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Screening for familial hypercholesterolaemia in childhood: Avon Longitudinal Study of Parents and Children (ALSPAC)

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ABSTRACT

Background and aims: Familial hypercholesterolaemia (FH) is an autosomal-dominant disease with frequency of 1/500 to 1/250 that leads to premature coronary heart disease. New approaches to identify FH mutation-carriers early are needed to prevent premature cardiac deaths. In a cross-sectional study of the Avon Longitudinal Study of Parents and Children (ALSPAC), we evaluated the biochemical thresholds for FH screening in childhood, and modelled a two-stage biochemical and sequencing screening strategy for FH detection.

Methods: From 5083 ALSPAC children with cholesterol measurement at age nine years, FH genetic diagnosis was performed in 1512 individuals, using whole-genome or targeted sequencing of known FH-causing genes. Detection rate (DR) and false-positive rate (FPR) for proposed screening thresholds (total-cholesterol > 1.53, or LDL-C > 1.84 multiples of the median (MoM)) were assessed.

Results: Six of 1512 sequenced individuals had an FH-causing mutation of whom five had LDL-C > 1.84 MoM, giving a verification-bias corrected DR of 62.5% (95% CI: 25–92), with a FPR of 0.2% (95% CI: 0.1–0.4). The DR for the TC cut-point of 1.53 MoM was 25% (95% CI: 3.2–65.1) with a FPR of 0.4% (95% CI: 0.2–0.6). We estimated 13 of an expected 20 FH mutation carriers (and 13 of the 20 parental carriers) could be detected for every 10,000 children screened, with false-positives reliably excluded by addition of a next generation sequencing step in biochemical screen-positive samples.

Conclusions: Proposed cholesterol thresholds for childhood FH screening were less accurate than previously estimated. A sequential strategy of biochemical screening followed by targeted sequencing of FH genes in screen-positive children may help mitigate the higher than previously estimated FPR and reduce wasted screening of unaffected parents.

1. Introduction

Familial hypercholesterolaemia (FH) is an inherited disorder with prevalence estimated from recent epidemiological[1] and sequencing studies[2,3] of 1 in 250, higher than historical estimates of 1 in 500. Autosomal dominant FH is caused by mutations in genes encoding the low-density lipoprotein receptor (*LDLR*) [4], apolipoprotein B (*APOB*) [5], and proprotein convertase subtilisin/kexin type 9 (*PCSK9*) [6]. Autosomal recessive FH is caused by mutations in the *LDLRAP1* gene[7]. Characteristics include elevated total cholesterol (TC) and LDL-C, cutaneous lipid deposition, and a

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high risk of premature coronary heart disease (CHD). Untreated FH patients exhibit a 13-fold excess risk of CHD compared to the general population[1], with FH men typically developing CHD in their 50s, and women in their 60s[8]. European guidelines recommend early high potency statin treatment to achieve maximal LDL-C reduction[9].

Pre-clinical screening of first-degree relatives of a patient (cascade testing) has been carried out effectively in Europe[10,11]. However, index cases are usually ascertained opportunistically (at clinical presentation) rather than systematically. Worldwide, fewer than 5% of FH individuals have a diagnosis, with only 12% known and treated in the UK [9].

New approaches are needed to identify affected individuals before they develop CHD. In 2007, Wald et al. proposed biochemical FH screening by measurement of TC and LDL-C in childhood (one to nine years of age), when the separation of the lipid distribution in affected and unaffected is greater than later in life[12]. The detection rate (DR), estimated for an LDL-C cut-point of 1.84 multiples of the median (MoM), or a TC of 1.53 MoM was 85% or 88% respectively, for a false positive rate (FPR) of 0.1%. It was estimated the affected parent could then be detected as the one with the higher cholesterol, with an FPR of 4%. A preliminary report based on ~200 children screened at the age of 15 months was published in 2011 [13], and the outcome of a 10,000 child screening study was reported in 2016[14].

However, in the primary report[12], the proposed biochemical thresholds were based on historic case-control data: lipid values in FH cases were ascertained from hospital clinic records, while those in controls were from unaffected siblings of patients, who may have been from a different age stratum to the cases, or from values in healthy population surveys from the same geographical region, conducted within 5 years of the case ascertainment. In addition, FH was confirmed either by clinical criteria or by mutation-detction methods that predated the more sensitive next generation DNA sequencing (NGS).

In the outcome study[14], a more relaxed case definition was adopted than in the prior meta-analysis of case-control data. A case was defined as *either* carriage of an FH mutation *or* a persistently high cholesterol, which risks mixing polygenic hypercholesterolaemia with monogenic FH. Using cholesterol both in the test and case definition also complicates assessment of screening performance. Finally, a 48 variant mutation detection panel was used rather than a sequencing approach, which runs the risk of missing cases with FH mutations not represented in the panel.

We, therefore, evaluated the performance of previously proposed LDL-C and TC thresholds for the detection of FH in a general population sample of children from the Avon Longitudinal Study of Parents and Children (ALSPAC) [15], using NGS of FH genes as the diagnostic standard.

2. Materials and methods

ALSPAC study description and the ethical approval are presented in the Supplementary Material.

2.1. Cholesterol measurement

At a mean (standard deviation, SD) age of 9.9 years (4 months), TC, LDL-C (calculated using the Friedewald equation) and other lipids and apolipoproteins were measured in non-fasting blood samples from 5083 ALSPAC children, using methods described previously[16]. Non-high-density-lipoprotein cholesterol (non-HDL-C) was calculated by subtracting HDL-C from TC.

2.2. DNA sequencing for FH-causing mutations

A 30% random sample of ALSPAC participants (N = 1503) were previously selected and 1497 (29.5%) successfully completed low-read depth whole genome sequencing (WGS, see Supplementary Material), as part of the UK10K project[3].

In addition, we conducted targeted high-read depth sequencing (see Supplementary Material) of the known FH genes (*LDLR*, *APOB*, *PCSK9,* and *LDLRAP1*) in 55 samples selected by stratified random sampling from each quartile of the LDL-C distribution, restricting to those that were also included in the UK10K project. A further 15 samples with an LDL-C>1.84 MoM, who also had a TC > 1.53 MoM, were selected for targeted NGS, giving a total of 70 with targeted sequencing data (Fig. 1), and 1512 samples with any sequencing data. No other clinical characteristics were included in the selection of these individuals. The variant interpretation methods are shown in the Supplementary Material.

2.3. Genotyping of common LDL-C-raising alleles

Since carriage of a high burden of common LDL-C-raising alleles can mimic the biochemical features of monogenic FH[17,18], and might contribute to a false positive FH diagnosis, we also obtained genotypes for six LDL-C-associated SNPs: rs629301 (*CELSR2*), rs1367117 (*APOB*), rs6544713 (*ABCG8*), rs6511720 (*LDLR*), rs429358 (*APOE*), rs7412 (*APOE*), in the whole cohort. The methods used for genotyping, generating and validating a weighted LDL-C genetic risk score were described previously[17,18].

2.4. Statistical analysis

Data were analysed using R (http://www.r-project.org) and WINPEPI[19]. We compared categorical and continuous variables using χ^2 and two sample t-tests respectively, generating *p*-values on the basis of the null hypothesis of no difference between the groups. To evaluate the performance of previously proposed LDL-C (1.84 MoM, 1.66 MoM and 1.58 MoM) and TC cut-offs (1.53 MoM, 1.42 MoM and 1.37 MoM) [12], we obtained estimates of DR, FPR, predictive value of a positive test (PPV), predictive value of a negative test (NPV), and odds of being affected given a positive test [20]. Since all samples with LDL-C >1.84 MoM or TC > 1.53 MoM underwent targeted sequencing but only a proportion of samples with LDL-C or TC below these values was sequenced, the study design was subject to verification bias. Information on the number of participants with FH mutations in the sampled group with LDL-C/TC below the pre-specified cut-points was therefore used to estimate the prevalence of FH mutations in the whole cohort by scaling. 95% confidence intervals were based on binomial probabilities.

The performance of the child-parent screening approach was evaluated with and without adjustment for verification bias and compared with previous estimates[12,14]. We also estimated the effect of misclassification of case status arising from imperfect accuracy of NGS screening performance, based on a 90% rather than 100% sensitivity of NGS, following the method of Greenland et al.[21]. The FPR of NGS was assumed to be equal to 0%, because all samples with mutations identified by NGS undergo confirmation by Sanger sequencing.

3. Results

Mean (SD) and median (interquartile range) values of LDL-C, TC, and a range of other variables were similar between the 1512 children who successfully underwent whole genome, targeted sequencing, or both, and the 3571 who did not (**Supplementary**