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Universal Screening for Familial Hypercholesterolemia in Children

Gašper Klančar, BSc,*† Urh Grošelj, MD,* Jernej Kovač, PhD,† Nevenka Bratanič, MD,* Nataša Bratina, MD,* Katarina Trebušak Podkrajšek, PhD,†‡ Tadej Battelino, MD*§

ABSTRACT

BACKGROUND Individuals with familial hypercholesterolemia (FH) who are untreated have up to 100-fold elevated risk for cardiovascular complications compared with those who are unaffected. Data for identification of FH with a universal screening for hypercholesterolemia in children are lacking.

OBJECTIVES This study sought genetic identification of FH from a cohort of children with elevated serum total cholesterol (TC) concentration, detected in a national universal screening for hypercholesterolemia.

METHODS Slovenian children born between 1989 and 2009 (n = 272) with TC >6 mmol/l (231.7 mg/dl) or >5 mmol/l (193.1 mg/dl) plus a family history positive for premature cardiovascular complications, identified in a national universal screening for hypercholesterolemia at 5 years of age were genotyped for variants in *LDLR*, *PCSK9*, *APOB*, and *APOE*.

RESULTS Of the referred children, 57.0% carried disease-causing variants for FH: 38.6% in *LDLR*, 18.4% in *APOB*, and none in *PCSK9*. Nine novel disease-causing variants were identified, 8 in *LDLR*, and 1 in *APOB*. Of the remaining participants, 43.6% carried the APOE E4 isoform. Estimated detection rate of FH in the universal screening program from 2009 to 2013 was 53.6% (95% confidence interval [CI]: 34.5% to 72.8%), peaking in 2013 with an upper estimated detection rate of 96.3%. Variants in *LDLR*, *APOB*, or the APOE E4 isoform occurred in 48.6%, 60.0%, and 76.5%, respectively, of patients with a family history negative for cardiovascular complications.

CONCLUSIONS Most participants who were referred from a national database of universal screening results for hypercholesterolemia had genetically confirmed FH. Data for family history may not suffice for reliable identification of patients through selective and cascade screening. (J Am Coll Cardiol 2015;66:1250–7) © 2015 by the American College of Cardiology Foundation. Published by Elsevier. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Individuals with untreated familial hypercholesterolemia (FH) have up to 100-fold increased risk for developing atherosclerosis and cardiovascular disease in early adulthood compared with unaffected individuals (1). Early diagnosis and management reduce this risk (2,3). The prevalence of FH in the general population is estimated to be between 1 in 200 (4,5) and 1 in 500 (4). FH is an autosomal dominant disorder clinically diagnosed by elevated concentration of serum total cholesterol (TC) and/or low-density lipoprotein (LDL) cholesterol, possible family history of premature cardiovascular complications, possible presence of xanthomas and corneal arcus, and/or causative variants in genes implicated in FH (4,6). Such variants are found predominantly in the gene encoding the LDL receptor (*LDLR*), which binds and clears LDL particles from the blood (7). A few patients have a common disease-causing variant of apolipoprotein B (*APOB*), p.Arg3527Gln, or disease-causing variants in the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene (8). APOB is an integral component of LDL particle

From the *Department of Pediatric Endocrinology, Diabetes and Metabolic Diseases, University Children's Hospital, UMC Ljubljana, Ljubljana, Slovenia; †Unit of Special Laboratory Diagnostics, University Children's Hospital, UMC Ljubljana, Ljubljana, Slovenia; ‡Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; and the §Department of Pediatrics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia. This work was supported in part by Slovenian National Research Agency, Bleiweisova cesta 30, SI-1000 Ljubljana, Slovenia, grants P3-0343, J3-4116, J3-6800, and J3-6798. The authors have reported that they have no relationships relevant to the contents of this paper to disclose.
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and a ligand for the LDL receptor (9), whereas PCSK9 regulates LDL receptor membrane concentration (10). Additionally, the interaction of common small-effect LDL cholesterol-elevating alleles in various genes may contribute to multifactorial disease development (11,12), with the apolipoprotein E (APOE) E4 isoform being the most recognized multivariant cause of hypercholesterolemia (13).

SEE PAGE 1258

Various screening strategies have been proposed to identify children with FH. Only implementations of partial (14) and selective screenings primarily on the basis of family history are reported (15). These approaches lack the strength of identifying patients without known family history. Therefore, the National Heart, Lung, and Blood Institute (16), the National Lipid Association Expert Panel (17), and the European Expert Panel (15) proposed universal screening as a preferred method of screening for hypercholesterolemia in primary prevention efforts. Slovenia (population 2 million) started universal screening for hypercholesterolemia in 5-year-old children in 1995 (15). Screening was gradually implemented throughout the whole country (18), reaching an estimated majority of 5-year-old children (approximately 20,000) during 2013. Data for the efficacy of a national universal screening for detection of FH are lacking.

We sought to identify individuals with FH from a cohort with elevated serum TC levels detected in the Slovenian national universal screening for hypercholesterolemia in 5-year-old children.

METHODS

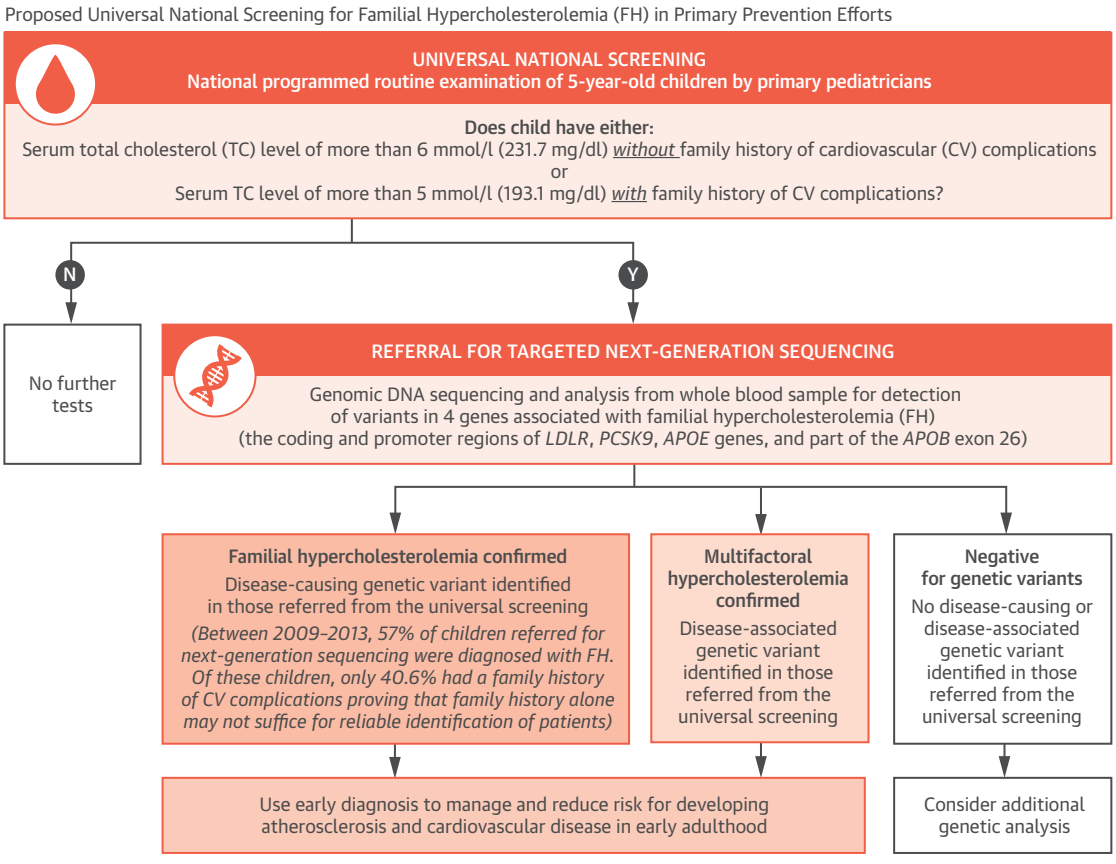
STUDY POPULATION AND DESIGN. Measurement of fasting serum TC was part of the national programmed routine examination of 5-year-old children by primary pediatricians (Slovenia national universal screening for hypercholesterolemia) (**Central Illustration**). According to national guidelines, participants with either a serum TC level of >6 mmol/l (231.7 mg/dl) without a family history of premature cardiovascular complications or with a TC level of >5 mmol/l (193.1 mg/dl) with a family history positive for premature cardiovascular complications (18) were referred after a consultation with their parents to a tertiary pediatric outpatient clinic. Data for participants' family histories were obtained in direct structured conversations with the parents. Participants' weight, body mass index, serum lipid profile (TC, LDL, high-density cholesterol [HDL], triglyceride [TG]), and whole-blood samples for targeted, next-generation sequencing (NGS) were obtained at the tertiary pediatric outpatient clinic. Simon Broome Register criteria (1) were used for assessment of family history. Positive family history was defined as myocardial infarction before 50 years of age in any second-degree relative or before 60 years of age in any first-degree relative or as TC >7.5 mmol/l (289.6 mg/dl) in any first- or second-degree relative (first-degree relation included parent, offspring or sibling; second-degree relation included grandparent, grandchild, nephew, niece, or half-sibling). Written informed consent was obtained from all parents or legal guardians. Principles of the Declaration of Helsinki were followed, and the Slovenian National Medical Ethics Committee approved the study (numbers 25/12/10 and 63/07/13).

ABBREVIATIONS AND ACRONYMS

APOB = apolipoprotein B
APOE = apolipoprotein E
FH = familial hypercholesterolemia
HDL = high-density lipoprotein
LDL = low-density lipoprotein
LDLR = low-density lipoprotein receptor
NGS = next-generation sequencing
PCSK9 = proprotein convertase subtilisin/kexin type 9
TC = total cholesterol
TG = triglyceride

DNA SEQUENCING AND ANALYSIS. Genomic DNA was isolated from whole-blood samples according to established laboratory protocols using FlexiGene isolation kit (Qiagen, Hilden, Germany). NGS is a high-throughput method based on simultaneous sequencing of numerous candidate genes or genomic intervals in a parallel fashion, effectively balancing cost and data quality for population-targeted sequencing studies (19). Samples for NGS were prepared following the manufacturer's protocol using ADH MASTR ready-to-use NGS-based molecular assay (Multiplicom NV, Niel, Belgium) for detection of variants in 4 genes associated with FH (encompassing coding and promoter regions of *LDLR*, *PCSK9*, *APOE* and part of *APOB* exon 26). Samples were sequenced using a MiSeq sequencer with MiSeq reagent kit version 2 (Illumina, San Diego, California) following the manufacturer's protocol, including recommendations for quality control parameters. The presence of copy number variations in *LDLR* was analyzed using NextGENe (Softgenetics, State College, Pennsylvania) and confirmed by Multiplex ligation-dependent probe amplification with an LDLR-P062 kit (MRC-Holland, Amsterdam, the Netherlands). Variants reported in disease-specific databases (20) and the Human Gene Mutation Database (HGMD) (Institute of Medical Genetics, School of Medicine, University of Cardiff, Cardiff, Wales) as unequivocally disease-causing were classified as pathogenic. In silico analysis of novel variants was performed with PolyPhen-2 (Harvard University, Cambridge, Massachusetts), SIFT (J. Craig Venter Institute, San Diego, California), and MutationTaster (University of Medicine, Berlin, Germany) bioinformatic tools. Variants declared to be disease-associated by at least 2 analytical algorithms were

CENTRAL ILLUSTRATION **Detection of Familial Hypercholesterolemia From a Universal National Screening for Hypercholesterolemia in Children**



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A national universal screening for hypercholesterolemia in children 5 years of age (referral criteria: serum total cholesterol [TC] level >6 mmol/l [231.7 mg/dl] without a family history of premature cardiovascular (CV) complications, or serum TC level >5 mmol/l [193.1 mg/dl] together with a family history of premature cardiovascular complications) identified a disease-causing genetic variant for familial hypercholesterolemia (FH) in 57.0% of children, with an additional one-fifth identified with the most common multifactorial form of hypercholesterolemia, amounting to >75.0% of referred participants carrying a disease-causing or disease-associated genetic variant. Assuming the commonly reported FH incidence numbers, the predicted detection rate from the data for the previous 5 years (2009 to 2013) was 53.6% (95% confidence interval [CI]: 34.5% to 72.8%), reaching its peak in 2013 (children born in 2008), with an upper estimated detection rate of 96.3% for an incidence of 1 in 500; nevertheless, when a greater incidence of 1 in 200 was assumed, the upper estimated detection rate was only 38.5%. Relative risk (RR) of having the disease-causing genetic variant when a participant had a CV-positive familial history was 1.53 (RR: 1.529; 95% CI: 1.25 to 1.88; p = 0.0001). *APOB* = presence of disease-causing variant in the apolipoprotein B gene; APOE E4 isoform = presence of disease-associated variant in the apolipoprotein E gene; *LDLR* = presence of disease-causing variant in the LDL receptor gene; Other = no presence of disease-causing or -associated variant in *LDLR*, *APOB*, *PCSK9*, or *APOE*.

classified as novel causative variants. Single-nucleotide variants and small duplications/deletions with potentially disease-causing effect were confirmed by targeted Sanger DNA sequencing.

STATISTICAL ANALYSIS. Descriptive statistics (means, standard deviations, and ratios) were used to characterize the investigated population. Assumption of equal proportions of detected FH population and total population enrolled in the national universal screening was made on the basis of the reported incidence, number of referred children, and number of live-born children publicly available at the Statistical Office of the Republic of Slovenia to estimate approximate number of children enrolled in the universal screening program in the previous 5 years (2009 to 2013), as exact data were not available. The detection rate of the universal screening in the studied population was evaluated by the number of children

TABLE 1 Clinical and Genetic Characteristics of the Study Cohort

		Familial Hypercholesterolemia* n = 155 (57.0%)		Multifactorial Hypercholesterolemia n = 117 (43.0%)	
	Study Cohort	*LDLR*	*APOB*	APOE E4 Isoform	Other†
Patients	272 (100.0)	105 (38.6)	50 (18.4)	51 (18.7)	66 (24.3)
Female	149 (54.8)	45 (42.9)	18 (36.0)	24 (47.1)	35 (53.0)
At universal screening for hypercholesterolemia at 5 years of age					
TC, mmol/l	7.0 ± 3.4	7.9 ± 1.2	7.2 ± 0.8	6.7 ± 0.8	6.6 ± 0.9
TC, mg/dl	258.7 ± 131.3	305.0 ± 46.3	278.0 ± 30.9	258.7 ± 30.9	254.8 ± 34.8
Family history‡					
Positive	90 (33.1)	46 (43.8)	17 (34.0)	11 (21.6)	13 (19.7)
Negative	168 (61.8)	51 (48.6)	30 (60.0)	39 (76.5)	51 (77.3)
Data NA	14 (5.1)	8 (7.6)	3 (6.0)	1 (2.0)	2 (3.0)
At first evaluation at the tertiary outpatient clinic					
Age, yrs	7.3 ± 3.1	7.0 ± 3.2	7.4 ± 3.4	7.6 ± 2.9	7.7 ± 2.9
BMI	0.6 ± 1.2	0.5 ± 1.2	0.3 ± 1.2	0.6 ± 1.3	0.7 ± 1.4
TC, mmol/l	6.5 ± 1.2	7.3 ± 1.2	6.6 ± 0.9	5.9 ± 0.7	5.7 ± 0.8
TC, mg/dl	251.0 ± 46.3	281.9 ± 46.3	354.8 ± 34.8	216.2 ± 27.0	220.1 ± 30.9
LDL, mmol/l	4.6 ± 1.2	5.4 ± 1.3	4.7 ± 0.8	3.8 ± 0.7	3.7 ± 0.8
LDL, mg/dl	177.6 ± 46.3	208.5 ± 50.2	181.5 ± 30.9	146.7 ± 27.0	142.9 ± 30.9
HDL, mmol/l	1.5 ± 0.4	1.4 ± 0.4	1.4 ± 0.3	1.5 ± 0.4	1.5 ± 0.3
HDL, mg/dl	57.9 ± 15.4	54.1 ± 15.4	54.1 ± 11.6	57.9 ± 15.4	57.9 ± 11.6
Non-HDL, mmol/l	5.1 ± 1.2	5.8 ± 1.2	5.2 ± 0.8	4.4 ± 0.7	4.2 ± 0.9
Non-HDL, mg/dl	196.9 ± 46.3	223.9 ± 46.3	200.8 ± 30.9	169.9 ± 27.0	162.2 ± 34.8
TG, mmol/l	1.2 ± 0.8	1.1 ± 0.6	1.2 ± 0.9	1.3 ± 0.9	1.2 ± 0.8
TG, mg/dl	106.2 ± 70.8	97.4 ± 53.1	106.2 ± 79.7	115.1 ± 79.7	106.2 ± 70.8

Values are n (%) or mean ± SD. Non-HDL was calculated as [total cholesterol − HDL]. SI conversion factors: to convert TC, LDL, HDL, and non-HDL to mg/dl, multiply by 38.61. To convert TG to mg/dl, multiply by 88.50. *No participants had disease-causing variants in the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene. †No presence of disease-causing or disease-associated variants in the *LDLR*, *APOB*, *PCSK9*, or *APOE* gene was found. ‡Simon Broome Register criteria (1) were used for assessment of family history. Positive family history was defined as myocardial infarction before 50 years of age in any second-degree relative or before 60 years of age in any first-degree relative or as serum TC concentration of >7.5 mmol/l (289.6 mg/dl) in any first- or second-degree relative (first-degree relatives included parent[s], offspring, or sibling; second-degree relatives includes grandparent, grandchild, nephew, niece, or half-sibling).

APOB = presence of disease-causing variant in the apolipoprotein B gene; APOE E4 isoform = presence of disease-associated variant in the apolipoprotein E gene; BMI = body mass index; HDL = high-density cholesterol; *LDLR* = presence of disease-causing variant in the LDL receptor gene; LDL = low-density cholesterol, NA = not available; non-HDL = non-high-density cholesterol; TC = total cholesterol; TG = triglyceride.

per generation referred to the tertiary outpatient clinic compared to the potential FH population estimated from the registry of live-born children and was reported as 1 in 500 and 1 in 200 incidence of FH (4,5). Data deviation from normal was evaluated with the D'Agostino and Pearson omnibus normality test. Differences in disease-associated variant accumulation between different groups of participants (sex and cardiovascular-positive family history), corresponding odds ratio (OR) and/or relative risk (RR), were evaluated using Fisher exact test for 2 × 2 contingency tables. Logistic regression model was applied (21) to calculate OR and RR with corresponding 95% confidence intervals (CIs). Genotype-associated differences among TC, LDL, HDL, and non-HDL levels and standard deviation score of body mass index were statistically evaluated using Kruskal-Wallis test with Dunn's multiple comparisons test. A p value <0.05 was used as an indicator of statistical significance. All statistical calculations were made in Prism version 6.01 software (GraphPad, Inc., La Jolla, California).

RESULTS

Altogether, 272 unrelated participants born between 1989 and 2009 were included in the study. Characteristics of participants are shown in Table 1. Serum TC levels at universal screening for hypercholesterolemia at age 5 and fasting lipid profile (TC, LDL, HDL, and TG levels) were measured at first admission to a tertiary pediatric outpatient clinic. Participants were referred to the tertiary pediatric outpatient clinic at 7.3 ± 3.1 years of age. A cardiovascular complications-positive family history according to Simone Broome Register criteria was identified in 33.1% of participants.

PREDICTION OF NATIONAL UNIVERSAL SCREENING DETECTION RATE. The national universal screening in Slovenia was introduced gradually, and the referral rate to the tertiary institution reached its expected level only in 2013. An estimated 33,000 to 70,000 of 96,690 live-born children were enrolled at

TABLE 2 Novel Heterozygous Disease-Causing Variants in *LDLR* and *APOB*

Gene	Number of Variants (%)	Nucleotide Change	Protein Change	Localization	Protein Domain	In Silico Novel Variant Analysis		
						SIFT Prediction (Score)	PolyPhen-2 Prediction (Score)	MutationTaster
LDLR	2 (1.9)	c.300C > G	p.Asp100Glu	Exon 3	LDL receptor class A2	Deleterious (0)	Probably damaging (0.982)	Disease causing
LDLR	1 (1.0)	c.408C > T	p.Asp136Asp‡	Exon 4	LDL receptor class A3	NA	NA	Disease causing
LDLR	1 (1.0)	c.827G > T	p.Cys276Phe	Exon 6	LDL receptor class A7	Deleterious (0)	Probably damaging (0.996)	Disease causing
LDLR	1 (1.0)	c.972C > G	p.Gly324Gly‡	Exon 7	EGF-like 1	NA	NA	Disease causing
LDLR	1 (1.0)	c.1470G > T	p.Trp490Cys	Exon 10	LDL receptor class B3	Deleterious (0)	Probably damaging (1)	Disease causing
LDLR	1 (1.0)	c.1587-1G > C*	NA	Intron 10	NA	NA	NA	NA
LDLR	1 (1.0)	c.1706-1G > C†	NA	Intron 11	NA	NA	NA	NA
APOB	1 (2.0)	c.10370C > G	p.Ser3457Cys	Exon 26	LDLR binding side	Deleterious (0.03)	Probably damaging (0.976)	Polymorphism
Double heterozygous								
LDLR	1 (1.0)	c.58G>A§	p.Gly20Arg	Exon 1				
		c.1487G>A	p.Gly496Asp	Exon 10	LDL-receptor class B3	Deleterious (0.02)	Probably damaging (0.998)	Disease causing

*Variant c.1587-1G > A at the same location is disease-causing according to a disease-specific database (19) and the Human Gene Mutation Database (HGMD; Institute of Medical Genetics, School of Medicine, University of Cardiff, Cardiff, Wales). †Variants c.1706-1G > A and c.1706-1G > T at the same location are disease-causing according to a disease-specific database (19) and the HGMD database. ‡Variant is predicted to have potential alterations on splicing by Human Splicing Finder. §Known disease-causing variant.

Abbreviations as in Table 1.

5 years of age in the national universal screening from 2009 to 2013. Assuming an FH incidence of 1 in 500, the predicted detection rate from the data for the previous 5 years (2009 to 2013) was 53.6% (95% CI: 34.5% to 72.8%), reaching its peak in 2013 (children born in 2008) with an upper estimated detection rate of 96.3%. Assuming a greater incidence of 1 in 200, the upper estimated detection rate was only 38.5%.

GENETIC CHARACTERIZATION. Of 272 participants, 105 (38.6%) had heterozygous disease-causing variants in *LDLR*, 50 (18.4%) in *APOB*, and none in *PCSK9*. Twenty-three known (Online Table 1) and 8 novel disease-causing variants (**Table 2**) were identified in *LDLR*. Six novel disease-causing variants were missense and 2 were intronic. Four participants had multiple variants, 2 combinations of known disease-causing variants (Online Table 1) and 1 combination of a known and a novel disease-causing variant (**Table 2**). Copy number variations were identified in 3 patients, 2 had a deletion of exons 2 to 18, and 1 had a deletion of the whole *LDLR* coding region (Online Table 1). Two known missense variants (Online Table 1) and 1 novel disease-causing variant (**Table 2**) were identified in *APOB*. In the remaining participants without disease-causing variants, 51 (18.7%) were carriers of the hypercholesterolemia-associated APOE E4 isoform, whereas 66 participants (24.3%) had conditions that remained genetically undiagnosed (**Table 1**). A cardiovascular complication-negative family history according to Simone Broome Register criteria (1) was associated with 48.6%, 60.0%, and 76.5% of patients with disease-causing or disease-associated variants in *LDLR*, *APOB*, or APOE E4 isoform, respectively (**Table 1**). Relative risk of having a disease-causing genetic variant when a participant had a positive family history was 1.53 (RR: 1.529; 95% CI: 1.25 to 1.88; $p = 0.0001$). The OR for having a disease-causing genetic variant in participants with a TC level >6 mmol/l (231.7 mg/dl) was 7.71 compared with an OR of 7.71 (95% CI: 1.75 to 33.35; $p = 0.0013$) for the participants with TC levels between 5 and 6 mmol/l (193.1 and 231.7 mg/dl, respectively). Among 33 patients with TC levels between 5 and 6 mmol/l (193.1 and 231.7 mg/dl, respectively), 2 had disease-causing variants in *LDLR* and none in *APOB*. Among 177 patients with TC levels between 6 and 8 mmol/l (231.7 and 308.9 mg/dl, respectively), 31.1% had disease-causing variant in *LDLR* and 23.7% in *APOB*. Among 62 patients with a TC level >8 mmol/l (308.9 mg/dl), 77.4% had disease-causing variants in *LDLR* and 12.9% in *APOB*. Carriers of *LDLR* and *APOB* disease-causing genetic variants had on average higher TC levels at universal screening for hypercholesterolemia than noncarriers (7.7 ± 1.2 mmol/l vs. 6.6 ± 0.9 mmol/l, respectively [297.3 ± 46.3 mg/dl vs. 254.8 ± 34.8 mg/dl, respectively]; $p < 0.0001$). Additionally, carriers of *LDLR* and *APOB* disease-causing genetic variants had on average higher concentrations of TC (7.0 ± 1.1 mmol/l vs. 5.8 ± 0.8 mmol/l, respectively [272.0 ± 43.7 mg/dl vs. 222.4 ± 29.6 mg/dl, respectively]; $p < 0.0001$), LDL (5.2 ± 1.2 mmol/l vs. 3.8 ± 0.8 mmol/l, respectively [198.9 ± 45.5 mg/dl vs. 145.4 ± 29.1 mg/dl, respectively]; $p < 0.0001$), and non-HDL (5.6 ± 1.2 mmol/l vs. 4.3 ± 0.8 mmol/l, respectively [217.3 ± 45.0 mg/dl vs. 164.9 ± 31.3 mg/dl, respectively]; $p < 0.0001$) upon initial evaluation at the tertiary pediatric outpatient clinic than noncarriers. Associations among

sex, serum TC levels, standard deviation score of body mass index, and presence of causative genetic variants in *LDLR* and *APOB* were not statistically significant.

DISCUSSION

This is the first study to evaluate genetic identification of FH by a national universal screening for hypercholesterolemia. Children were screened at 5 years of age, yet the mean referral age to the tertiary pediatric outpatient clinic was 7.3 years of age, indicating the requirement of additional educational intervention to promote more rapid referral of patients from primary care institutions for further clinical evaluation, along with a record of children not referred or whose parents refused referral. Serum TC level discriminates best between people with and without FH in the interval from 1 to 9 years of age, making it the optimal period for universal screening for hypercholesterolemia. Serum TC can identify 88.0% to 96.0% of cases, with a false positive rate between 0.1% and 1.0% (21). In addition, once an affected child is identified, 96.0% of parents with the disorder could be detected by identifying the parent with the higher cholesterol level (child-parent screening strategy) (22). All parents and older siblings from children identified as having FH in our study were referred for evaluation. The simulated detection rate of FH in our national universal screening for hypercholesterolemia on the basis of commonly reported incidence of 1 in 500 was more than 96.0% in the last year and allowed for a reliable identification of the risk population. Nevertheless, when an incidence rate of 1 in 200 is assumed, only 38.5% of the at-risk population was detected. An estimation of the total population enrolled in the national universal screening from 2009 to 2013 indicated that only approximately one-half of the total population was reached. Consequently, continuation and further improvements of screening and referral strategies will provide more data for the real FH incidence in the Slovenian population and improve clinical algorithms in FH risk management. The optimal age for a universal screening for hypercholesterolemia remains to be determined (15). Early dietary and lifestyle intervention along with cholestyramine assessment at 6 years of age (23) and use of statins after 8 years of age (24) in FH are beneficial, and therefore a diagnosis and intervention before pubertal age seems prudent (4,15,18).

A disease-causing genetic variant for FH was identified in 57% of participants referred from the universal screening, with an additional one-fifth identified with the most common multifactorial form of hypercholesterolemia, amounting to >75.0% of referred participants carrying a disease-causing or disease-associated genetic variant. Proportions of disease-causing variants in the present study were on the lower end of reported population data for *LDLR* and on the higher end in the same reports for *APOB*. For Czech (25), Polish (26), French (27), Spanish (28), and Italian (29) populations, the reported disease-causing variant frequencies were 23.9%, 45.0%, 73.9%, 96.4%, and 97.4%, respectively, and were 11.8%, 6.0%, 6.6%, 3.5%, and 2.2%, respectively, for *APOB*. Lack of the disease-causing *PCSK9* variant in the Slovenian population was similar to that in the Finnish (30) and Greek (31) populations. The *LDLR* and *APOB* spectra of disease-causing variants are diverse. In large countries such as Italy (29), Spain (28), France (27), or Poland (25), more than 100 disease-causing variants contribute to a heterogeneous pool, whereas in populations such as those in Finland (30) or Iceland (32), the diversity of disease-causing variants is smaller, with less than 10 disease-causing variants present. Thirty-three disease-causing variants were identified in *LDLR* in the Slovenian cohort, 6 of which explained 55.2% of *LDLR*-positive cases. Two disease-causing variants were identified in *APOB*. One of these (p.Arg3527Gln) explained 96.0% of *APOB*-positive cases, probably due to its central European origin and a known migration patterns of carriers (33). Approximately 6.0% of disease-causing variants in *LDLR* and *APOB* were novel, confirming the wide variety of variants associated with the disease in other populations (4).

Almost one-fifth of the total cohort was negative for disease-causing variants in *LDLR*, *APOB*, and *PCSK9* but carried the APOE E4 isoform, emphasizing the potential contribution of *APOE* genetic variants in the multifactorial cause of hypercholesterolemia (13). The remaining 24.3% of patients in the present study had no disease-causing or disease-associated variants identified, which is in accordance with other reports in which 10.0% to 40.0% (depending on the inclusion criteria) of those with clinical diagnosis of FH were found not to be carriers of disease-causing genetic variants in *LDLR*, *APOB*, or *PCSK9* and were likely to have a polygenic or multifactorial type of hypercholesterolemia (28,34).

A significant advantage in cost reduction (approximately 3-fold decrease for the central European region) and timely identification of causative genetic variants (approximately 7-fold reduction in turnaround time in our laboratory) with the NGS compared with more widely used genetic screening methods (35) (e.g., denaturing high-pressure liquid chromatography or high resolution DNA melting analysis and Sanger sequencing) were observed.

Moreover, NGS reduced the possibility of human errors due to reduced hands-on time per sample (36).

A 56.0% diagnostic yield and a significant association of tendon xanthoma and LDL-cholesterol level with the disease-causing *LDLR* or *APOB* variant has been demonstrated in an adult cohort (34). Comparable diagnostic yield in the adult population with clinical presentation of hypercholesterolemia (34) and our pediatric population without any clinical signs indicates that a universal screening may be an efficacious strategy for early detection of FH and prevention of cardiovascular complications. Importantly, almost one-half of participants with a TC level <6 mmol/l (231.7 mg/dl) carried a disease-causing or disease-associated genetic variant. Furthermore, a cardiovascular complications-negative family history according to Simone Broome Register criteria (1) was observed in one-half to three-quarters of patients with a disease-causing or disease-associated variant in *LDLR*, *APOB*, or the APOE E4 isoform. The RR of having a disease-causing variant with a positive family history was significant but relatively low. Data for a family history may not always be a reliable indicator for identification of potential FH patients (37).

STUDY LIMITATIONS. This study was limited by a relatively small Slovenian population, as well as gradual implementation of the universal national screening for hypercholesterolemia. More data are required to optimize and evaluate the universal screening criteria and referral strategy. Additionally, a cascade screening for parents and siblings of identified affected children should be envisaged.

CONCLUSIONS

Results from the present study demonstrated for the first time that a national universal screening for hypercholesterolemia at 5 years of age identified genetically confirmed FH in more than one-half of the referred participants. Moreover, a genetic variant in *APOE* associated with hypercholesterolemia was detected in an additional one-fifth of the referred participants. Data for family history may not suffice for reliable identification of patients through selective screening. Our findings support the latest recommendations by professional forums for a universal screening for hypercholesterolemia in children. Coupled with a cascade screening for family members, universal screening for hypercholesterolemia could be a powerful approach for FH detection and prevention of cardiovascular complications. Additional studies are warranted to confirm the results of this study.

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REPRINT REQUESTS AND CORRESPONDENCE: Dr. Tadej Battelino, University Children's Hospital, Department of Pediatric Endocrinology, Diabetes and Metabolic Diseases, Bohoričeva 20, SI-1000 Ljubljana, Slovenia. E-mail: tadej.battelino@mf.uni-lj.si.

PERSPECTIVES

COMPETENCY IN SYSTEMS-BASED PRACTICE: Screening for hypercholesterolemia in children at 5 years of ages for familial hypercholesterolemia (FH) identified disease-causing variants in genes for LDLR and APOB in almost two-thirds of participants, and one-fifth were carriers of disease-associated variant encoding APOE E4 isoform. These data support proposals by the National Heart, Lung, and Blood Institute, National Lipid Association Expert Panel in the United States, and the European Expert Panel for universal screening as a preferred method of identifying FH as part of comprehensive primary prevention efforts.

TRANSLATIONAL OUTLOOK: Larger confirmatory studies are needed to determine the optimum timing and strategy for universal screening for FH using an expanded panel of FH-associated genes, and to define long-term efficacy and cost-effectiveness of this population-based approach.

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KEY WORDS cholesterol, familial hypercholesterolemia, next-generation sequencing, universal screening

APPENDIX For supplemental tables, please see the online version of this article.