



Early diagnosis and treatments in childhood are associated with better prognosis in patients with familial hypercholesterolemia

Hayato Tada^{a,*}, Nobuko Kojima^a, Kan Yamagami^a, Akihiro Nomura^a, Atsushi Nohara^b,
Soichiro Usui^a, Kenji Sakata^a, Masayuki Takamura^a, Masa-aki Kawashiri^a

^a Department of Cardiovascular Medicine, Graduate School of Medical Sciences, Kanazawa University, 13-1 Takara-Machi, Kanazawa 920-8641, Japan

^b Department of Clinical Genetics, Ishikawa Prefectural Central Hospital, Kanazawa, Japan

ARTICLE INFO

Keywords:

Familial hypercholesterolemia
LDL cholesterol
Genetics
LDL receptor
Children

ABSTRACT

Objective: The early diagnosis and treatment initiation for children with familial hypercholesterolemia (FH) has been recommended in guidelines. However, there is limited data on the impact of early treatments on the prognosis of children with FH. To investigate if the early initiation of lipid-lowering therapies among Japanese pediatric patients with FH reduced the occurrence of cardiovascular disease (CVD) events in them.

Methods: We retrospectively investigated the occurrence of CVD events (myocardial infarction, unstable angina, or coronary artery revascularization) in patients with FH ($N = 1050$, male/female = 490/560), including 106 children below 20 years. We compared a variety of phenotypes, including genetic backgrounds, other complications, LDL cholesterol, medical therapies, and their prognoses between the patients' diagnoses before the age of 20 years (children, mean age = 15 years) and after that age (adults, mean age = 52 years). Overall, 290 patients (27.6%) had a history of prior CVD events.

Results: The median follow-up duration was 12.6 [9.5–17.9] years. The baseline LDL cholesterol level, 239 mg/dL, dropped to 112 mg/dL with the treatments. The Achilles tendon thickness was significantly lower in children than that of adults (7.2 vs. 8.9 mm, $P < 0.001$). Over the follow-up duration, 119 CVD events were observed. Importantly, no CVD event was observed in children despite their median LDL cholesterol level at follow-up being significantly higher than that of adults (122 vs. 111 mg/dL, $P < 0.001$).

Conclusion: The likelihood of CVD events in those with FH diagnosed and treated in childhood is low.

1. Introduction

Familial hypercholesterolemia (FH), one of the most common autosomal dominant lipoprotein disorders, is caused by a pathogenic mutation in the LDL receptor (*LDLR*) or its associated genes, including apolipoprotein B (*APOB*), proprotein convertase subtilisin/kexin type 9 (*PCSK9*), and *LDLR* adaptor protein 1 (*LDLRAP1*) [1]. This is characterized by extremely elevated LDL cholesterol from birth, which leads to premature cardiovascular diseases (CVDs) [2]. Currently, the reported prevalence of this condition is 1/300 in the general population [3,4]. Lipid-lowering therapies such as statins, ezetimibe, colestimide, and PCSK9 inhibitors can effectively reduce LDL cholesterol levels [5]. Among these drugs, statins are the most essential therapy for patients with FH, including children [6]. The American College of Cardiology-American Heart Association and the European

Atherosclerosis Society consensus panel recommend starting statin therapy for pediatric patients with FH from the age of 8–10 years [7,8]. In Japan, the Japan Atherosclerosis Society guideline for patients with pediatric FH also recommends starting statin therapy from the age of 10 years [9]. Although there is evidence suggesting that statins effectively reduce LDL cholesterol levels in pediatric FH, there is a paucity of data on the clinical outcomes of the patients involved when followed up to their adulthood. Luirink et al. demonstrated that pediatric patients with FH in whom statin therapy was initiated at the age of 13 years had significantly better prognoses than their parents who started medical therapy much later [10]. This study aimed to investigate whether the early initiation of lipid-lowering therapy in Japanese pediatric patients with FH had the beneficial effect of reducing their CVD event rates.

* Corresponding author.

E-mail address: ht240z@sa3.so-net.ne.jp (H. Tada).

<https://doi.org/10.1016/j.ajpc.2022.100434>

Received 3 September 2022; Received in revised form 12 November 2022; Accepted 18 November 2022

Available online 19 November 2022

2666-6677/© 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

2. Methods

2.1. Ethical considerations

This study was approved by the Ethics Committee of Kanazawa University. All procedures were conducted per the ethical standards of the Human Research Committee (institutional and national) and the Helsinki Declaration (1975, revised in 2008). Informed consent for genetic analyses was obtained from all the study participants.

2.2. Study population

We assessed the data of 2011 patients with FH diagnosed clinically using the Japan Atherosclerosis Society (JAS) 2017 criteria for adults or children [9,11] at Kanazawa University Hospital between 1990 and 2020. All patients aged ≥ 15 years fulfilled at least two of the three essential clinical criteria stipulated by the JAS for FH diagnosis, which are as follows: 1) LDL cholesterol level ≥ 180 mg/dL, 2) tendon xanthoma on the backs of the hands, elbows, knees, or other areas; Achilles tendon hypertrophy or Achilles tendon thickness on X-ray ≥ 9 mm; or xanthoma tuberosum, and 3) family history of FH or premature coronary artery disease diagnosed in a first-degree or second-degree relative. We used the criteria for pediatric FH for the patients aged < 15 years [9]: 1) LDL cholesterol level ≥ 140 mg/dL, and 2) family history of FH or premature coronary artery disease diagnosed in a first-degree or second-degree relative. Nine hundred and sixty-one patients were excluded due to missing data, lost to follow-up, or patients with homozygous FH. Finally, 1050 patients (aged 6 to 82 years) were included in this study (Supplemental Fig. 1).

2.3. Clinical data assessment

Hypertension was defined as a systolic blood pressure of ≥ 140 mmHg, and/or diastolic blood pressure ≥ 90 mmHg, or the use of antihypertensive medications. We used the Japan Diabetes Society's definition of diabetes [12]. The smoking status was defined as the current smoking status. CVD was defined as myocardial infarction, unstable angina, or coronary artery revascularization. Serum levels of total cholesterol, triglycerides, and HDL cholesterol were determined enzymatically via automated instrumentation. LDL cholesterol levels were calculated using the Friedewald formula if the triglyceride level was < 400 mg/dL; otherwise, it was determined enzymatically. The LDL cholesterol year score was calculated as LDL cholesterol $\max \times$ [age at diagnosis/statin initiation] + LDL cholesterol at inclusion \times [age at inclusion - age at diagnosis/statin initiation] [13]. LDL cholesterol levels during LDL apheresis therapy was calculated as follows, $C_{\text{average}} = C_{\text{min}} + 0.73 (C_{\text{max}} - C_{\text{min}})$, where C_{average} is the mean concentration during biweekly LDL-apheresis therapy, C_{max} and C_{min} are the concentration just before and after single session of LDL-apheresis [14]. We used LDL cholesterol level 2 weeks after the use of PCSK9 inhibitor for the patients under the use of this drug.

2.4. Genetic analysis

We assessed genotypes using a next-generation sequencer. The coding regions of *LDLR*, *APOB*, *PCSK9*, and *LDLRAP1* were sequenced as described in a previous study [15]. Further, copy number variations at the *LDLR* were assessed using the eXome Hidden Markov Model software program as described in an earlier study [16]. We evaluated the pathogenicity of the genetic variants according to the standard American College of Medical Genetics and Genomics criteria [17].

2.5. Statistical analysis

Normally distributed continuous variables were reported as means \pm standard deviations while those with skewed data distributions were

reported as medians and interquartile ranges. Categorical variables were reported as frequencies and percentages, and they were compared using Fisher's exact test or the chi-square test. Mean values of continuous variables were compared using Student's *t*-test for independent variables, and median values were compared using the non-parametric Wilcoxon rank-sum test. Cumulative Kaplan-Meier survival curves starting at baseline were constructed to compare time-lapses to first CVD events. For the outcome of time-lapses to first CVD events, follow-up started at birth and ended for each participant at the date of the first CVD events or the date of the visit, whichever came first. The probability of disease by time *t* was estimated by $F(t) = 1 - S(t)$, where $S(t)$ is the survivor function, calculated by the *survfit* function from the R survival package. All statistical analyses were conducted using R statistics (<https://www.r-project.org>). P-values of < 0.05 were considered statistically significant.

3. Results

3.1. Clinical characteristics

The clinical characteristics of the study participants are shown in Table 1. The mean age of patients was 49 years, and almost half of the patients were men. The median LDL cholesterol level was 239 mg/dL. A total of 776 patients (73.9%) had a family history of FH and/or premature CVD. Furthermore, 290 patients (27.6%) had a history of CVD. When we divided the patients into two groups based on their age at diagnosis, we observed several differences in variables, such as age, complications, including hypertension, diabetes, smoking, triglycerides, HDL cholesterol, percentage of patients diagnosed via cascade screening, family history of FH and/or premature CVD, FH pathogenic variants, Achilles tendon thickness, and history of prior CVD between the groups. Similar differences were observed when we divided the

Table 1
Baseline characteristics.

Variables	All (N = 1050)	Children (N = 106)	Adults (N = 944)	P-value
Age (years)	49 \pm 16	15 \pm 4	52 \pm 15	$< 2.2 \times 10^{-16}$
Male (%)	490 (46.7%)	44 (41.5%)	446 (47.2%)	0.31
Hypertension (%)	250 (23.8%)	0 (0.0%)	136 (14.4%)	5.4×10^{-5}
Diabetes (%)	83 (7.9%)	0 (0.0%)	83 (8.8%)	0.003
Smoking (%)	224 (21.3%)	0 (0.0%)	128 (14.6%)	0.0001
Total cholesterol (mg/dL)	318 [268–365]	312 [291–354]	319 [287–360]	0.56
Triglyceride (mg/dL)	113 [76–177]	90 [66–138]	126 [84–173]	2.3×10^{-5}
HDL cholesterol (mg/dL)	47 [43–51]	55 [45–60]	47 [40–56]	9.8×10^{-5}
LDL cholesterol (at baseline, mg/dL)	239 [208–279]	237 [206–276]	240 [207–283]	0.61
LDL cholesterol (at follow-up mg/dL)	112 [96–120]	122 [109–143]	111 [93–133]	2.4×10^{-6}
Diagnosed via cascade screening (%)	498 (47.4%)	97 (91.5%)	401 (42.5%)	$< 2.2 \times 10^{-16}$
Family history of FH and/or premature CVD (%)	776 (79.9%)	106 (100%)	670 (71.0%)	7.8×10^{-6}
FH pathogenic variants (%)	777 (74.0%)	93 (87.7%)	684 (72.5%)	0.001
Achilles tendon thickness (mm)	8.7 [7.6–10.0]	7.2 [6.3–8.6]	8.9 [7.6–10.0]	$< 2.2 \times 10^{-16}$
History of prior CVD (%)	290 (27.6%)	0 (0.0%)	290 (30.7%)	4.3×10^{-11}
Median Follow-up duration (years)	12.6 [9.5–17.9]	12.4 [9.1–18.4]	12.7 [9.2–17.4]	0.33

FH: familial hypercholesterolemia, CVD: cardiovascular disease.

patients into 4 groups (quartile of age, Supplemental Table 1). Mean age of initiating medical treatments in children was 13 years, and that in adults was 49 years. The medical treatments administered during follow-up are summarized in Table 2. Statin therapy, frequently followed by ezetimibe and colestimide therapy, was administered to most adult patients, while most patients diagnosed during childhood were treated with statins alone. We identified 89 pathogenic variants in 777 patients (Supplemental Table 2).

3.2. LDL cholesterol level at their follow-up

Based on their ages at diagnosis, we divided the patients into the following two groups: children (diagnosed before the age of 20 years) and adults (diagnosed after the age of 20 years). The histogram of density showed a similar distribution of baseline LDL cholesterol between these two groups (Fig. 1). We found that the median LDL cholesterol level at baseline did not differ significantly between these two groups (237 vs. 240 mg/dL, $P = 0.61$, Table 1 and Fig. 2A), while the LDL cholesterol level of the adult group was significantly lower than that of children during follow-up under lipid-lowering therapies (Fig. 2B).

3.3. LDL cholesterol year score

Despite the fact that the LDL cholesterol level assessed cross-sectionally did not differ significantly between the two groups, the LDL cholesterol year score at baseline, which accounts for the accumulation of long-term exposure to elevated LDL cholesterol among adults, was significantly higher in adults than in children (Fig. 3). In addition, we observed essentially the same trend in the LDL cholesterol year score at follow-up (Supplemental Fig. 2). Interestingly, we found that the mean LDL cholesterol year score at the first MACE among patients with FH was 10,190 mg/dL \times years when we focused on the patients without prior CVD event ($N = 760$).

3.4. Difference in the prognosis between adults and children

We compared age-dependent probabilities for MACE between adults and children and found that none of the patients with FH diagnosed during childhood had experienced CVD events, as all of such events were observed among patients diagnosed during adulthood (Fig. 4). In addition, we observed similar trends when we divided the patients into age groups (quartiles of age, Supplemental Fig. 3). When we focused on children and their parents ($N = 97$ for each), we observed similar difference between these 2 groups, although we did not see statistical significance due to small sample size (Supplemental Fig. 4).

4. Discussion

In this study, we investigate if the early initiation of lipid-lowering

Table 2
Medical therapies.

Lipid-lowering therapies	All ($N = 1050$)	Children ($N = 106$)	Adults ($N = 944$)	<i>P</i> -value
Statins (%)	1025 (97.6%)	95 (89.6%)	930 (98.5%)	8.4×10^{-8}
Ezetimibe (%)	644 (61.3%)	10 (9.4%)	634 (67.2%)	$<2.2 \times 10^{-16}$
Colestimide (%)	243 (23.1%)	4 (3.8%)	239 (25.3%)	1.1×10^{-6}
Probolol (%)	2 (0.2%)	0 (0.0%)	2 (0.2%)	1.0
PCSK9 inhibitor (%)	45 (4.3%)	0 (0.0%)	45 (4.8%)	0.04
LDL apheresis (%)	2 (0.2%)	0 (0.0%)	2 (0.2%)	1.0
Fibrates (%)	6 (0.6%)	0 (0.0%)	6 (0.6%)	0.89
n-3 PUFAs (%)	10 (1.0%)	0 (0.0%)	10 (1.1%)	0.59

PCSK9: proprotein convertase subtilisin/kexin type 9, PUFA: polyunsaturated fatty acid.

therapies among Japanese pediatric patients with FH had the beneficial effect of reducing their CVD event rates. We found that patients with FH diagnosed during childhood exhibited much better prognoses than did those diagnosed during adulthood despite having higher LDL cholesterol levels under milder lipid-lowering therapies.

We, and others, have postulated a cholesterol-accumulation hypothesis where the long-term exposure to elevated LDL cholesterol appears to lead to a high risk for CVD [13,18]. In other words, earlier diagnosis and treatment initiation should resolve this situation. We have shown that patients with FH diagnosed via cascade screening at the age of 39 years had much better prognoses despite receiving milder treatments than the probands who seek medical care at the age of 57 [19]. To the best of our knowledge, there is only one study that specifically investigated this issue in children so far [10]. Luirink et al. demonstrated that pediatric patients with FH in whom statin therapy was started when they were 13 years old exhibited significantly better prognoses than their parents who started medical therapies much later in their own lives. It is quite interesting that the only pediatric patient who developed angina had stopped taking statins. In this study, we have confirmed that patients with FH diagnosed during childhood had significantly better prognoses than those diagnosed during adulthood despite their lower LDL cholesterol levels achieved under more intensive lipid-lowering therapies. These facts indicate that the “the earlier, the better” slogan appears to apply in the prevention of CVD among patients with FH. We also confirmed that LDL cholesterol year scores, which reflect the life-long exposure to LDL cholesterol among children, were much lower than those of adults. In fact, we found that the mean LDL cholesterol year score at the first MACE among patients with FH was 10,190 mg/dL \times years. This observation suggests that patients with FH whose LDL cholesterol was 250 mg/dL will likely to suffer from MACE at the age of 40 years. On the other hand, we can put it off until the age of 100 years if their LDL cholesterol level could be lowered to 100 mg/dL on average. However, these results raise further clinical questions; 1) Is the effect of cholesterol accumulation linear? In other words, is there any specific threshold age to start treatment for patients with FH? 2) Is there any “legacy effect” of LDL-lowering therapies where the early initiation of LDL-lowering therapies could have long-lasting beneficial effects? Further studies are needed to answer these questions and help us brush up on our clinical guidelines for FH across the world.

Another aspect from this study includes the problem of under-diagnosis of FH, especially among children. We were actually surprised to see that most of the patients with FH were actually diagnosed at their adulthood in Japan. In fact, most of the patients with FH aged < 20 years in this study were diagnosed via cascade screening from their parents. Accordingly, we did not see any changes in the ways of screening nor the numbers of diagnosis during the study period.

It is also noteworthy that almost half of our study subjects had nonsense mutation in *LDLR*, which we believe are associated with severe form of FH. In fact, we have shown that phenotype of FH with nonsense mutation in *LDLR* is severer than that of FH with missense mutation [20].

This study has several limitations. First, it is a retrospective study conducted in a single center; therefore, its findings may not be applicable to other patients. However, our institute has a long history of treating patients with FH and has one of the largest databases in Japan. Second, we could not account for treatments, including dose of statins administered during follow-up, nor changes of prescription pattern during the study period and this may affect our results. Third, many patients were excluded from the study because of missing data or loss to follow-up. This exclusion may also affect the results. Fourth, in this study, functional analyses were not performed to validate the pathogenicity of genetic variants. Fifth, it may be rather unfair to compare children with adults, because children are much younger and with fewer years of exposure to FH and thus less likely to have events. The results from an observational nature of this study comparing much younger children with adults with FH needs to be carefully interpreted, and while

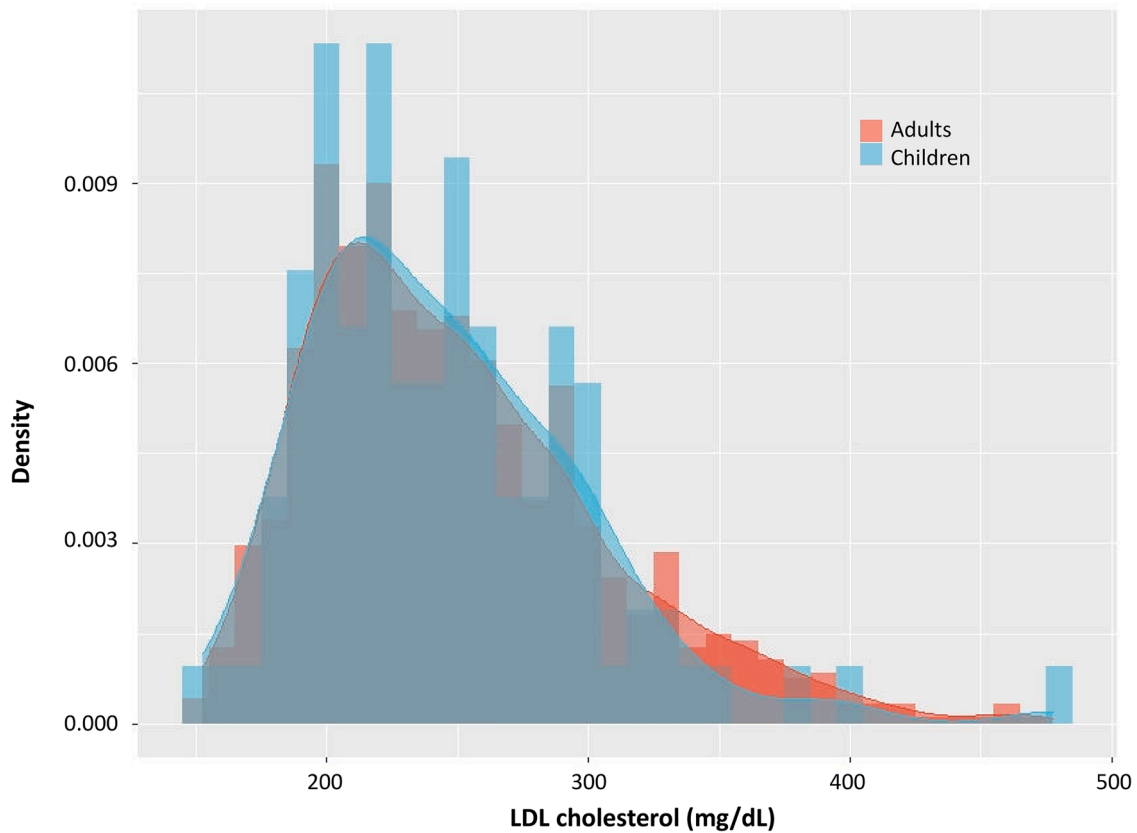


Fig. 1. Histograms with density. Red indicates adults. Blue indicates children. The X-axis represents LDL cholesterol (mg/dL). The Y-axis represents density.

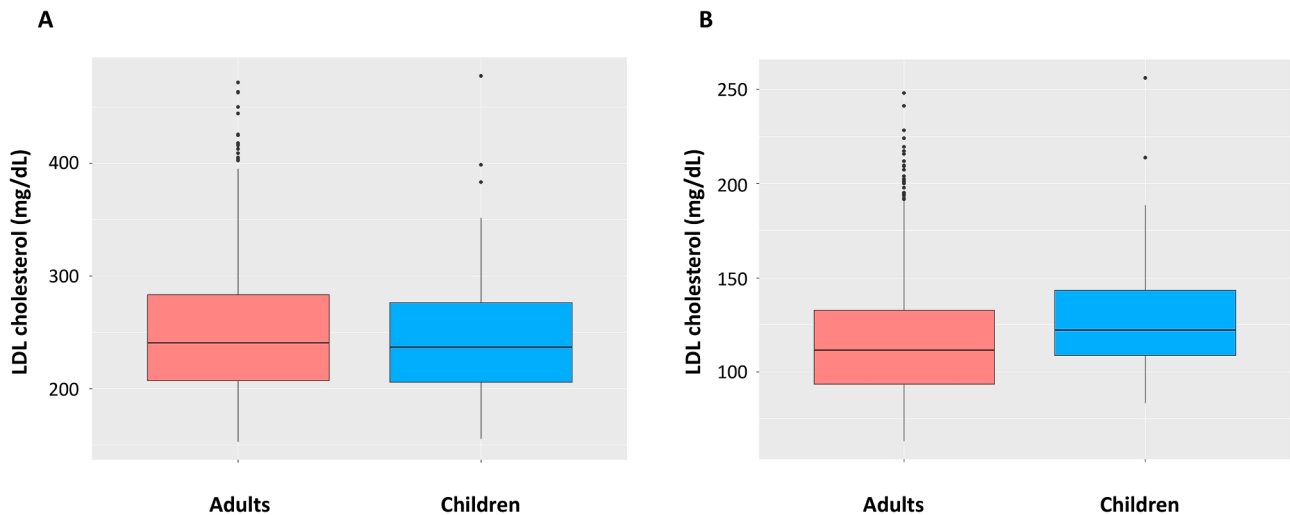


Fig. 2. LDL cholesterol levels at baseline and during follow-up (A) At baseline: Red indicates adults. Blue indicates children. (B) During follow-up: Red indicates adults. Blue indicates children.

supportive of guidelines to begin treatment as early as possible, does not prove such early treatment will result in better outcomes.

In conclusion, we found that patients with FH diagnosed during childhood exhibited significantly better prognoses than those diagnosed during adulthood despite their having lower LDL cholesterol levels under more intensive lipid-lowering therapies. Our study strongly supports the current clinical guidelines of FH that recommend the initiation of LDL-lowering therapy during childhood.]

Author contributions

H.T. and M.K. conceived the presented idea. H.T., N.K., K.Y., A.N., A. N., S.U., K.S., K.H., N.F., M.T., and M.K. collected clinical data. H.T., N. K, K.Y., and A.N. performed genetic analyses. H.T. performed statistical analyses. M.T. and M.K. supervised the findings of this work. All authors contributed to the writing of the manuscript. All authors discussed the results and contributed to the final manuscript.

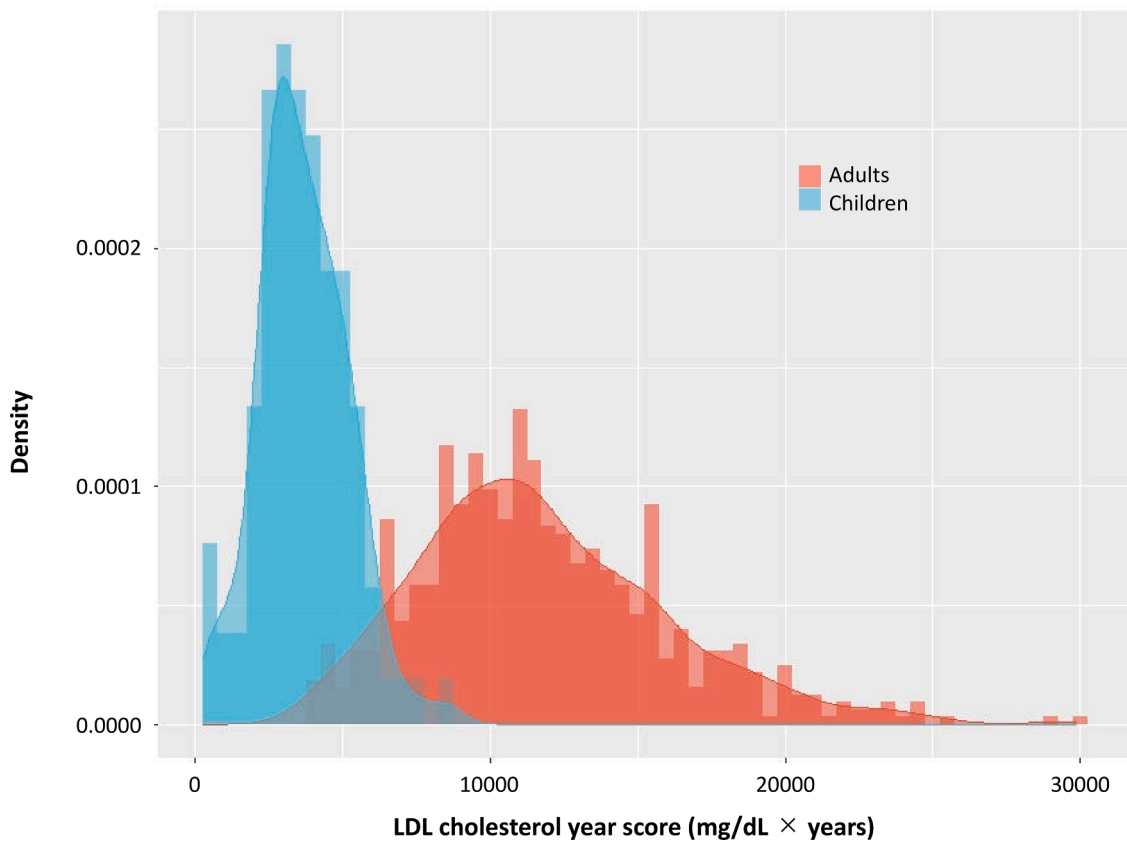


Fig. 3. Histograms with density (the LDL cholesterol year score at baseline). Red indicates adults. Blue indicates children. The X-axis represents the LDL cholesterol year score (mg/dL × years). The Y-axis represents density.

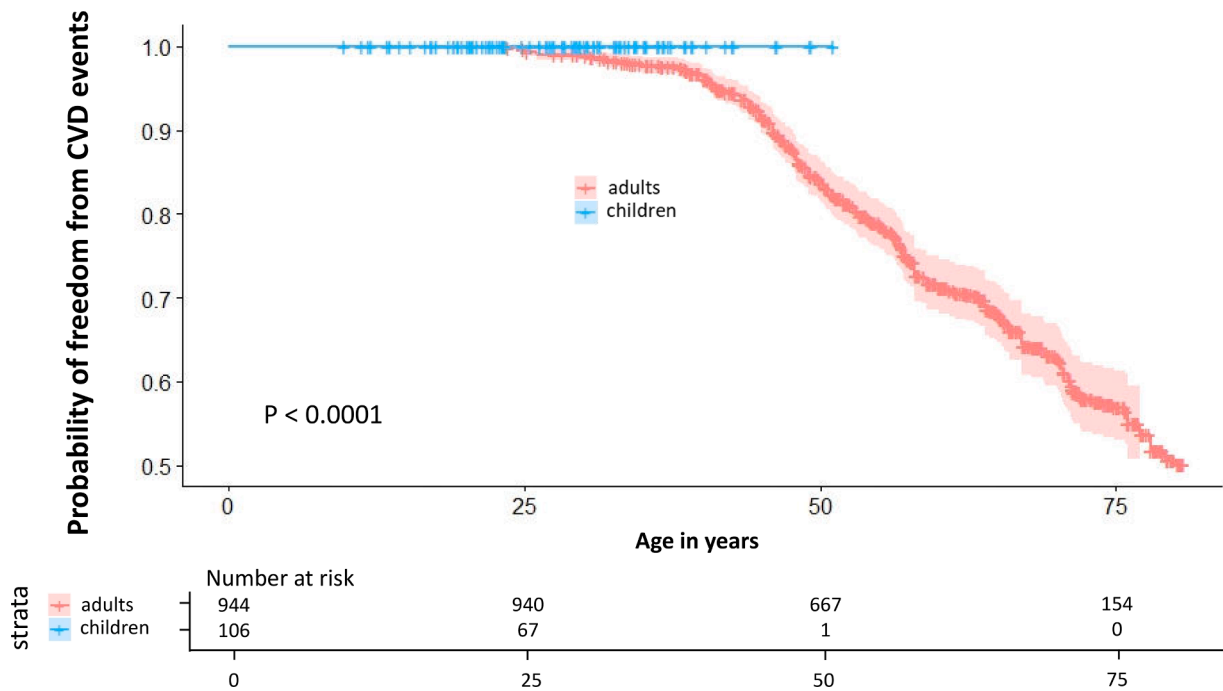


Fig. 4. Age-dependent probability of MACE. We quantified the age-dependent probability of MACE in children and adults. Red indicates adults. Blue indicates children.

Declaration of Competing Interest

The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

Previous presentations

None.

Sources of support

This work has been supported by JSPS KAKENHI (20H03927, 21H03179, and 22H03330), a grant from the Ministry of Health, Labour and Welfare (MHLW) of Japan (Science Research Grant for Research on Rare and Intractable Diseases), the Japanese Circulation Society (project for genome analysis in cardiovascular diseases) and also the Japan Agency for Medical Research and Development (20314864, and 22672854) to Dr. Hayato Tada.

Disclaimers

None.

Acknowledgments

We are thankful to Ms. Kazuko Honda and Mr. Sachio Yamamoto for their outstanding technical assistance.

Data availability statement

Requests to access the datasets should be directed to Dr. Hayato Tada.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.ajpc.2022.100434](https://doi.org/10.1016/j.ajpc.2022.100434).

References

- [1] Mabuchi H. Half a century tales of familial hypercholesterolemia (FH) in Japan. *J Atheroscler Thromb* 2017;24:189–207.
- [2] Nohara A, Tada H, Ogura M, Okazaki S, Ono K, Shimano H, et al. Homozygous familial hypercholesterolemia. *J Atheroscler Thromb* 2021;28:665–78.
- [3] Beheshti SO, Madsen CM, Varbo A, Nordestgaard BG. Worldwide prevalence of familial hypercholesterolemia: meta-analyses of 11 million subjects. *J Am Coll Cardiol* 2020;75:2553–66.
- [4] Hu P, Dharmayat KI, Stevens CAT, Sharabiani MTA, Jones RS, Watts GF, 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.
- [5] Raal FJ, Hovingh GK, Catapano AL. Familial hypercholesterolemia treatments: guidelines and new therapies. *Atherosclerosis* 2018;277:483–92.
- [6] Anagnostis P, Vaitis K, Kleitsiotti P, Mantsiou C, Pavlogiannis K, Athyros VG, et al. Efficacy and safety of statin use in children and adolescents with familial hypercholesterolemia: a systematic review and meta-analysis of randomized-controlled trials. *Endocrine* 2020;69:249–61.
- [7] Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: a report of the American College of Cardiology/American Heart Association task force on clinical practice guidelines. *Circulation* 2019;139:e1082–143.
- [8] Wiegman A, Gidding SS, Watts GF, Chapman MJ, Ginsberg HN, Cuchel M, et al. Familial hypercholesterolemia in children and adolescents: gaining decades of life by optimizing detection and treatment. *Eur Heart J* 2015;36:2425–37.
- [9] Harada-Shiba M, Ohta T, Ohtake A, Ogura M, Dobashi K, Nohara A, et al. Guidance for pediatric familial hypercholesterolemia 2017. *J Atheroscler Thromb* 2018;25:539–53.
- [10] Luijckx IK, Wiegman A, Kusters DM, Hof MH, Groothoff JW, de Groot E, et al. 20-year follow-up of statins in children with familial hypercholesterolemia. *N Engl J Med* 2019;381:1547–56.
- [11] Harada-Shiba M, Arai H, Ishigaki Y, Ishibashi S, Okamura T, Ogura M, et al. Guidelines for diagnosis and treatment of familial hypercholesterolemia. *J Atheroscler Thromb* 2018;25:751–70.
- [12] Araki E, Goto A, Kondo T, Noda M, Noto H, Origasa H, et al. Japanese clinical practice guideline for diabetes 2019. *J Diabetes Investig* 2020;11:1020–76.
- [13] Tada H, Okada H, Nohara A, Yamagishi M, Takamura M, Kawashiri MA. Effect of cumulative exposure to low-density lipoprotein-cholesterol on cardiovascular events in patients with familial hypercholesterolemia. *Circ J* 2021;85:2073–8.
- [14] Kroon AA, van't Hof MA, Demacker PN, Stalenhoef AF. The rebound of lipoproteins after LDL-apheresis. Kinetics and estimation of mean lipoprotein levels. *Atherosclerosis* 2000;152:519–26.
- [15] Tada H, Kawashiri MA, Nomura A, Teramoto R, Hosomichi K, Nohara A, et al. Oligogenic familial hypercholesterolemia, LDL cholesterol, and coronary artery disease. *J Clin Lipidol* 2018;12:1436–44.
- [16] Yamamoto T, Shimojima K, Ondo Y, Imai K, Chong PF, Kira R, 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.
- [17] Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical genetics and genomics and the association for molecular pathology. *Genet Med* 2015;17:405–24.
- [18] Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis society. *Eur Heart J* 2013;34:3478–90.
- [19] Tada H, Okada H, Nomura A, Nohara A, Yamagishi M, Takamura M, et al. Prognostic impact of cascade screening for familial hypercholesterolemia on cardiovascular events. *J Clin Lipidol* 2021;15:358–65.
- [20] Tada H, Kojima N, Yamagami K, Nomura A, Nohara A, Usui S, et al. Effects of different types of pathogenic variants on phenotypes of familial hypercholesterolemia. *Front Genet* 2022;13:872056.