Genetic insights, clinical efficacy and practical implications of genetic screening for familial hypercholesterolemia

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Chapter 3

Review of first 5 years of screening for familial hypercholesterolaemia in The Netherlands

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Abstract

Introduction
Familial hypercholesterolaemia is a common lipid disorder that predisposes for premature cardiovascular disease (CVD). We set up a screening programme in the Netherlands in 1994 to: establish the feasibility of active family screening supported by DNA diagnostics; assess whether or not active identification of these patients with familial hypercholesterolaemia would lead to more cholesterol-lowering treatment; and compare diagnosis by DNA analysis with that by cholesterol measurement.

Methods
Both DNA analysis and measurement of cholesterol concentrations were used to screen families in which a functional mutation in the LDL-receptor gene had been detected.

Results
In the first 5 years, 5442 relatives of 237 people with familial hypercholesterolaemia were screened; 2039 individuals were identified as heterozygous by LDL-receptor gene mutation analysis. At the time of examination, 667 of these adults with familial hypercholesterolaemia (39%) received some form of lipid-lowering treatment; 1 year later, this percentage had increased to 93%. In addition, laboratory analysis showed that for carriers as well as non-carriers 18% would have been misdiagnosed by cholesterol measurement alone, with sex-specific and age-specific 90th percentiles of the general Dutch population as diagnostic criteria.

Discussion
Targeted family screening with DNA analysis proved to be highly effective in identifying patients with hypercholesterolaemia. Most of the identified patients sought treatment and were successfully started on cholesterol-lowering treatment to lower the risk of premature CVD. Our findings could have wider relevance for the screening of other prevalent genetic disorders in the population at large.
Introduction

Familial hypercholesterolaemia is an inherited disorder of lipoprotein metabolism caused by mutations in the LDL-receptor gene. In heterozygous familial hypercholesterolaemia, only 50% of these receptors are functional, which increases plasma cholesterol concentrations to between 7.5 and 16 mmol/L. Characteristically, familial hypercholesterolaemia results in premature cardiovascular disease (CVD) and untimely death. In patients with hypercholesterolaemia the age-standardised and sex-standardised mortality ratios are four to five times higher than in the general population.

Lowering LDL-cholesterol concentrations results in a large decrease in cardiovascular morbidity and mortality, especially in patients at highest risk, and lipid-lowering treatment of patients with familial hypercholesterolaemia could prevent premature death. In western countries although hypercholesterolaemia is a common disorder most patients are not diagnosed or do not receive proper treatment. With the elucidation of the molecular basis of the disorder, an unequivocal diagnosis has now become available. The World Health Organization has recommended that if treatment is started early maximum health benefit can be obtained.

In clinical practice, raised cholesterol concentrations, data from personal and family histories, and physical examination are the main criteria for the diagnosis of familial hypercholesterolaemia. The sensitivity and specificity of raised cholesterol concentrations as well as the use and definition of appropriate cut-off points has been studied extensively. However, most studies have been done in small numbers of adults and children in a lipid clinic setting.

We have established a programme in the Netherlands to assess whether identification of substantial numbers of people with heterozygous familial hypercholesterolaemia by active family screening and DNA analysis is feasible. We also aimed to assess whether identification of these new patients would improve their degree of preventive care, and the specificity and sensitivity of cholesterol measurement. We report the first 5 years of this ongoing screening programme in the Netherlands.

Methods

Identification of index cases

We did the clinical diagnoses at the lipid clinic according to a uniform diagnostic protocol,
with criteria such as LDL cholesterol concentrations, physical signs, and personal and family history in a scoring system.\textsuperscript{13} Family history was judged as positive when there had been signs of CVD in men before the age of 55 years and in women before 60 years. We analysed DNA samples of patients with familial hypercholesterolaemia for the presence of an LDL-receptor gene mutation. Once a mutation had been identified in a patient who had been clinically diagnosed, this patient was classified as an index case. The mutations tested in the screening programme had all been described previously and were known to be functional. Individuals with inherited hyper-cholesterolaemia due to familial defective apolipoprotein B, caused by mutations in the apolipoprotein B gene, were excluded from this study.\textsuperscript{14}

**Family investigation by genetic fieldwork**

First-degree relatives of index cases were contacted after written consent had been obtained from the patient. A specialised nurse visited the relatives at home for written consent, blood sampling, and collection of personal and family data. All blood samples of relatives were tested for the mutation causing familial hypercholesterolaemia in the index case. If carrier status was confirmed, the first-degree relatives of this newly identified patient were contacted. Second-degree relatives, with a risk for the condition of 25%, were included in the screening programme only if examination of the first-degree relative was not possible, because this individual had died or had not been willing to participate. All participants were informed of the findings of the DNA analysis in writing, and carriers of a mutation causing the disorder were referred to lipid specialists.

We isolated genomic DNA from the leukocyte fraction of 10 mL of freshly collected blood, and did PCR and restriction enzyme analysis.\textsuperscript{5}

**Lipoprotein variables**

After an overnight fast, blood samples were taken and concentrations of plasma cholesterol, HDL cholesterol, and triglycerides lipoprotein(a) were measured by commercially available kits (Boehringer Mannheim, Mannheim, Germany). LDL-cholesterol concentrations were calculated by the Friedewald formula only if triglyceride concentrations were below 4.5 mmol/L.\textsuperscript{14,15} As reference values for lipoproteins, age-specific and sex-specific percentiles for total cholesterol were calculated by use of data from the MORGEN-cohort, a large random sample of the general Dutch population.\textsuperscript{16}
Statistical analysis

The $\chi^2$ test was used to compare characteristics between patients with familial hypercholesterolaemia and their non-affected relatives in the age range of 20-65 years. Index cases and individuals on lipid-lowering medication were excluded from the analyses. To select the total cholesterol percentile that keeps the proportion of false negatives and false positives to a minimum, we plotted a receiver operating characteristic curve. The diagnostic value of total cholesterol concentrations was analysed by looking at pre-test and post-test probabilities and was expressed as the post-test likelihood of having the condition (odds).

Results

Between January, 1994, and January, 1999, family screening was initiated in 237 families of which 5442 members participated in the screening programme. The participation rate was 90% over the duration of the study. 277 individuals declined genetic testing because of: fear of negative effects on employment or insurance (124 [45%]); negative advice from general practitioner (69 [25%]); general lack of interest (51 [18%]); treatment for

Characteristics of mutation carriers and non-carriers identified through the national screening programme

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Carriers (n=2039)</th>
<th>Non-carriers (n=3403)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age distribution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40 years</td>
<td>1189 (58%)</td>
<td>1489 (44%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>40-59 years</td>
<td>559 (27%)</td>
<td>1260 (37%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Additional data in adults</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive history of CVD</td>
<td>186 (11%)</td>
<td>189 (6%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Previously known total cholesterol &gt;7.5 mmol/L</td>
<td>875 (51%)</td>
<td>289 (9%)</td>
<td>†</td>
</tr>
<tr>
<td>Treatment with statins</td>
<td>667 (39%)</td>
<td>160 (5%)</td>
<td>†</td>
</tr>
<tr>
<td>Lipoproteins*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>7.43 (1.65)</td>
<td>5.49 (1.34)</td>
<td>†</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>5.62 (1.59)</td>
<td>3.56 (1.11)</td>
<td>†</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.09 (0.35)</td>
<td>1.20 (0.37)</td>
<td>†</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.47 (1.08)</td>
<td>1.66 (1.10)</td>
<td>†</td>
</tr>
</tbody>
</table>

Lipoprotein values were determined in 278 carriers and in 851 non-carriers. *Values are mean (SD). †By selection on functional mutations in the LDL-receptor gene.
hypercholesterolaemia already in place or no offspring (23 [8%]); and no reason given (10 [4%]). Of the 5442 individuals screened, 945 men and 1094 women had an LDL-receptor gene mutation (table). Patients with familial hypercholesterolaemia were slightly younger with a mean age of 37.6 years versus 42.9 in the non-carrier group. After correction for age, no significant differences were reported between patients and non-carriers with regard to smoking habits, incidence of diabetes or hypertension, or family history for CVD (data not shown).

At screening, about half the adult patients with familial hypercholesterolaemia reported previous knowledge of the condition (defined as a total cholesterol level higher than 7.5 mmol/L) and 667 patients (39%) received a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (statin). A small number of patients with hypercholesterolaemia showed clinical manifestations of CVD. These patients had a mean age of 60.4 years, whereas those without CVD had a mean age of 35.3 years. At the time of screening 147 (79%) patients with CVD and 623 (34%) patients without CVD, received statins.

One year after screening, 93% of patients identified with hypercholesterolaemia in our programme had visited a physician and had started lipid-lowering medication. In the first 1129 consecutive individuals who did not receive cholesterol-lowering treatment

A receiver operating characteristic curve for plasma total cholesterol in patients with identified LDL-receptor mutations causing familial hypercholesterolaemia.

Dots represent age- and sex-specific percentiles obtained from the general Dutch population.
lipoprotein concentrations were also measured. The table shows the cholesterol concentrations in carriers of an LDL-receptor mutation and in non-carriers. The figure shows that the best available cut-off point to diagnose the disorder in relatives by total cholesterol concentration is the age-specific and sex-specific 90th percentile. A total cholesterol concentration below these percentiles was reported in 18% of patients (false negatives; 95% CI 13-22%). These patients would have been missed if only cholesterol concentrations had been measured. On the other hand, the proportion of false positives was also 18% (95% CI 16-21%) when the 90th percentile was used as a cut-off point. In this subanalysis, the frequency of mutations in the LDL-receptor gene was 0·25 among relatives. Given a cholesterol concentration above the 90th percentile, the post-test likelihood of having familial hypercholesterolaemia was 1·52 (1·22-1·78), corresponding to a probability of 0·60 (0·55-0·64). For cholesterol concentrations below the 90th percentile, the similar post-test likelihood (odds) of having the disorder was 0·08 (0·05-0·10).

Discussion

We have shown that familial hypercholesterolaemia is frequently underdiagnosed and that many patients who had been identified previously on clinical grounds were not being treated appropriately. Our first research goal was to establish whether or not active identification—ie, by our method of directly approaching family members of index cases was effective in identifying large numbers of still symptomless, quite young, and untreated patients with familial hypercholesterolaemia. On average, about 20 relatives of one index case could be traced, of which 8 (37%) were diagnosed by their carrier status. However, the detection rate of relatives was lower than the expected rate of 50% and could be explained by the fact that offspring of the first-degree relatives (with an a priori risk of being carrier of 50%), who had died or were not available for testing, had been included in the screening—which was done to confirm or exclude transmission of the mutation via the (not available) first-degree relative.

We identified 2039 patients with familial hypercholesterolaemia over a 5-year period, which suggests that public-health interventions to identify and help individuals with this disorder are feasible. Perhaps such screening could be extended to other disorders, such as the long QT syndrome and Marfan's disease. The participation rate in our study was very high, perhaps availability of effective and safe medication, fully covered by insurance in this patient group, contributed to this high percentage. Moreover, before
testing, participants were informed by a brochure that at least 50% of individuals tested are not at risk, will never develop the disorder, and will not pass it to their children. Screening programmes for inherited disorders, such as Huntington's chorea for which no treatment is available have much lower participation rates.\textsuperscript{19} Negative consequences of large-scale genetic screening are to be expected, especially ethical, psychological, and social issues. 10% of possible participants declined genetic testing, mainly out of fear for social consequences, such as negative effects with regard to employment and insurance.

It is remarkable that at the time of examination only 39% of adult patients with familial hypercholesterolaemia initially identified were receiving some form of cholesterol-lowering treatment, whereas 1 year later this percentage had risen to 93%. Although longer-term compliance will be continually assessed as part of the programme, the 1-year data represent an important public-health milestone. In Dutch patients with familial hypercholesterolaemia more than 130 different LDL-receptor gene mutations have now been identified. These mutations account for about 80% of patients of Dutch origin.\textsuperscript{5} However, we only screened relatives of patients in whom an LDL-receptor mutation was already confirmed. Therefore, the diagnosis of participants was established in our DNA laboratory and not during a lipid clinic consultation. Our finding of hypercholesterolaemia in some people without a mutation agrees with reports of other risk factors in families attending lipid clinics.\textsuperscript{20} In these families, a genetic predisposition besides the LDL-receptor mutation could have caused a disproportional increase in dyslipidaemia and CVD, which may have led to referral to the lipid clinic. Therefore, we could conclude that in such screening programmes molecular methods as well as measurement of cholesterol concentrations are required to identify as many persons at high CVD risk as possible.

**Acknowledgments**

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References
