Child–Parent Familial Hypercholesterolemia Screening in Primary Care


BACKGROUND
Child–parent screening for familial hypercholesterolemia has been proposed to identify persons at high risk for inherited premature cardiovascular disease. We assessed the efficacy and feasibility of such screening in primary care practice.

METHODS
We obtained capillary blood samples to measure cholesterol levels and to test for familial hypercholesterolemia mutations in 10,095 children 1 to 2 years of age during routine immunization visits. Children were considered to have positive screening results for familial hypercholesterolemia if their cholesterol level was elevated and they had either a familial hypercholesterolemia mutation or a repeat elevated cholesterol level 3 months later. A parent of each child with a positive screening result for familial hypercholesterolemia was considered to have a positive screening result for familial hypercholesterolemia if he or she had the same mutation as the child or, if no mutations were identified, had the higher cholesterol level of the two parents.

RESULTS
The use of a prespecified cholesterol cutoff value of 1.53 multiples of the median (MoM, corresponding to a percentile of 99.2) identified 28 children who had positive screening results for familial hypercholesterolemia (0.3% of the 10,095 children; 95% confidence interval [CI], 0.2 to 0.4), including 20 with a familial hypercholesterolemia mutation and 8 with a repeat cholesterol level of at least 1.53 MoM. A total of 17 children who had a cholesterol level of less than 1.53 MoM also had a familial hypercholesterolemia mutation. The overall mutation prevalence was 1 in 273 children (37 in 10,095; 95% CI, 1 in 198 to 1 in 388). The use of an initial cholesterol cutoff value of 1.35 MoM (95th percentile) plus a mutation, or two cholesterol values of at least 1.50 MoM (99th percentile), identified 40 children who had positive screening result for familial hypercholesterolemia (0.4% of the 10,095 children, including 32 children who had a familial hypercholesterolemia mutation and 8 who did not have the mutation) and 40 parents who had positive screening results for familial hypercholesterolemia.

CONCLUSIONS
Child–parent screening was feasible in primary care practices at routine child immunization visits. For every 1000 children screened, 8 persons (4 children and 4 parents) were identified as having positive screening results for familial hypercholesterolemia and were consequently at high risk for cardiovascular disease. (Funded by the Medical Research Council.)
Population-based child–parent screening has been proposed to detect familial hypercholesterolemia. The method screens two generations; the child provides the screening entry point, at an age when the measurement of cholesterol is most discriminatory. If a child with familial hypercholesterolemia is identified, the parent with familial hypercholesterolemia may then be identified. Adults 20 to 39 years of age who were classified as having familial hypercholesterolemia were found to have a risk of a coronary heart disease event at a young age that was 100 times that of a person who did not have familial hypercholesterolemia. Identification of children and most parents before the onset of overt cardiovascular disease provides an opportunity to initiate preventive medication. Statins can be offered to parents immediately and to affected children once they become adolescents since adolescence is a period at which evidence of benefit has been reported in a randomized trial. Furthermore, it is obvious that children potentially benefit if premature death is averted in one of their parents.

A measure of the value of a screening test is the detection rate (sensitivity) for a given false positive rate, but estimation of the detection rate associated with familial hypercholesterolemia screening is not straightforward. Familial hypercholesterolemia can be defined by identification of a familial hypercholesterolemia mutation, but not all mutations are known. Furthermore, some people who have a familial hypercholesterolemia mutation do not have high cholesterol levels. These issues could be addressed by defining familial hypercholesterolemia on the basis of high cholesterol levels and a familial hypercholesterolemia mutation. However, when a disorder is defined on the basis of measures used to screen for it, a tautology is created that tends to overestimate the assessment of screening performance because the disorder is then defined on the basis of the results of the screening test.

On the basis of a meta-analysis, we previously estimated that a total cholesterol cutoff value of 1.53 multiples of the median (MoM), corresponding to the 99.9th percentile, would identify 88% of children 1 to 9 years of age who had familial hypercholesterolemia. Because the studies in that meta-analysis used the cholesterol level, at least in part, to define familial hypercholesterolemia, the results had the limitations noted above. We sought to overcome these limitations and assess the feasibility and efficacy of child–parent familial hypercholesterolemia screening in a large study in primary care practices by analyzing data on both cholesterol level and familial hypercholesterolemia mutation.

## METHODS

### STUDY DESIGN AND PROCEDURES

From March 2012 through March 2015, at 92 general medical practices in the United Kingdom, the parents of 13,097 children approximately 13 months of age were asked if they would like their children to participate in familial hypercholesterolemia screening, which would take place at the time of the child’s immunization (e.g., hemophilus influenza type B immunization). A total of 11,010 parents (84%) agreed to their children’s participation in the screening study. At the time that the immunization was administered, a heel-stick capillary blood sample was obtained for measurement of cholesterol and for testing for familial hypercholesterolemia mutations. Satisfactory samples were obtained from 10,118 children; an overall 8% test failure rate occurred because of either slow collection of capillary blood that resulted in clotting or an insufficient sample, but among staff who collected at least 200 blood samples, the test failure rate was 4%. Total cholesterol levels (hereafter referred to as cholesterol levels), high-density lipoprotein cholesterol levels, and triglyceride levels were measured with the use of the Cholestech LDX point-of-care analyzer (Alere). Monthly coefficients of variation were calculated for low standard samples (median, 159 mg per deciliter [4.11 mmol per liter]) and high standard samples (median, 240 mg per deciliter [6.21 mmol per liter]); median coefficients of variation were 4.7% (10th to 90th percentile, 3.7 to 8.4) and 4.7% (10th to 90th percentile, 3.2 to 8.3), respectively. Low-density lipoprotein (LDL) cholesterol levels, which were initially estimated with the use of the Friedewald equation, were later independently calculated at the study center; 23 incorrect results (<0.3%), which were found to be a result of transcription errors, were identified and were excluded from the statistical analyses. The statistical analyses are based on data from the remaining 10,095 children.

We converted cholesterol levels (reported as
milligrams per deciliter) to multiples of the median for all children who were screened; we initially used a median value from a pilot study and updated the median value after every 2000 measurements. The use of multiples of the median helps to overcome analytic differences among instruments and avoids imprecision in the estimation of extreme percentile cutoffs in new populations.

All the children were tested for 48 familial hypercholesterolemia mutations (FH48; see Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org, for a complete list of the 48 mutations), including the most common 46 LDL receptor (LDLR) mutations that were identified in the Regional Genetics Laboratory between 2001 and 2010 in patients who underwent DNA analysis for suspected familial hypercholesterolemia. The children were also tested for the c.10580G→A (p.Arg3527Gln) mutation in APOB and the c.1120G→T (p.Asp374Tyr) mutation in PCSK9. DNA was extracted with the use of the QuickGene-810 (AutoGen) from approximately 200 μl of blood and was analyzed by means of TaqMan single-nucleotide polymorphism (SNP) genotyping (Life Technologies) on the Fluidigm Biomark platform. Identified mutations were verified by means of sequencing. In children who did not have an FH48 mutation but had a cholesterol level of at least 1.53 MoM (230 mg per deciliter [5.95 mmol per liter]), Sanger sequencing of LDLR (all exons, boundary and promoter regions), APOB (exon 26), and PCSK9 (exon 7) was performed with the use of the BigDye Terminator, v. 3.1, Cycle Sequencing Kit (Applied Biosystems). Mutation Surveyor software (SoftGenetics) aligned traces to the reference sequence and called variants. Multiplex ligation-dependent probe amplification (MLPA PO62-C2; MRC Holland) for large LDLR duplications or deletions was also performed (Sanger sequencing and MLPA are hereafter collectively referred to as DNA sequencing). Children who had a cholesterol level of at least 1.53 MoM but did not have either an FH48 mutation or a mutation that was found on the basis of DNA sequencing underwent a repeat cholesterol measurement at least 3 months later.

Children who had a cholesterol level of at least 1.53 MoM and also had either a familial hypercholesterolemia mutation or a cholesterol level of at least 1.53 MoM on the repeat test were considered to have positive screening results for familial hypercholesterolemia. The parent of each child with a positive screening result for familial hypercholesterolemia was considered to have a positive screening result for familial hypercholesterolemia if he or she had the same familial hypercholesterolemia mutation as the child. If no mutation was identified, the parent who had the higher cholesterol level of the two parents was classified as having a positive screening result for familial hypercholesterolemia, on the assumption that the parent with the higher cholesterol level had a nondetectable mutation. The validity of using the higher cholesterol level of each parent as a cutoff value corresponding to the 95th percentile with the goal of formulating a practical population-screening policy.

Parents who had positive screening results for familial hypercholesterolemia completed a questionnaire that assessed whether the screening was worthwhile. The effect of screening on immunization rates was assessed by comparing rates in the year before screening and after the second year of screening in a sample of 24 medical practices.

**STUDY OVERSIGHT**

The Central London Research Ethics Committee approved the study protocol, available at NEJM.org. Written informed consent was obtained from each participating parent, and consent for screening a child was provided by one or both parents. The steering committee (see the Supplementary Appendix) designed the study, and all the authors collected and analyzed the data. The first and last authors wrote the first draft of the manuscript. All the authors contributed to subsequent drafts, agreed to submit the manuscript for publication, and vouch for the accuracy and completeness of the data and for the fidelity of the study to the protocol.

**RESULTS**

**CHILDREN**

Demographic and clinical characteristics of the children and their parents at baseline are shown in Table 1. Figure 1 shows the cholesterol level...
and familial hypercholesterolemia mutation status of the children who participated in the study. The cholesterol level was at least 1.53 MoM in 92 children; 13 of these children had an FH48 mutation, and 7 had a familial hypercholesterolemia mutation on the basis of DNA sequencing (a ratio of 2:1), a finding that indicates the incremental value of sequencing. Among 10,003 children who had a cholesterol level of less than 1.53 MoM, 17 had an FH48 mutation. The 37 familial hypercholesterolemia mutations are listed in Table S2 in the Supplementary Appendix; the list of mutations is ranked by the child’s associated cholesterol levels and also shows cholesterol levels in the parent with the mutation. All the children were heterozygous for the familial hypercholesterolemia mutation.

The prevalence of a familial hypercholesterolemia mutation was 37 in 10,095 children, or 1 in 273 (95% confidence interval [CI], 1 in 198 to 1 in 388), and the prevalence of a familial hypercholesterolemia mutation or two cholesterol values of at least 1.53 MoM was 45 in 10,095 children, or 1 in 224 (95% CI, 1 in 168 to 1 in 308). Among children with an initial cholesterol level of at least 1.53 MoM, 28 children had positive screening results for familial hypercholesterolemia (20 with a familial hypercholesterolemia mutation and 8 with a repeat cholesterol value of ≥1.53 MoM), which equated to a positive rate among the 10,095 children in the total analysis cohort of 0.3% (95% CI, 0.2 to 0.4).

Figure 2 shows cholesterol levels and percentile values in children with and those without an FH48 mutation. The cutoff value of 1.53 MoM corresponded to a percentile of 99.2, which was close to the percentile of 99.9 that was previously predicted; of the 30 children who had an FH48 mutation, 13 (43%; 95% CI, 25 to 63) had cholesterol levels at or above this percentile. A total of 14 children (47%; 95% CI, 28 to 66) had a cholesterol level at or above the 99th percentile (≥1.50 MoM), 7 (23%; 95% CI, 10 to 42) had a cholesterol value between the 95th and 99th percentiles (1.35 to 1.50 MoM), and 9 (30%; 95% CI, 15 to 49) had a cholesterol value that was below the 95th percentile (5 of the 30 children [17%] had cholesterol values that were less than or equal to the median cholesterol level). The distribution of FH48 mutations was similar with respect to LDL cholesterol levels (15 of the 30 children had an LDL cholesterol value at or above the 99th percentile, 6 of 30 children had a value between the 95th and 99th percentiles, and 9 of 30 children had a value below the 95th percentile) (see Fig. S1 in the Supplementary Appendix).

Table 1. Baseline Characteristics of the Study Population.*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Children</strong></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>10,095</td>
</tr>
<tr>
<td>Male sex — no. (%)</td>
<td>5213 (52)</td>
</tr>
<tr>
<td>Median age (IQR) — mo</td>
<td>12.7 (12.4–13.3)</td>
</tr>
<tr>
<td>Family history of premature myocardial infarction — no. (%)†</td>
<td>1094 (11)</td>
</tr>
<tr>
<td>Median cholesterol level (IQR) — mg/dl‡</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>152 (135–171)</td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol</td>
<td>85 (70–102)</td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol</td>
<td>36 (30–44)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>59 (41–84)</td>
</tr>
<tr>
<td><strong>Parents§</strong></td>
<td></td>
</tr>
<tr>
<td>Median age of mothers (IQR) — yr</td>
<td>31 (27–35)</td>
</tr>
<tr>
<td>Median age of fathers (IQR) — yr</td>
<td>34 (30–38)</td>
</tr>
</tbody>
</table>

* IQR denotes interquartile range. † Myocardial infarction occurred in a first- or second-degree relative younger than 50 years of age. ‡ To convert the values to millimoles per liter, multiply by 0.02586. § Data on age were available for 10,094 mothers and 10,087 fathers.

Figure 3 shows cholesterol levels and percentiles plotted against that in the parent without the familial hypercholesterolemia mutation on the basis of DNA sequencing. Among 10,003 children who had a cholesterol value of ≥1.53 MoM, 28 children had positive screening results for familial hypercholesterolemia (20 with a familial hypercholesterolemia mutation and 8 with a repeat cholesterol value of ≥1.53 MoM), which equated to a positive rate among the 10,095 children in the total analysis cohort of 0.3% (95% CI, 0.2 to 0.4).

The results indicate that the presence of a familial hypercholesterolemia mutation alone did not adequately define familial hypercholesterolemia because not all persons with the disorder had hypercholesterolemia. Consequently, reliance on a familial hypercholesterolemia mutation as a screening outcome yields a misleading assessment of the detection rate and false positive rate of screening (rates are shown in Table S3 in the Supplementary Appendix).

**PARENTS**

Cholesterol levels in the parents of 32 of the 37 children with a familial hypercholesterolemia mutation (for 5 children, a parent either declined or was unavailable for testing) are shown in Figure 3. The cholesterol level in the parent with the familial hypercholesterolemia mutation was plotted against that in the parent without the familial hypercholesterolemia mutation. In 27 of the 32 parents, the parent with the higher cholesterol level had the familial hypercholesterolemia mutation, a rate of 84% (95% CI, 67 to 95),
The result was similar with respect to LDL cholesterol levels: 88% (95% CI, 73 to 96), as compared with the predicted estimate of 96% (see Fig. S2 in the Supplementary Appendix).

For each child with a positive screening result for familial hypercholesterolemia, the parent who was determined to have a positive screening result for familial hypercholesterolemia was identified either on the basis of having a positive test for the familial hypercholesterolemia mutation that was found in the child or, if no mutation was found in the child, on the basis of having the higher cholesterol level of the two parents. This method sometimes identified a parent who had a familial hypercholesterolemia mutation but who did not have a high cholesterol level, although 90% of the parents who had positive screening results for familial hypercholesterolemia had cholesterol values that were above the 75th percentile (Table S4 in the Supplementary Appendix). Among parents who had positive screening results for familial hypercholesterolemia, of whom none was receiving treatment with statins, 25 of 28 (90%) subsequently started treatment with statins (2 were pregnant and planned to start later and 1 could not be contacted); all the parents indicated that they thought the screening was worthwhile and none reported negative effects. Screening did not reduce immunization rates; the median rate was 76% in the year before screening and 85% after the second year.
**CHILD–PARENT SCREENING POLICY**

Figure 4 shows the results of the application of our findings to the screening of a typical population of 10,000 children on the basis of an initial cholesterol cutoff value of 1.35 MoM (95th percentile) instead of a value of 1.53 MoM and with the use of “reflex” DNA testing (i.e., obtaining the blood sample but testing for mutations only if the child’s cholesterol level is ≥1.35 MoM).

The use of a cutoff value of 1.35 MoM also identified 12 more parents who had positive screening results for familial hypercholesterolemia (40 instead of the 28 identified when the cholesterol cutoff value of 1.53 MoM was used), approximately half of whom had a cholesterol level higher than 272 mg per deciliter (7.03 mmol per liter; see Table S4 in the Supplementary Appendix). Figure 4 shows that an estimated 80 persons who had positive screening results for familial hypercholesterolemia (40 children and 40 parents) were identified, which represents a rate of 8 cases per 1,000 children screened.

**DISCUSSION**

In the current study, 28 of 10,095 children (0.3%) were considered to have positive screening results for familial hypercholesterolemia because of a very high cholesterol level (≥1.53 MoM, which is equivalent to a percentile of 99.2 in this study) and either a familial hypercholesterolemia mutation or a very high cholesterol level on repeat testing. The use of a cholesterol cutoff value of 1.35 MoM (95th percentile) resulted in the identification of 40 children (0.4%) who had positive screening results for familial hypercholesterolemia. The parent with the higher cholesterol level had the same familial hypercholesterolemia mutation that was present in his or her child in 5 of 6 cases, which was similar to what was predicted.1 The population prevalence of children found to have a familial hypercholesterolemia mutation was approximately 1 in 270, which is nearly double that usually reported (1 in 500).16 Approximately one third of the children in our study who had an FH48 mutation had cholesterol levels that were below the 95th percentile, which showed that the presence of a familial hypercholesterolemia mutation alone is insufficient to characterize a familial hypercholesterolemia phenotype. Finally, child–parent screening was feasible in 92 primary care immunization clinics.

The medical consequences of familial hypercholesterolemia are driven by the cholesterol level.4 A person who has a familial hypercholesterolemia mutation but does not have a raised cholesterol level is unlikely to have an excess risk of cardiovascular disease. Low cholesterol levels in persons with a familial hypercholesterolemia mutation have been reported,17 but the population prevalence of such findings was uncertain because, in most studies, testing for familial hypercholesterolemia mutations was sought only in families with high cholesterol levels.18–22 Our general population study showed that approximately one in three persons with a familial hyper-
cholesterolemia mutation did not have a high cholesterol level, and one in six persons had cholesterol levels below the median. Moreover, the same familial hypercholesterolemia mutation can be associated with either high or low cholesterol levels. In our study, persons with the APOB c.10580G→A mutation had cholesterol levels between 273 mg per deciliter (7.06 mmol per liter; 99.9th percentile) and 143 mg per deciliter (3.70 mmol per liter; 36th percentile), which indicates that interactions with other factors are necessary to express the effect on cholesterol. The presence of a familial hypercholesterolemia mutation is, therefore, insufficient to explain the high cholesterol levels that lead to the clinically significant disorder—a premature event of cardiovascular disease. In this respect, heterozygous familial hypercholesterolemia differs from the homozygous form, in which all persons have a high cholesterol level and are expected to die before 30 years of age if treatment is not initiated.4

Defining familial hypercholesterolemia on the basis of a high cholesterol level rather than on the basis of a familial hypercholesterolemia mutation acknowledges that familial hypercholesterolemia mutations can be benign. However, defining a disorder, at least in part, on the basis of its screening test will overestimate screening performance (100% detection rate for a 0% false positive rate, in this case, if cholesterol levels alone were used). Most previous assessments of screening for familial hypercholesterolemia,26,23 including our own meta-analysis,1 suffer from this limitation.

These various problems can be avoided by regarding heterozygous familial hypercholesterolemia, however specified, not as the disorder but rather as a positive screening test for the development of premature cardiovascular disease. This approach is analogous to regarding cholesterol as a screening test for cardiovascular disease rather than as regarding hypercholesterolemia itself as a disorder,24 or to regarding the BRCA1 mutation as a risk factor for breast cancer and ovarian cancer and not as a medical disorder.25 Regarding familial hypercholesterolemia as a risk factor for cardiovascular disease rather than as a disorder recognizes that cardiovascular disease is not inevitable in persons who have familial hypercholesterolemia and that lowering of cholesterol levels reduces exposure to the main cause of cardiovascular disease rather than treats an existing disease.

The population-based screening policy illustrated in Figure 4 identified children who had either a high cholesterol level (≥1.35 MoM) and a familial hypercholesterolemia mutation or two very high cholesterol measurements (≥1.50 MoM) taken several months apart. This approach largely excludes persons who have a chance high cholesterol level and includes persons with a presumed unknown mutation, thereby identifying persons who have the greatest risk of an event of cardiovascular disease owing to an inherited high cholesterol level.

In our study, blood samples for testing of cholesterol levels and familial hypercholesterolemia mutations were obtained simultaneously and were tested in all children, but in a standard service screening program, a mutation test for

Figure 3. Total Cholesterol and Pairs of Parents with and Parents without a Familial Hypercholesterolemia Mutation.

Shown are total cholesterol levels in the pairs of parents of 32 of the 37 children with a familial hypercholesterolemia mutation (for 5 children, a parent either declined or was unavailable for testing). The total cholesterol level in the parent with the familial hypercholesterolemia mutation is plotted against that in the parent without the familial hypercholesterolemia mutation. The diagonal line represents identical levels in the parent with and the parent without the mutation. The presence of the familial hypercholesterolemia mutation in parents was determined on the basis of either the FH48 panel or DNA sequencing. To convert the total cholesterol values to millimoles per liter, multiply by 0.02586.
familial hypercholesterolemia is required only if a child has a cholesterol level that is at least 1.35 MoM (1 in 20 children). If instituted, “reflex” DNA testing would avoid the need to recall children for a second blood test — in this case, for a familial hypercholesterolemia mutation test. If no familial hypercholesterolemia mutation is detected, the cholesterol test can be repeated approximately 3 months later or, if the medical record is flagged, can be undertaken at the next scheduled immunization visit.

Screening children at routine immunization visits, at 1 to 2 years of age, avoids a separate clinic visit and offers screening at a time when parents are particularly receptive to preventing disease in their child. Previous studies showed that the discrimination of cholesterol levels in persons with and those without familial hypercholesterolemia was best at 1 to 9 years of age, with a suggestion that it may be best at 1 to 2 years of age, and was worse at older ages and in neonates.

Screening younger children identifies par...
nts earlier, which enables parents to start statin therapy earlier if needed. Advances in DNA sequencing have lowered costs, which makes SNP panels (such as FH48) redundant. Consequently, the evaluation proposed in Figure 4 can be based upon familial hypercholesterolemia mutation testing that uses sequencing alone. An illustration of the cost of screening if, for example, cholesterol testing costs $7 and DNA sequencing costs $300 per sample (in U.S. dollars) is $2,900 per person identified as having positive screening results for familial hypercholesterolemia, without an additional service delivery cost when screening is combined with immunization (on the basis of approximate U.K. costs of cholesterol testing and DNA sequencing, which could be lower if screening is performed on a large scale).

In conclusion, our study shows the feasibility and efficacy of child–parent familial hypercholesterolemia screening in primary care. The results suggest that familial hypercholesterolemia is better regarded as a marker that indicates an increased risk of premature cardiovascular disease rather than as a separate medical disorder. Regardless of which conceptual view is adopted, the conclusion remains that child–parent familial hypercholesterolemia screening is a simple, practical, and effective way of screening the population to identify and prevent a relatively common inherited cause of premature cardiovascular disease.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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REFERENCES


