Tani *et al. Rare Dis Orphan Drugs J.* 2025;4:11
DOI: 10.20517/rdodj.2024.50

Rare Disease and Orphan Drugs Journal

Original Article

Open Access

Check for updates

Potential barriers to reverse cascade screening combined with universal cholesterol screening for paediatric familial hypercholesterolemia: focus on second-degree relatives of a proband

Ryosuke Tani[1], Keiji Matsunaga[1], Tomoko Inoue[1], Katsufumi Nishioka[2], Kanako Irie[2], Sonoko Kondo[2], Takashi Iwase[2], Hai Ying Fu[3], Shigeru Ito[4], Tsuyoshi Sasaki[5], Sumiko Yoshida[6], Ichiro Yokota[7], Yoichi Hoshikawa[8], Katsunori Yokoyama[8], Takuji Fujisawa[9], Hayato Tada[10], Masayuki Takamura[10], Takashi Kusaka[2], Tetsuo Minamino[1]

[1]Department of Cardiorenal and Cerebrovascular Medicine, Faculty of Medicine, Kagawa University, Miki, Kagawa 761-0793, Japan.
[2]Department of Paediatrics, Faculty of Medicine, Kagawa University, Miki, Kagawa 761-0793, Japan.
[3]Department of Biochemistry, Hyogo Medical University, Nishinomiya 663-8501, Japan.
[4]Department of Paediatrics, Kagawa Prefectural Central Hospital, Takamatsu 760-8557, Japan.
[5]Department of Paediatrics, Mitoyo General Hospital, Mitoyo 769-1695, Japan.
[6]Department of Clinical Research, National Hospital Organization Shikoku Medical Centre for Children and Adults, Zentsuji 765-8507, Japan.
[7]Department of Paediatrics, Division of Paediatric Endocrinology and Metabolism, National Hospital Organization Shikoku Medical Center for Children and Adults, Zentsuji 765-8507, Japan.
[8]Department of Health and Welfare, Kagawa Prefectural Government, Takamatsu 760-8570, Japan.
[9]Fujisawa Paediatric Clinic, Takamatsu 761-8043, Japan.
[10]Department of Cardiovascular Medicine, Graduate School of Medical Sciences, Kanazawa University, Kanazawa 920-8640, Japan.

Correspondence to: Dr. Tetsuo Minamino, Department of Cardiorenal and Cerebrovascular Medicine, Faculty of Medicine, Kagawa University, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan. E-mail: minamino.tetsuo.gk@kagawa-u.ac.jp

How to cite this article: Tani R, Matsunaga K, Inoue T, Nishioka K, Irie K, Kondo S, Iwase T, Fu HY, Ito S, Sasaki T, Yoshida S, Yokota I, Hoshikawa Y, Yokoyama K, Fujisawa T, Tada H, Takamura M, Kusaka T, Minamino T. Potential barriers to reverse cascade screening combined with universal cholesterol screening for paediatric familial hypercholesterolemia: focus on second-degree relatives of a proband. *Rare Dis Orphan Drugs J.* 2025;4:11. https://dx.doi.org/10.20517/rdodj.2024.50

Received: 26 Nov 2024 **First Decision:** 8 Apr 2025 **Revised:** 21 Apr 2025 **Accepted:** 6 May 2025 **Published:** 9 May 2025

Academic Editor: Daniel Scherman **Copy Editor:** Ping Zhang **Production Editor:** Ping Zhang

Abstract

Aim: Familial hypercholesterolaemia (FH), an autosomal-dominant disorder, requires early diagnosis to prevent atherosclerosis in children and coronary artery disease (CAD) in their parents. This study aimed to evaluate the

effectiveness and barriers of reverse cascade screening (RCS) combined with universal cholesterol screening (UCS) for paediatric FH.

Methods: We performed RCS combined with UCS for paediatric FH between January 2018 and July 2023. Family pedigree was evaluated in second-degree relatives, using a child with a genetic diagnosis of FH as the proband. Based on the 2022 Japan Atherosclerosis Society clinical guidelines, cases with suspected FH were classified into four categories: "Definite", "Probable", "Possible", or "Unlikely". Those who did not complete the diagnostic process were evaluated based on the reasons, including challenges such as "Untested lipids in children", "Bereavement", "CAD with unspecified details", "Dyslipidaemia with unspecified low-density lipoprotein cholesterol (LDL-C) levels", and "No information on CAD or dyslipidaemia".

Results: Of 252 patients suspected of having FH, 94 completed the diagnostic process. Among them, 49 were classified as "Definite" FH cases, predominantly among first-degree relatives. In contrast, 158 patients did not complete the diagnostic process, with the most common barriers being "Dyslipidaemia with unspecified LDL-C levels" and "No information on CAD or dyslipidaemia", particularly among second-degree relatives.

Conclusion: RCS combined with UCS for paediatric FH using genetic testing is effective for the early diagnosis of FH in asymptomatic cases. However, addressing barriers that hinder FH diagnosis, such as difficulties in approaching second-degree relatives, is critical for improving diagnosis rates.

Keywords: familial hypercholesterolemia, reverse cascade screening, universal cholesterol screening, low-density lipoprotein cholesterol, genetic testing, cardiovascular disease, paediatric screening

INTRODUCTION

Familial hypercholesterolaemia (FH) is a common autosomal dominant genetic disorder that affects approximately 1 in 300 individuals globally[1]. It is characterised by elevated low-density lipoprotein cholesterol (LDL-C) levels from birth, which leads to an increased risk of premature coronary artery disease (CAD) if left untreated[2]. Despite the significant CAD events associated with FH, global diagnosis rates remain alarmingly low[3]. Therefore, the early diagnosis and treatment of FH are crucial to reduce CAD.

Screening strategies for FH include universal cholesterol screening (UCS), cascade screening, and opportunistic screening, which are widely recommended to enhance early detection rates[4-6]. Although atherosclerosis develops early, most cases of paediatric FH are asymptomatic[7]. Therefore, UCS is essential for detecting patients with paediatric FH who could benefit from early medication. Moreover, UCS provides an opportunity to identify affected family members through reverse cascade screening (RCS), a process that involves testing relatives with clinically or genetically confirmed FH using biochemical and/or genetic testing[8]. RCS is particularly beneficial because it allows the identification of cases with FH before the onset of clinical symptoms or CAD, facilitates the diagnosis of affected relatives who may not have been tested, and enables earlier intervention, improving health outcomes and reducing healthcare costs for both individuals and society[8,9].

In Kagawa Prefecture, Japan, RCS is combined with UCS, covering more than 7,000 children aged 9-10 years annually[10,11]. Children with LDL-C levels ≥ 140 mg/dL are further evaluated, and if other causes are ruled out, they are referred for genetic testing to confirm FH[10]. Over 450 cases of FH have been identified using this approach, demonstrating the potential of RCS as an effective tool for the diagnosis of FH within families. However, although this approach has been successfully implemented in several countries, it remains underutilised globally, and RCS is relatively understudied.

Therefore, the present study aimed to evaluate the effectiveness and barriers to RCS combined with UCS for paediatric FH. Using genetic testing, this study ultimately demonstrated that RCS combined with UCS for paediatric FH is effective for the early diagnosis of FH in asymptomatic cases.

METHODS

Study population

This single-centre retrospective cohort observational study was conducted at Kagawa University between January 2018 and July 2023. We implemented RCS using genetically confirmed FH as a proband through the "Kagawa health checkups for preventing lifestyle-related diseases in children"[10]. Data were collected using the electronic medical record system of Kagawa University. Family pedigree was evaluated in second-degree relatives, using a child with a genetic diagnosis of FH as the proband. Relatives suspected of having FH were classified into two groups: those who completed the FH diagnostic process based on the flow chart of the 2022 Japan Atherosclerosis Society (JAS) clinical guidelines and those who did not complete the FH diagnostic process based on these guidelines[12,13]. Among those who completed the process, cases were further classified into the following four categories based on the 2022 JAS guidelines[12,13]: "Definite", "Probable", "Possible", or "Unlikely". These guidelines provide distinct diagnostic algorithms for adults and children, which are shown in Supplementary Figures 1 and 2, respectively. For adults, "Definite" is diagnosed when two or more of the following are present: elevated LDL-C (≥ 180 mg/dL), presence of tendon or cutaneous xanthomas, and a family history of FH or CAD in first-degree relatives. Even if fewer than two criteria are met, a diagnosis of "Probable" can be made when LDL-C is ≥ 250 mg/dL, or when two criteria are met with LDL-C ≥ 160 mg/dL. If only one criterion is met and there is a family history of elevated LDL-C or CAD, the diagnosis is considered "Possible". Cases not meeting any of these conditions are classified as "Unlikely". For children, "Definite" is diagnosed when LDL-C is ≥ 140 mg/dL along with a family history of FH or CAD, or when LDL-C is ≥ 180 mg/dL with a relevant family history, or when LDL-C is ≥ 250 mg/dL regardless of family history. Additionally, confirmation of a pathogenic variant of FH also qualifies as "Definite". "Probable" applies when LDL-C is between 140-179 mg/dL with a family history, or 180-249 mg/dL without such history. "Possible" includes cases with LDL-C 100-139 mg/dL with a relevant family history. Cases with LDL-C < 100 mg/dL despite a family history, or < 140 mg/dL with no family history, are considered "Unlikely". Those who did not complete the FH diagnostic process were classified into the following five categories: "Untested lipids in children", "Bereavement", "CAD with unspecified details", "Dyslipidaemia with unspecified LDL-C levels", and "No information on CAD or dyslipidaemia". Specifically, the category "Untested lipids in children" includes cases in which blood testing could not be performed due to fear of needles or lack of parental consent, both of which are often influenced by the child's age. "Bereavement" refers to cases, particularly involving grandparents, where family history could not be obtained due to the death of the relative. "Unspecified LDL-C levels" include cases where blood was drawn, but LDL-C values were not recorded in the medical records. "Unspecified CAD history" refers to cases where the presence or absence of coronary artery disease could not be confirmed through medical records or patient reports. Finally, "No information available" describes cases where no relevant medical information could be obtained at all, making risk evaluation impossible. In this study, the family history recorded during the explanation of genetic test results was defined as the initial visit. After that, patients received ongoing treatment, primarily lipid-lowering therapy, provided by local primary care physicians, while annual follow-up visits were conducted at our institution. At each follow-up, we reviewed and updated the family history. If new at-risk relatives were identified, further evaluation was performed. During the initial visit, both parents were interviewed and tested for LDL-C to assess the likelihood of FH. If one parent was suspected of having FH, we investigated second-degree relatives on that side of the family according to the 2022 JAS clinical guidelines. If both parents were suspected, both family lines were investigated.

Effectiveness and barriers assessment

To obtain a clear demonstration and understanding of how patients were distributed among those diagnosed with FH, effectiveness was evaluated by categorising patients who completed the FH diagnostic process based on the flowchart of the 2022 JAS clinical guidelines. In contrast, barriers were evaluated based on the reasons provided for incomplete diagnostic processes, including challenges such as "Untested lipids in children", "Bereavement", "CAD with unspecified details", "Dyslipidaemia with unspecified LDL-C levels", and "No information on CAD or dyslipidaemia". We also evaluated the number of new FH diagnoses or reasons for incomplete diagnosis within second-degree relatives and documented the overall progression of the diagnostic process.

Genetic analysis

All children underwent genetic analysis at Kanazawa University, where their genotypes were examined using a next-generation sequencing platform[14]. The coding regions of *LDLR* (NM_000527.5), *APOB* (NM_000384.3), *PCSK9* (NM_174936.4), and *LDLRAP1* (NM_015627.3) were sequenced, following the established protocols. Additionally, copy number variations at the *LDLR* locus were assessed using the eXome Hidden Markov Model[15]. Adhering to the guidelines of the American College of Medical Genetics and Genomics, the pathogenic variant of FH was determined during multidisciplinary meetings involving specialists in genetics.

Ethical considerations

This study was approved by the Ethics Committee of Kagawa University (H30-059). All procedures adhered to the ethical standards set by the Institutional and National Human Research Committees and conformed to the Declaration of Helsinki (1975, revised in 2008). Written informed consent for the genetic testing of the children was obtained from at least one parent.

Statistical analysis

Patients who completed the FH diagnostic process were classified into four categories-"Definite", "Probable", "Possible", or "Unlikely"-according to the 2022 JAS clinical guidelines. Those who did not complete the FH diagnostic process were classified into five categories: "Untested lipids in children", "Bereavement", "CAD with unspecified details", "Dyslipidaemia with unspecified LDL-C levels", and "No information on CAD or dyslipidaemia". Using these categories, we evaluated the effectiveness and barriers of RCS in the diagnostic process. To evaluate whether the distribution between first-degree and second-degree relatives differed significantly within each diagnostic group, each group was compared with all other groups combined using the chi-squared test. Fisher's exact test was used when > 20% of the expected cell frequencies were less than five. Statistical analyses were conducted using IBM SPSS Statistics version 29 (IBM Corp., Armonk, NY, USA), with the significance level set at $P < 0.05$.

RESULTS

In this study, 68 of 151 children referred to our facility had genetically confirmed FH [Figure 1]. However, 14 cases were excluded due to the absence of family pedigree (4 cases), parental divorce (8 cases), or a previous diagnosis of FH through cascade screening (2 cases). Consequently, 48 families with FH, including 6 sets of siblings, were ultimately enrolled in the study, with a total of 252 suspected cases of FH. Of these 252 suspected cases, 94 completed the FH diagnostic process based on the flowchart of the 2022 JAS clinical guidelines (81 cases in first-degree relatives and 13 cases in second-degree relatives), whereas 158 patients did not complete the diagnostic process (25 cases in first-degree relatives and 133 cases in second-degree relatives). The chi-square test showed a statistically significant difference in diagnostic completion rates between first- and second-degree relatives ($\chi^2 = 120.11$, $P < 0.001$).

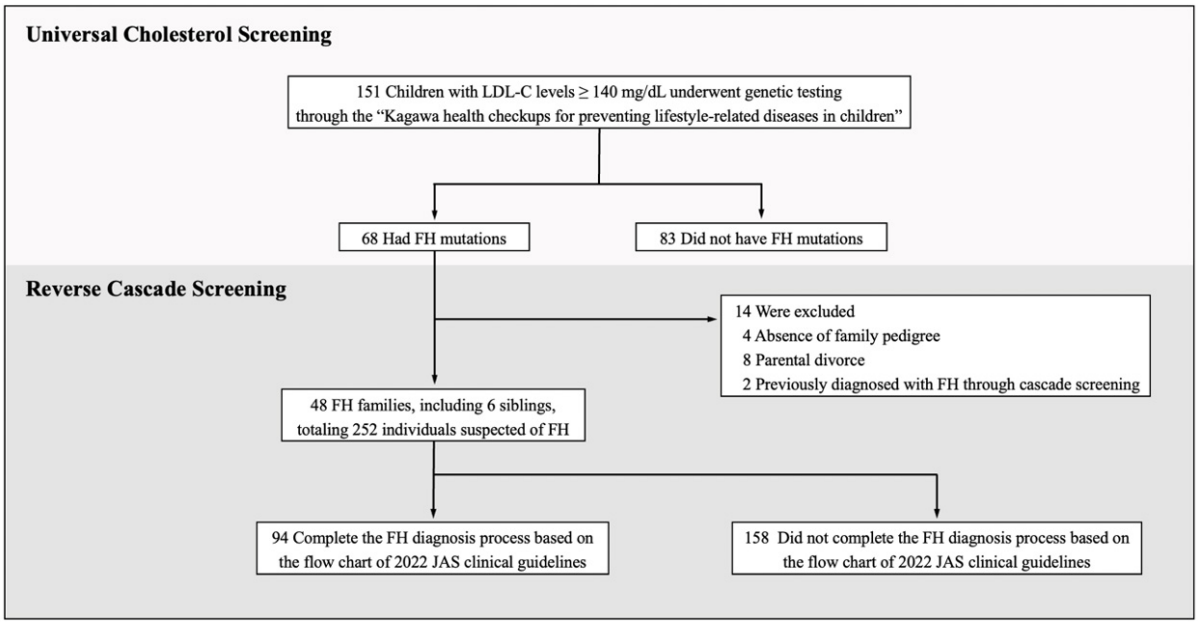

Figure 1. Study flowchart of the process of reverse cascade screening combined with universal cholesterol screening for paediatric FH. This flowchart shows the process used to identify FH cases through the "Kagawa health checkups for preventing lifestyle-related diseases in children" in Kagawa Prefecture. FH: Familial hypercholesterolaemia; LDL-C, low-density lipoprotein cholesterol; JAS, Japan atherosclerosis society.

Table 1 presents the results of the pathogenic FH variants in children who underwent genetic testing. Of the 48 children, 47 and 1 were identified as having heterozygous and homozygous variants, respectively. Among those with heterozygous variants, 40 children had pathogenic variants in *LDLR*, 6 children had pathogenic variants in *PCSK9*, and 1 child had a pathogenic variant in *APOB*. The one child was found to carry double heterozygous variants in both the *LDLR* and *PCSK9* genes.

Table 2 shows the distribution of the 94 patients who completed the FH diagnostic process. These patients were classified as follows: 49 "Definite" cases, with 47 cases in first-degree relatives and 2 cases in second-degree relatives; 5 "Probable" cases, with all 5 cases being in first-degree relatives; zero "Possible" cases; and 40 "Unlikely" cases, with 30 cases in first-degree relatives and 10 cases in second-degree relatives. 49 FH patients were identified from 48 families, corresponding to 1.02 diagnosed individuals per index FH case. Among the "Definite" first-degree relative cases, 9 were siblings and 38 were parents. All sibling cases were untreated and had no history of CAD. In contrast, of the 38 parent cases, 13 were undergoing treatment for dyslipidaemia and 1 had a prior CAD event. Regarding the two "Definite" second-degree relative cases, one case was undergoing treatment for dyslipidaemia and both had no history of a CAD event.

Table 3 shows the distribution of the 158 patients who did not complete the FH diagnostic process. These patients were classified as follows: 13 cases of "Untested lipids in the children", all involving first-degree relatives; 19 cases of "Bereavement", all in second-degree relatives; 6 cases of "CAD with unspecified details", all involving second-degree relatives; 52 cases of "Dyslipidaemia with unspecified LDL-C levels", with 10 cases in first-degree relatives and 42 in second-degree relatives; and 68 cases of "No information on CAD or dyslipidaemia", with 2 cases in first-degree relatives and 66 in second-degree relatives.

Table 1. Pathogenic variants of familial hypercholesterolemia in children who underwent genetic testing

Gene	Region	Variant type	Number	ACMG classification	Judgement
Heterozygous variant			47		
LDLR	c.301G > A	Missense	1	PM1/PP2/PP3/PP4/PP5 Likely pathogenic	Pathogenic
	c.361T > C	Missense	1	PM1/PM2/PM5/PP2/PP3/PP5/PS4 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.1056C > A	Missense	1	PVS1/PM2/PP5/PS4 Pathogenic	Pathogenic
	c.1187-10G > A	Splice-site	1	PP3/PP4/PS3 Likely pathogenic	Pathogenic
	c.1207T > C	Missense	10	PM1/PM2/PM5/PP3 Likely pathogenic	Pathogenic
	c.1252G > A	Missense	3	PM1/PM2/PM3/PP2/PP3/PP5 Likely pathogenic	Pathogenic
	c.1502C > T	Missense	1	PM1/PM2/PM3/PM5/PP2/PP3/PP5 Likely pathogenic	Pathogenic
	c.1702C > G	Missense	5	PM2/PP1/PP3/PP5 Likely pathogenic	Pathogenic
	c.1705+1G > C	Splice-site	4	PVS1/PM2/PM4/PP4 Pathogenic	Pathogenic
	c.1706A > G	Missense	1	PM2/PM5/PP2/PP3 Likely pathogenic	Likely pathogenic
	c.1747C > T	Missense	1	PM1/PM2/PM3/PM5/PP1/PP2/PP3/PP5/PS3 Pathogenic	Pathogenic
	c.1783C > T	Missense	3	PM1/PM2/PP3/PP4 Likely pathogenic	Pathogenic
	c.1845+2T > C	Missense	1	PVS1/PM2/PS4/PP5 Pathogenic	Pathogenic
	c.2054C > T	Missense	1	PM1/PM2/PM3/PM5/PP1/PP2/PP3/PP5/PS3 Pathogenic	Pathogenic
	c.2431A > T	Missense	1	PVS1/PM2/PM3/PP1/PP5 Pathogenic	Pathogenic
	c.2579C > T	Missense	2	PVS1/PM2/PM4/PP4 Pathogenic	Pathogenic
PCSK9	c.94G > A	Missense	6	PS1/PS3/PP4/PP5 Pathogenic	Pathogenic
APOB	c.10596_10597insGCATT	Insertion	1	PVS1/PM2 Likely pathogenic	Likely pathogenic
Double heterozygous variants			1		
LDLR	c.1195G > A			PM1/PM2/PM5/PP2/PP3/PP5/PS3/PS4/BS3 Pathogenic	Pathogenic
PCSK9	c.94G > A	Missense		PS1/PS3/PP4/PP5 Pathogenic	Pathogenic

FH: Familial hypercholesterolaemia; LDLR: low-density lipoprotein receptor; PCSK9: proprotein convertase subtilisin/kexin type 9; APOB: apolipoprotein B; ACMG: american college of medical genetics and genomics.

Figure 2 shows the progression of the number of cases that completed and did not complete the FH diagnostic process through the family-based follow-up. A chi-square test showed a statistically significant association between the number of follow-up visits and diagnostic completion ($\chi^2 = 8.89$, $P = 0.012$). This

Table 2. Classification of 94 cases that completed the familial hypercholesterolemia diagnostic process according to the 2022 Japan Atherosclerosis Society clinical guidelines, by first- and second-degree relatives

	First-degree relatives (*N* = 82)		Second-degree relatives (*N* = 12)		*P* value
	Parents (*N* = 40)	Siblings (*N* = 42)	Grandparents (*N* = 7)	Aunts/Uncles (*N* = 5)	
Definite	35	12	2	0	0.0120
Probable	5	0	0	0	1.0000
Possible	0	0	0	0	1.0000
Unlikely	0	30	5	5	0.0035

Table 3. Classification of 158 cases that did not complete the familial hypercholesterolemia diagnostic process according to the 2022 Japan Atherosclerosis Society clinical guidelines, by first- and second-degree relatives

	First-degree relatives (*N* = 25)		Second-degree relatives (*N* = 133)		*P* value
	Parents (*N* = 5)	Siblings (*N* = 20)	Grandparents (*N* = 84)	Aunts/Uncles (*N* = 49)	
Untested lipids in children	0	13	0	0	< 0.0001
Bereavement	0	0	17	2	0.0450
CAD with unspecified details	0	0	5	1	0.5910
Dyslipidaemia with unspecified LDL-C levels	5	5	28	14	0.4870
No information on CAD or dyslipidaemia	0	2	34	32	< 0.0001

CAD: Coronary artery disease; LDL-C: low-density lipoprotein cholesterol.

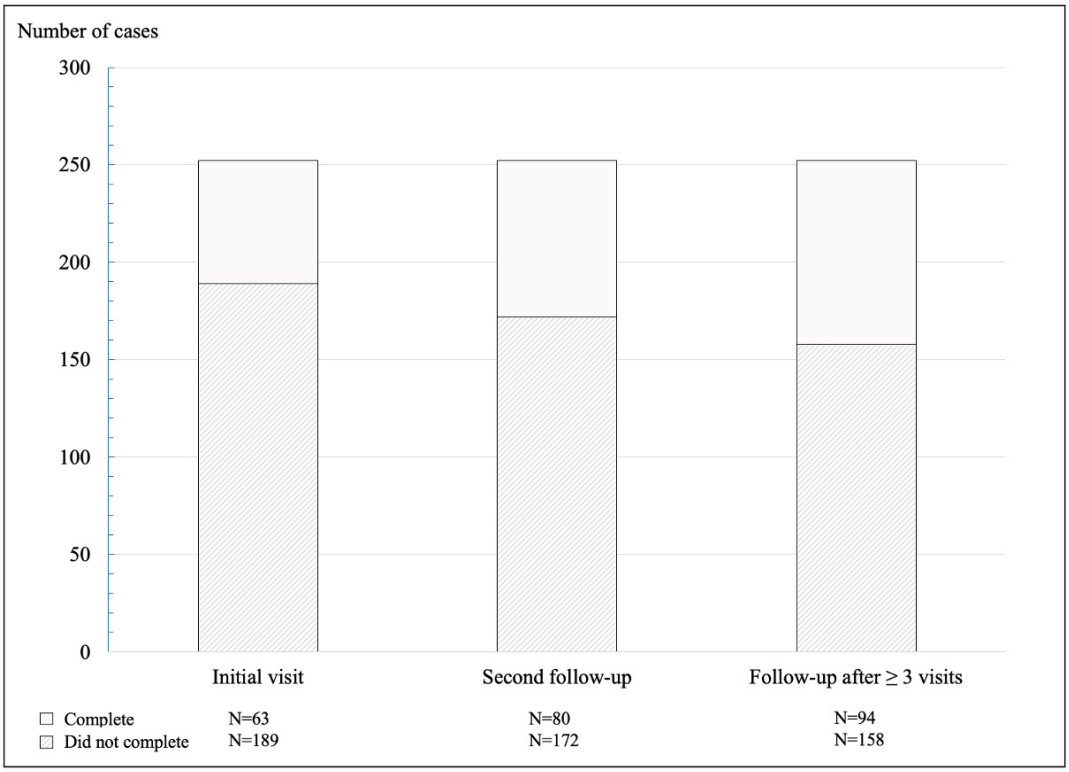

Figure 2. Progression of the number of cases that completed or did not complete the FH diagnostic process at each stage of family-based follow-up. A statistically significant association was observed between the number of follow-up visits and diagnostic completion (χ^2 = 8.89, *P* = 0.012), suggesting that repeated follow-up visits contributed to a higher diagnostic completion rate.

trend is also observed in Figure 3, which shows the changes associated with an increase in the number of visits in each category of patients who completed the FH diagnostic process. Among the 49 "Definite" cases, 36 cases were diagnosed when the probands were identified with genetically confirmed FH and 13 cases were diagnosed through family-based follow-up. Conversely, Figure 4 demonstrates the changes associated with an increase in the number of visits in each category of patients who did not complete the FH diagnostic process. Notably, as the number of family-based follow-up visits increased, the "Untested lipids in children" and "Bereavement" categories remained stable, while "CAD with unspecified details" and "Dyslipidaemia with unspecified LDL-C levels" showed an increasing trend and "No information on CAD or dyslipidaemia" showed a decreasing trend.

DISCUSSION

The effectiveness of reverse cascade screening

This study diagnosed 49 patients from 48 families with FH. The number of diagnosed cases per index patient was 1.02, which is comparable to the range reported in previous studies (mean 1.65, range 0.22-8.0)[16]. We found that RCS, including genetic testing, can lead to the diagnosis of FH in relatives. Given that FH is an autosomal dominant genetic disorder, it can be diagnosed in at least one parent. Therefore, this approach can facilitate the diagnosis of FH not only in children but also in their parents[17]. Thus, RCS has the potential to identify patients with FH who have not yet been diagnosed and are asymptomatic. FH is an autosomal dominant disorder, and when a child is genetically diagnosed with FH, it is highly likely that one of the parents also has FH. Furthermore, family members are also likely to be affected. Therefore, the diagnosis of FH in these relatives should not be considered of limited significance, but rather as an important finding that indicates the broader presence of FH within the family. Since second-degree relatives also include the first-degree relatives of the proband's parent, we believe that not only cascade screening for first-degree relatives but also the continuous updating of family history across all blood relatives is essential for improving FH detection. Regarding cost-effectiveness, we have previously evaluated the economic impact of this screening approach using a simulation model, which demonstrated its potential utility in clinical practice[18].

Moreover, this study demonstrated the importance of family-based follow-up, which includes regularly updating family histories[19]. This implementation identified 13 additional FH diagnoses after the initial diagnosis of 36 cases. Importantly, regularly updating the family histories of children diagnosed with FH could improve their adherence to treatment, introduce essential lifestyle changes, and lead to the diagnosis of new FH cases.

Barriers to reverse cascade screening

The number of patients diagnosed with FH was lower among second-degree relatives than among first-degree relatives (47 cases in first-degree relatives and 2 cases in second-degree relatives). Conversely, the number of suspected FH cases for which the screening flowchart could not be completed was higher among second-degree relatives than among first-degree relatives (25 cases in first-degree relatives and 133 cases in second-degree relatives). This discrepancy may be due to the absence of the second-degree relative during the medical consultation, leading to "CAD with unspecified details", "Dyslipidaemia with unspecified LDL-C levels", and "No information on CAD or dyslipidaemia" among second-degree relatives. When constructing family information, documenting negative findings is crucial because "No information on CAD or dyslipidaemia" may suggest a lack of clarity regarding whether the relevant data were collected. Thus, documenting negative findings ensures the accuracy of the clinical records and aids in more effective risk assessment and management. Additionally, the lack of information on CAD or dyslipidaemia is a significant issue that can be addressed by raising awareness regarding FH among healthcare professionals through educational initiatives. This is important as the early detection and treatment of FH depend

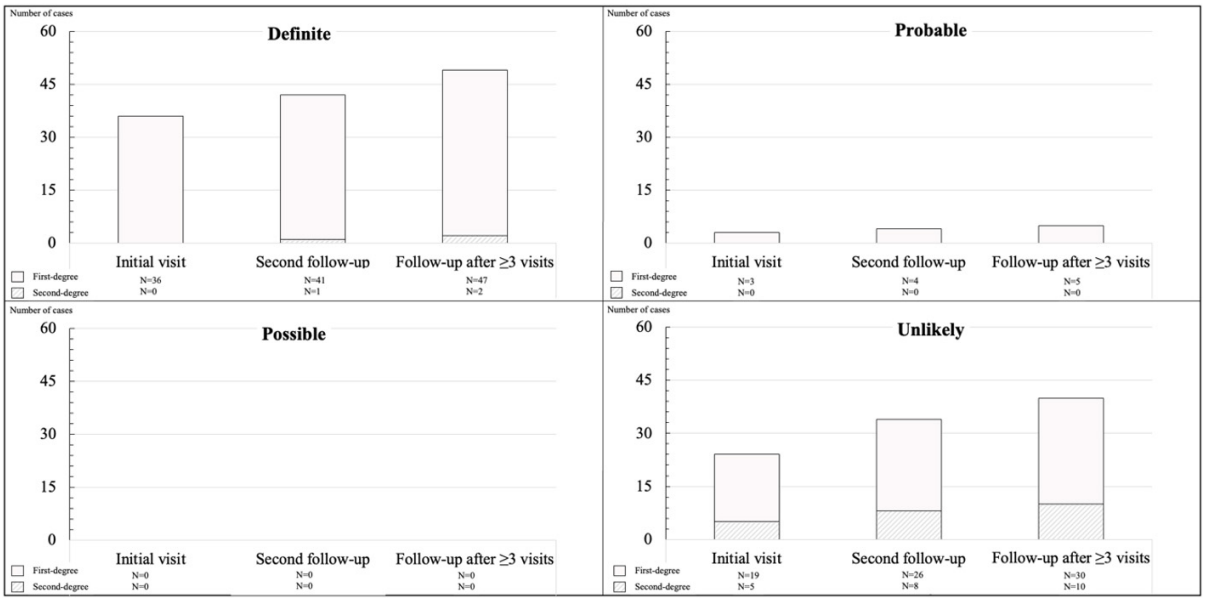

Figure 3. The association between family-based follow-up frequency and completion of the familial hypercholesterolaemia diagnostic process. An increasing trend in completed cases is observed across all categories from the initial visit, through the second follow-up, and to the follow-up after three or more visits.

significantly on healthcare professionals. Given that the rate of diagnosis of FH is reported to be below 10% in Asian populations, increasing awareness may lead to more frequent medical consultations and, consequently, higher rates of diagnosis[20]. In addition, raising awareness among the public is crucial for addressing issues such as "CAD with unspecified details" or "Dyslipidaemia with unspecified LDL-C levels", and it is therefore important to foster interest regarding FH among the public and encourage families to take a proactive approach to FH diagnosis. In fact, public education campaigns have been shown to significantly increase awareness and engagement in FH screening programmes, which could improve the early diagnosis and management of FH[21].

The category "Untested lipids in children" also represented a barrier to FH diagnosis. In children who are unable to undergo lipid testing due to fear of needles or lack of parental consent, non-invasive genetic testing using a buccal swab may be a helpful alternative. Previous studies have reported its successful use in paediatric FH screening and its potential to improve cascade testing uptake[22]. To minimize the barrier posed by invasive procedures, the development of non-invasive testing methods, including buccal swab-based genetic testing, is warranted.

The results of this study also demonstrated that family-based follow-up could reduce "No information on CAD or dyslipidaemia" and improve the diagnosis of FH. Previous studies suggest that direct contact or home visits to families of probands can significantly enhance the diagnosis of FH[20]. However, these methods are limited as healthcare providers are not always able to make direct contact (calls or letters from the FH service) or other contact (all alternative methods besides direct or indirect contact with adults) with these individuals, which may result in patient-centred approaches failing to encourage relatives to seek medical evaluation. Therefore, utilising Information and Communication Technology (ICT) instead of other contact methods might address the issue of an incomplete FH diagnostic process[23]. The use of ICT, such as electronic health records and machine learning algorithms, to efficiently identify potential FH cases has shown promise, with several studies reporting the effectiveness of such approaches[24-26]". To address the

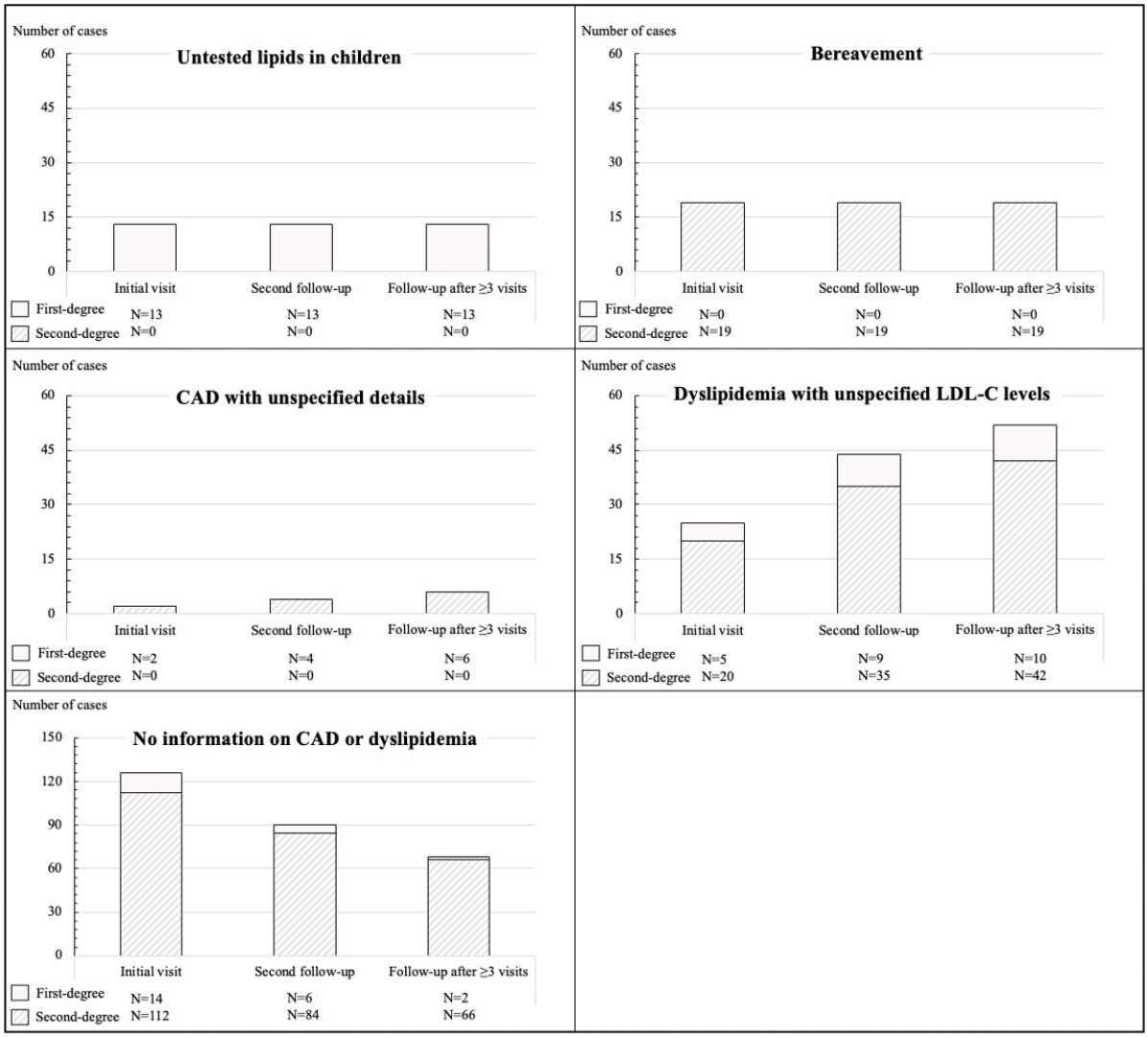

Figure 4. The association between family-based follow-up frequency and incompletion of the familial hypercholesterolaemia diagnostic process. As the number of visits increased, the proportion of cases with "No information on Premature coronary artery disease (CAD) or dyslipidemia" decreased, suggesting that follow-up helped clarify missing clinical information. LDL-C: low-density lipoprotein cholesterol.

missing information observed in this study, integrating electronic health record systems and using standardized questionnaires may help improve data quality and reduce information gaps. In Kagawa Prefecture, the "K-MIX R" regional health information network connects all medical institutions via electronic health records. Through this network, the results of universal screening are shared to enhance the diagnostic accuracy of FH and optimise family-based treatment. This initiative employs an opt-in system that enables data sharing based on patient consent, ensuring privacy protection and addressing ethical considerations. Currently, information from other hospitals is gathered through written communication, which can be inefficient and delay the diagnostic process. If healthcare facilities were connected through an integrated network, it would enable easier and more timely access to the detailed data necessary for FH diagnoses, such as accurate LDL-C levels and CAD events. Such a network could significantly enhance diagnostic efficiency and improve the coordination between medical institutions. Furthermore, utilising ICT to create an interconnected health information system would facilitate the sharing of necessary

diagnostic data and streamline the overall screening process, potentially increasing FH diagnosis rates.

Limitations

This study was based on data from a single medical institution in Japan, which may limit the generalisability of the findings. Differences in cultural factors, such as family health information sharing willingness, socioeconomic factors, cultural contexts, and healthcare infrastructure, may also affect the applicability of our results to other regions in Japan and international settings. These factors may be potential reasons for the low diagnosis rate of second-degree relatives. Therefore, targeted measures such as community-based health education or economic incentives might improve awareness and participation. Future studies involving multicenter data are warranted to verify our conclusions' broader relevance and applicability. Part of the family history and medical history was obtained through self-reports by patients or their family members, which may have introduced recall bias and information bias related to self-reported data. Annual in-person follow-up visits were conducted to update family histories; however, alternative methods such as telephone calls, home visits, or electronic platforms were not utilised. Since previous studies have reported improved FH diagnosis rates through telephone or home visit strategies, integrating these approaches warrants consideration in future implementation[27]. This study is a limited use of genetic testing in relatives of FH probands with a known pathogenic variant of FH. In Japan, predictive genetic testing for asymptomatic individuals is not generally recommended in the guidelines[12]. Therefore, we focused on relatives with clear clinical indicators. Moving forward, there is a need to promote appropriate and ethical use of genetic testing. A key limitation of this study is its focus on the diagnostic stage, without evaluating post-diagnosis intervention effects such as medication adherence or reduction in cardiovascular events. We acknowledge the importance of long-term follow-up data and recommend that future research, in collaboration with academic societies and government agencies, incorporate clinical outcome indicators to comprehensively assess the value of screening programmes, including treatment effectiveness, patient adherence, and clinical endpoints.

Conclusions

This study demonstrated that RCS combined with UCS for paediatric FH, utilising genetic testing, is effective for the early diagnosis of FH in asymptomatic cases. However, barriers such as difficulties in approaching second-degree relatives hinder the diagnosis of FH in their families. To overcome these barriers, family-based follow-ups, increased awareness, and an efficient system for the accurate and accessible collection of family medical information are crucial.

DECLARATIONS

Acknowledgements

The authors would like to extend heartfelt thanks to the officials in Kagawa Prefecture and the 17 municipalities (including Takamatsu City, Marugame City, Sakaide City, Zentsuji City, Kanonji City, Sanuki City, Higashikagawa City, Mitoyo City, Tonosho Town, Shodoshima Town, Miki Town, Naoshima Town, Utazu Town, Ayagawa Town, Kotohira Town, Tadotsu Town, and Manno Town) for their valuable co-operation in the research. The authors also express sincere appreciation to the members of the Review Committee of Kagawa Health Checkups for Childhood Lifestyle-Related Disease Prevention, Kagawa Medical Association, and Kagawa Paediatric Association for their invaluable support.

Authors' contributions

Performed data collection and manuscript writing and editing: Tani R
Performed data collection and manuscript editing and review: Matsunaga K
Performed data collection and manuscript review: Inoue T, Nishioka K, Irie K, Kondo S, Iwase T, Fu HY, Ito S, Sasaki T, Yoshida S, Yokota I, Hoshikawa Y, Yokoyama K, Fujisawa T, Tada H, Takamura M, Kusaka T

Performed data collection, manuscript review, and final approval: Minamino T

Availability of data and materials

Raw data were generated at Kagawa University. The derived data supporting the findings of this study are available from the corresponding author upon reasonable request.

Financial support and sponsorship

This study received support from various sources, including a research grant from the Japan Agency for Medical Research and Development (AMED) under grant number 22gk0110065h0001, JSPS KAKENHI (Grant Number JP 24K11192, JP 24K11241, JP 23K15159, 21FC1009, 20K08474).

Conflicts of interest

Tada H is the Guest Editor of the Special Issue "Advancements in Diagnosis and Treatment of Familial Hypercholesterolemia" in the journal *Rare Diseases and Orphan Drugs*. Tada H was not involved in any steps of the editorial process, including reviewer selection, manuscript handling, and decision making. The other authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

This study was approved by the Ethics Committee of Kagawa University (H30-059). All procedures adhered to the ethical standards set by the Institutional and National Human Research Committees and conformed to the Declaration of Helsinki (1975, revised in 2008). Written informed consent for genetic testing was obtained from at least one parent.

Consent for publication

Not applicable.

REFERENCES

1. Hu P, Dharmayat KI, Stevens CAT, et al. Prevalence of familial hypercholesterolemia among the general population and patients with atherosclerotic cardiovascular disease: a systematic review and meta-analysis. *Circulation.* 2020;141:1742-59. DOI PubMed
2. Gulizia MM, Maggioni AP, Abrignani MG, et al; POSTER Investigators. Prevalence of familial hypercholesterolaemia (FH) in italian patients with coronary artery disease: the POSTER study. *Atherosclerosis.* 2020;308:32-8. DOI PubMed
3. Sturm AC, Knowles JW, Gidding SS, et al; Convened by the Familial Hypercholesterolemia Foundation. Clinical genetic testing for familial hypercholesterolemia: *JACC* scientific expert panel. *J Am Coll Cardiol.* 2018;72:662-80. DOI PubMed
4. Gidding SS, Wiegman A, Groselj U, et al. Paediatric familial hypercholesterolaemia screening in Europe: public policy background and recommendations. *Eur J Prev Cardiol.* 2022;29:2301-11. DOI PubMed
5. McKay AJ, Hogan H, Humphries SE, Marks D, Ray KK, Miners A. Universal screening at age 1-2 years as an adjunct to cascade testing for familial hypercholesterolaemia in the UK: a cost-utility analysis. *Atherosclerosis.* 2018;275:434-43. DOI PubMed
6. Brett T, Qureshi N, Gidding S, Watts GF. Screening for familial hypercholesterolaemia in primary care: time for general practice to play its part. *Atherosclerosis.* 2018;277:399-406. DOI PubMed
7. Tada H, Okada H, Nomura A, et al. Prognostic impact of cascade screening for familial hypercholesterolemia on cardiovascular events. *J Clin Lipidol.* 2021;15:358-65. DOI PubMed
8. Lokkesmoe R, Hamilton L. The role of reverse cascade screening in children with familial hypercholesterolemia: a literature review and analysis. *Curr Atheroscler Rep.* 2024;26:427-33. DOI PubMed
9. Kusters DM, Wiegman A, Kastelein JJ, Hutten BA. Carotid intima-media thickness in children with familial hypercholesterolemia. *Circ Res.* 2014;114:307-10. DOI PubMed
10. Matsunaga K, Mizobuchi A, Ying Fu H, et al. Universal screening for familial hypercholesterolemia in children in Kagawa, Japan. *J Atheroscler Thromb.* 2022;29:839-49. DOI PubMed PMC
11. Fu HY, Matsunaga K, Inoue T, et al. Improved efficiency of the clinical diagnostic criteria for familial hypercholesterolemia in children: a comparison of the Japan atherosclerosis society guidelines of 2017 and 2022. *J Atheroscler Thromb.* 2024;31:1048-57.

DOI PubMed PMC

12. Harada-Shiba M, Arai H, Ohmura H, et al. Guidelines for the diagnosis and treatment of adult familial hypercholesterolemia 2022. *J Atheroscler Thromb.* 2023;30:558-86. DOI PubMed PMC
13. Harada-Shiba M, Ohtake A, Sugiyama D, et al. Guidelines for the diagnosis and treatment of pediatric familial hypercholesterolemia 2022. *J Atheroscler Thromb.* 2023;30:531-57. DOI PubMed PMC
14. Tada H, Kawashiri MA, Nomura A, et al. Oligogenic familial hypercholesterolemia, LDL cholesterol, and coronary artery disease. *J Clin Lipidol.* 2018;12:1436-44. DOI
15. Yamamoto T, Shimojima K, Ondo Y, et al. Challenges in detecting genomic copy number aberrations using next-generation sequencing data and the eXome Hidden Markov Model: a clinical exome-first diagnostic approach. *Hum Genome Var.* 2016;3:16025. DOI PubMed PMC
16. Lee C, Rivera-Valerio M, Bangash H, Prokop L, Kullo IJ. New case detection by cascade testing in familial hypercholesterolemia: a systematic review of the literature. *Circ Genom Precis Med.* 2019;12:e002723. DOI PubMed PMC
17. Ibarretxe D, Rodríguez-Borjabad C, Feliu A, Bilbao JÁ, Masana L, Plana N. Detecting familial hypercholesterolemia earlier in life by actively searching for affected children:the DECOPIN project. *Atherosclerosis.* 2018;278:210-6. DOI PubMed
18. Matsunaga K, Harada-Shiba M, Yamashita S, et al. A cost-effectiveness analysis for the combination of universal screening at 9-10 years old and reverse cascade screening of relatives for familial hypercholesterolemia in Japan. *J Atheroscler Thromb.* 2025;32:000-000. DOI
19. Wilemon KA, Patel J, Aguilar-Salinas C, et al; Representatives of the Global Familial Hypercholesterolemia Community. Reducing the clinical and public health burden of familial hypercholesterolemia: a global call to action. *JAMA Cardiol.* 2020;5:217-29. DOI
20. Cox E, Faria R, Saramago P, et al. Challenges and opportunities for identifying people with familial hypercholesterolemia in the UK: evidence from the national FH PASS database. *J Clin Lipidol.* 2024;18:e1046-54. DOI
21. Campbell-Salome G, Jones LK, Masnick MF, et al. Developing and optimizing innovative tools to address familial hypercholesterolemia underdiagnosis: identification methods, patient activation, and cascade testing for familial hypercholesterolemia. *Circ Genom Precis Med.* 2021;14:e003120. DOI PubMed PMC
22. Lentz M, Benoy M, Zhang X, Peterson AL. Cascade screening for familial hypercholesterolemia from pediatric index cases diagnosed through universal screening. *J Clin Lipidol.* 2024;18:e620-4. DOI
23. Monaco A, Maggi S, De Cola P, Hassan TA, Palmer K, Donde S. Information and communication technology for increasing healthy ageing in people with non-communicable diseases: identifying challenges and further areas for development. *Aging Clin Exp Res.* 2019;31:1689-93. DOI PubMed PMC
24. Osei J, Razavi AC, Otchere B, et al. A scoping review of electronic health records-based screening algorithms for familial hypercholesterolemia. *JACC Adv.* 2024;3:101297. DOI
25. Page C, Zheng H, Wang H, et al. A comparison of the Netherlands, Norway and UK familial hypercholesterolemia screening programmes with implications for target setting and the UK's NHS long term plan. *PLOS Glob Public Health.* 2023;3:e0001795. DOI PubMed PMC
26. Reeskamp LF, Annink ME, Schonck WAM. Familial hypercholesterolemia detection through machine learning algorithms: a low-hanging fruit. *JACC Adv.* 2024;3:101181. DOI PubMed PMC
27. Baldry E, Redlinger-Grosse K, MacFarlane I, et al. Outcomes from a pilot genetic counseling intervention using motivational interviewing and the extended parallel process model to increase cascade cholesterol screening. *J Genet Couns.* 2022;31:164-75. DOI