose FH. Moreover, when considering the risk of arteriosclerosis in parents, physicians should not hesitate to use genetic testing for early diagnosis, especially if in the "Friedewald ≥ Martin" group. Despite their utility as noninvasive methods, the time-consuming calculations involved highlight the need for applications that facilitate easier and more efficient to use. Such improvements would contribute to overcoming the clinical inertia of FH diagnosis.

Study Limitations

First, the study relied on retrospective data from a single center within a distinct geographic region and included a small sample size. This limitation could potentially restrict the generalizability of the findings to broader populations, as the unique characteristics of the local area and limited number of participants may not accurately reflect all populations. Among children with LDL-C ≥180 mg/dL, the presence of pathogenic variants of FH was not significantly different between the "Friedewald ≥ Martin" and "Friedewald < Martin" groups, likely owing to the small sample size. Second, the study did not account for potential differences between the testing kits used for data collection. Variability in the sensitivity and specificity of testing kits could introduce bias, affecting the reliability of the conclusions. Third, the TG levels in children without pathogenic variants of FH were higher than those in children with pathogenic variants. In this study, the groups without pathogenic variants of FH may have included individuals with polygenic hypercholesterolemia, which could explain the elevated TG levels observed in this group. Polygenic hypercholesterolemia has distinct metabolic characteristics compared with monogenic FH, with a reported association with higher TG levels.[27–29] Because a detailed analyses of polygenic hypercholesterolemia was not conducted in this study, future studies should further investigate the genetic factors associated with polygenic hypercholesterolemia. In addition, because TG positively correlates with non-HDL-C,[30] its effect on the adjustable factor, as determined by the Martin method, appeared to be minimal. Nonetheless, we cannot exclude that postprandial hypertriglyceridemia may influence the adjustable factor. Fourth, the distribution of pathogenic variants of FH has been reported to vary significantly by region, with certain mutations being more prevalent in specific geographic areas.[31–32] This study found a distribution similar to that reported for Japan, where the prevalence of *APOB* variants is lower, and *PCSK9* variants are more common compared with other regions worldwide.[33] Discrepancies between the Friedewald and Martin equations cannot be ruled out for each pathogenic variant of FH, particularly given the small sample size, which included only 2 cases of *PCSK9* variants. This limitation highlights the need for further investigation to better understand the effect of these variants. In total, these limitations highlight the importance of cautiously interpreting the outcomes of the study, and suggest improvements in future studies.

Conclusions

Discrepancies in LDL-C levels measured with the Friedewald and Martin equations could serve as a useful predictive marker of the presence of pathogenic variants of FH, especially in cases of LDL-C <180 mg/dL where it is often challenging to diagnose.

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Disclosures

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Ethical Approval

The Ethics Committee of Kagawa University (H30-059) approved this study.

Data Availability

The raw data and analysis results generated during this study will be made available. The study protocol and derived data supporting the findings of this study will also be accessible. Data will become available immediately upon publication of the manuscript and remain accessible for 5 years. Researchers can request the data from the corresponding author (T.M.) for any type of analysis. The data will be provided as Excel files and shared via email.

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