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Universal screening for familial hypercholesterolemia in children: The Slovenian model and literature review

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ABSTRACT

Background and aims: Familial hypercholesterolemia (FH) is arguably the most common monogenic disorder in humans, but severely under-diagnosed. Individuals with untreated FH have an over 10-fold elevated risk of cardiovascular complications as compared to unaffected individuals; early diagnosis and timely management substantially reduce this risk.
Slovenia has gradually implemented the program of universal FH screening in pre-school children, consisting of a two step approach: (1) universal hypercholesterolemia screening in pre-school children at the primary care level; (2) genetic FH screening in children referred to the tertiary care level according to clinical guidelines (with additional cascade screening of family members). The program is presented in detail.
Methods: We analyzed retrospective data (2012–2016), to assess the efficiency of the universal FH screening program. In that period, 280 children (59.3% female) were referred to our center through the program for having TC > 6 mmol/L (231.7 mg/dL) or >5 mmol/L (193.1 mg/dL), with a positive family history of premature cardiovascular complications at the universal hypercholesterolemia screening.
Results: 170 (57.1% female) of them were fully genotyped, 44.7% had an FH disease-causing variant (28.8% in *LDLR* gene, 15.9% in *APOB*, none in *PCSK9*), one patient was LIPA positive, and 40.9% of the remaining patients carried an ApoE4 isoform; genetic analysis is still ongoing for one-third of the referred patients. For almost every child with confirmed FH, one parent had highly probable FH.
Conclusions: FH was confirmed in almost half of the referred children, detected through the universal screening for hypercholesterolemia.

1. Introduction

Individuals with heterozygous familial hypercholesterolemia (FH) have over 10-fold increased relative risk for developing atherosclerosis and cardiovascular disease (CVD) compared to unaffected individuals [1,2]. Early diagnosis and timely management substantially reduce this risk [3,4]. In the general population, the prevalence of FH is estimated to be 1/500–1/200, making it not only the most common inborn error of metabolism, but arguably also the most common monogenic disorder in humans [4,5]. It is an autosomal dominant disorder clinically diagnosed by elevated serum total cholesterol (TC) and/or LDL-cholesterol (LDL-C), possible family history of premature cardiovascular complications, possible presence of xanthomas and corneal arcus, and/or causative variants in genes implicated in FH [5,6]. Most patients are carriers of disease-causing variants in the LDL-C receptor (*LDLR*) gene [7,8].

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The minority of patients have a common disease-causing variant of apolipoprotein B (*APOB*) or disease-causing variants in proprotein convertase subtilisin/kexin type 9 (*PCSK9*) [8,9]. Additionally, the interaction of common small-effect LDL-C-elevating alleles in various genes may contribute to the polygenic disease development [10]. Up to 40% (depending on the inclusion criteria) of those with clinically diagnosed FH have not confirmed disease-causing genetic variants in *LDLR, APOB,* or *PCSK9* and are likely to have polygenic or multifactorial type of hypercholesterolemia [6,11].

Due to severe under diagnosis and under treatment of this extremely high-risk condition, urgent worldwide diagnostic FH screening with early and aggressive treatment was recommended [5]. Various screening strategies are proposed to identify FH patients, most data is existing on cascade FH screening [12]. Recently, a very promising universal child-parent screening at the immunizations was shown to be very effective in a pilot study [13]. Slovenia is currently, to the best of our knowledge, the only country with successfully implemented universal hypercholesterolemia screening program to detect FH [14]. The polygenic form of hypercholesterolemia might be present in FH patients without a detectable mutation in known FH genes. Calculating weighted scores of multiple LDL-C-raising common single-nucleotide polymorphisms (SNPs) can explain elevated LDL-C levels in individuals negative for LDLR, APOB, or PCSK9 mutation [15,16]. ApoE conformation is an also an important factor contributing to polygenic hypercholesterolemia [15–17].

In the first part of this article, we aimed at presenting the current Slovenian model of universal FH screening in pre-school children. In the second part, we performed a retrospective analysis of the universal screening efficacy for the period 2012–2016.

2. Materials and methods

2.1. Slovenian model of universal familial hypercholesterolemia screening in pre-school children

Slovenia (population 2 million) started universal screening for hypercholesterolemia in 5-year-old children in 1995. Measuring the TC at the age of 5 was than formally mandated by the official leaflet of Republic of Slovenia, as an obligatory part of the blood check-up at the programmed visit of all 5-year old children at the primary care pediatrician [18]. Despite the legal requirement, the hypercholesterolemia screening was only gradually implemented through the whole country and only in the last few years, after investing vast efforts to better inform primary care pediatricians, now reaching the majority of 5-year-old children (approximately 20,000/year) [15]. In 2011, routine FH genetic diagnostic was introduced at the University Children's Hospital (UCH) Ljubljana genetics laboratory, which enabled more accurate diagnostics of FH and further improved implementation of universal FH screening program [14].

The universal FH screening in Slovenia consists of a two step approach:

(1) universal hypercholesterolemia screening in pre-school children (5- or 6-year old) at their programmed visit at the primary care pediatricians (Fig. 1).
(2) genetic FH screening in children referred to the tertiary care level (lipid clinic at the UCH Ljubljana) according to clinical guidelines, with additional cascade screening of family members (Fig. 2).

The program of genetic FH screening is currently still funded by hospital genetics funds and/or research projects, but was in February 2018 approved by the Slovenian National Council of Pediatrics, which is a precondition for obtaining regular funding by health insurance which we expect in a near future.

2.2. Retrospective analysis of familial hypercholesterolemia screening program efficacy for the period 2012–2016

2.2.1. Patients and methods

Altogether, 280 children born between 2007 and 2010 were included into the retrospective analysis. Only the children referred through the universal screening of TC at the primary care level were included. Serum TC level at universal screening for hypercholesterolemia at the age 5 and lipid profile (TC, LDL-C, HDL-C, and TG) at first admission to the tertiary pediatric outpatient clinic were measured in the fasting state. The data on family history were recorded for all the participants and the Simon Broome criteria were used to evaluate it [1]. For the family history to be positive one of these criteria had to be fulfilled: myocardial infarction before 60 years in first degree relatives (parent, offspring or sibling); myocardial infarction before 50 years in any second degree relatives (grandparents, grandchildren, nephew, niece, uncle, aunt, or half-sibling); TC > 7.5 mmol/L (289.6 mg/dL) in any first or second degree relative. Written informed consent was obtained from all parents or legal guardians. The principles of the Declaration of Helsinki were followed, and the Slovenian National Medical Ethics Committee approved the study (#25/12/10 and #63/07/13).

Genomic DNA was isolated from the whole blood sample according to the established laboratory protocols using FlexiGene isolation kit (Qiagen, Germany). Samples for NGS were prepared following the manufacturer's protocol using ADH MASTR v2 ready to use NGS based molecular assay (Multiplicom, Belgium) for detection of variants in 4 genes associated with FH (the coding and promoter regions of LDLR, PCSK9, APOE genes, and part of the APOB exon 26). Samples were sequenced on MiSeq sequencer with MiSeq Reagent kit v3 (both Illumina, USA) following the manufacturer's protocol including recommendations for quality control parameters. A variant in LIPA gene was detected by targeted Sanger DNA sequencing. Variants reported in the disease-specific database [19] and HGMD Professional database (www.biobase-international.com/product/hgmd) as unequivocally disease-causing were classified as pathogenic. *In silico* analysis of novel variants was performed with PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org/www/SIFT_enst_submit.html), CADD (http://cadd.gs.washington.edu/) and MutationTaster (www.mutationtaster.org) bioinformatic tools. Variants declared as a disease associated with at least two analytical algorithms and CADD phred score >20 were classified as novel potentially causative variants. Single nucleotide variants and small duplications/deletions with potentially disease-causing effect were confirmed by targeted Sanger DNA sequencing.

Descriptive statistics (mean, 95% confidence interval [95%CI], standard deviation and ratio) was used to characterize the analyzed population. The detection rate of the universal screening in the studied population was evaluated by the number of participants per generation referred to the tertiary outpatient clinic due to the positive screening test result compared to the potential FH population estimated from the National Registry of live-born children publicly available at the Statistical Office of the Republic of Slovenia (http://www.stat.si/eng/index.asp). To visualize the TC difference between the participants with the identified *LDLR*, *APOB* causative variants and those without identified genetic cause, the population distribution was plotted and least-square fit non-linear regression was performed. Three different models (Gaussian, log (Gaussian) and Sum of two Gaussian distributions) of non-linear regression were tested to optimally represent the TC-level distribution in different disease subpopulations. Optimal model and the difference