Systematic Family Screening for Familial Hypercholesterolemia in Iceland

Bolli Thorsson, Gunnar Sigurdsson, Vilmundur Gudnason

Objective—This study compares a novel approach using systematic family screening for patients in Iceland who have familial hypercholesterolemia (FH) with conventional proband screening and assesses the sensitivity and specificity of diagnosing FH by cholesterol measurements compared with mutational testing of family members.

Methods and Results—Probands with the I4T+2C mutation were traced to common ancestors. A downtracing of each family lineage was performed back to the oldest living offspring (key individuals); these individuals were recruited for cholesterol measurement and mutation testing. The sensitivity and specificity of cholesterol measurements was assessed against mutational analysis. Eleven probands clustered into 4 families. There were 364 key individuals identified among their descendants. Eighty-four percent responded, and 11% were positive for the mutation. There were 78 offspring of the positive key individuals, and 40 of those were carriers. Compared with use of the conventional first-degree relative approach, an additional 19% of FH individuals, including key individuals and their descendants, were identified. As diagnostic criteria, cholesterol measurements in the families had 95% specificity and 94% sensitivity.

Conclusions—Tracing FH probands to common ancestors and screening the oldest offspring in each family lineage adds considerably to the conventional method of FH screening (testing first-degree relatives). This may have relevance in other founder populations. (Arterioscler Thromb Vasc Biol. 2003;23:335-338.)

Key Words: familial hypercholesterolemia ■ preventive medicine ■ family screening ■ mutational analysis ■ screening enrichment

Familial hypercholesterolemia (FH) is an autosomal-dominant inherited disease that is caused by mutations in the LDL receptor gene and has an estimated prevalence of 1 in 500 for heterozygotes in most populations.1 It is characterized by lifelong elevation of plasma cholesterol, leading to premature coronary heart disease and to cholesterol deposits that form tendon xanthomas, arcus cornealis, and xanthelasma palpebrarum.1 A report from the World Health Organization shows the mean age of onset for coronary heart disease in untreated individuals to be 45 to 48 years in males and 55 to 58 years in females.2 It has been demonstrated that cholesterol-lowering drugs are effective in reducing coronary stenosis assessed by coronary angiography in patients with FH,3 and there is evidence for improved survival of patients with FH in recent years, especially after the introduction of statin therapy.4 Thus, early identification of FH patients is of great importance.

The main aim of the present study was to evaluate genealogical tracing compared with the conventional first-degree relative approach for identification of new FH individuals in Iceland. Population screening for high serum cholesterol levels to identify FH patients is generally not considered cost effective;5 however, a screening of first-degree relatives in patients with known FH is known to be very cost effective.5 In the present study, we used the extensive genealogical information available in Iceland to identify large FH pedigrees for screening close as well as distant relatives of the probands carrying the previously identified Icelandic LDL receptor mutation (I4T+2C). The results were then used to evaluate sex- and age-standardized serum cholesterol percentiles versus genetic testing for FH diagnosis in the families.

Methods

Individuals attending the Lipid Clinic of the University Hospital of Iceland with a clinical and biochemical diagnosis of definite FH form the basis for the present study (index cases/probands). The diagnostic criteria used to identify FH probands were as follows: total serum cholesterol >8.5 mmol/L in the proband and in a first-degree relative, tendon xanthoma in the proband or in a first-degree relative, and myocardial infarction in the proband or in a first-degree relative before the age of 55 years.6

For the evaluation of the genetic screening, only probands with the common mutation identified in Iceland (I4T+2C)6 were included. These probands were genealogically traced to common ancestors by The Icelandic Genetic Council’s family-tracing office. The family tracing was performed through a partly computerized database derived from censuses (first carried out in Iceland in 1703), church
records, and birth and marriage certificates. Once a common ancestor had been identified, a list of all descendants was produced. The oldest individual alive in each family lineage was identified as a key individual and was contacted for cholesterol measurements and for genetic testing (Figure 1). If positive for the common mutation, his or her offspring were recruited for testing. Relatives of the key individuals who were negative for the common mutation were not recruited. All participants in the present study were invited to the Lipid Clinic, where fasting blood lipids were measured, and blood was drawn for DNA extraction.

DNA was isolated by a rapid method,7 amplified by polymerase chain reaction, and digested with NlaIII restriction endonuclease, as previously described.8 The concentration of total serum cholesterol and serum triglycerides was measured enzymatically by using kits based on enzymatic colorimetric testing from Boehringer-Mannheim. HDL was isolated from serum by phosphotungstic acid–magnesium precipitation.

An age- and sex-matched cholesterol percentile was calculated for each participant in the study who was aged 15 years, and this was compared with a random population mean, excluding those on lipid-lowering medication. The sensitivity and specificity of using the cholesterol percentile as a diagnostic tool for FH in the families was assessed. The 95th percentile was the cutoff value. Sensitivity was defined as the number of affected individuals classified by the cholesterol percentile divided by the total number of those affected according to the DNA test. Specificity was defined as the number of individuals who were classified as unaffected by using the cholesterol percentile divided by the total number of unaffected individuals according to the DNA test.

The study protocol was approved by the Data Protection Commission and the National Bioethics Committee, and the participants gave their informed consent.

Results

Fourteen probands positive for the common mutation were genealogically traced to 4 family clusters: 1 cluster with 4 probands, 1 cluster with 3 probands, and 2 clusters with 2 probands each. The ancestors for the clusters were born in the late 18th century and early 19th century and were traced back for 3 and 4 generations. Three of the probands could not be linked to any other proband.

The tracing revealed 2201 living individuals in the 4 family clusters, and of these, 364 (17%) key individuals were identified (Figure 1). Three hundred six (84%) of the key individuals responded. Eleven percent of the key individuals were living abroad or could not be reached for other reasons, and the remaining 5% refused to participate. Thirty-five (11%) of the 306 key individuals who responded were positive for the common mutation (nearly 1 in every 9 key individuals tested). This yield is a 56-fold enrichment from the 1 in 500 yield produced by screening the general population. No homozygotes were detected. Of the 35 positive key individuals, 7 had not been previously diagnosed.

The genealogical tracing revealed 78 living offspring of the positive key individuals. Sixty-eight could be recruited, and 40 (59%) of those were positive. Twenty-one offspring had already been diagnosed with FH, but 19 had not been diagnosed previously and were not receiving lipid-lowering therapy. Seven of these 19 individuals were offspring of the key individuals that had not been previously diagnosed. Therefore, 14 individuals (7 key individuals and 7 offspring [19%]) of the 75 FH patients identified in the families were from previously unknown family lineages.

The overall yield of the screening was 20%, ie, an 11% yield among the key individuals and a 59% yield among first-degree relatives of the positive key individuals (Table 1).

The blood lipid profile of the FH patients is shown in Table 2. The mean total serum cholesterol level was 9.5 mmol/L for males and 9.4 mmol/L for females. Mean HDL cholesterol and triglyceride levels were near the mean of the normal Icelandic population9-11.

### Table 1. Number of FH Cases Identified by the Screening (Excluding the 11 Probands)

<table>
<thead>
<tr>
<th>Number of Individuals Identified</th>
<th>Number of Individuals Tested</th>
<th>Number of Positives</th>
<th>Percentage of Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Key individuals</td>
<td>364</td>
<td>306</td>
<td>35</td>
</tr>
<tr>
<td>Descendants of positive key individuals</td>
<td>78</td>
<td>68</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>442</td>
<td>374</td>
<td>75</td>
</tr>
</tbody>
</table>

### Table 2. Mean Blood Lipid Levels (±SD) of FH Family Members (Excluding 14 Individuals Taking Blood Lipid–Lowering Drugs and 9 Children Under the Age of 15 Years)

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Affected (n=37)</td>
<td>Nonaffected (n=125)</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>9.5±1.9</td>
<td>5.6±1.0</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.0±0.3</td>
<td>1.2±0.5</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.4±0.9</td>
<td>1.4±0.8</td>
</tr>
</tbody>
</table>

*All values are in mmol/L.*
Age- and sex-standardized percentiles for cholesterol of the family members are shown in Figure 2. All affected females were above the 95th percentile, as were 9 of the 135 unaffected females. All affected males were above the 95th percentile except for 4 men. The sensitivity of using the 95th percentile for cholesterol as a diagnostic tool in the families was 93% (59 of 63). The specificity was 95% (244 of 259). Further analysis revealed that the 95th cholesterol percentile gave 11% false negatives for men but no false negatives among women. All the false-negative men were young (aged 15 to 20 years). False positives were 6% for women and 5% for men.

**Discussion**

The main aim of the present study was to evaluate the additional value of genealogical tracing compared with the conventional first-degree relative approach for identification of new FH individuals in Iceland. Compared with the first-degree relative approach, this new approach identified 19% additional new patients. The conventional approach to identify new FH patients is to ask FH index patients for consent to contact first-degree relatives. This is the method recommended by the Med Ped project (Make Early Diagnosis—Prevent Early Deaths in Medical Pedigrees). The main advantage of such an approach is the high probability of diagnosing family members carrying the LDL receptor mutation when contacting close relatives. The probability is 50% for first-degree relatives of the index patient (parent, sibling, and child) and then declines by about half for each meiosis. In the Netherlands, experience from FH screening using a similar first-degree relative approach revealed 2039 individuals identified with heterozygous FH of 5442 relatives tested. The main disadvantage of this approach is that the doctor needs to rely on consent from the patient to contact his/her relatives and that pedigrees based on information from the patients are seldom complete for >1 or 2 generations back. Therefore, the search for new patients can reach a blind end. The present study shows that by using a computerized genealogical database to obtain complete information regarding names, identification numbers, times of birth, and times of death of all members of the extended families, some of these limitations can be abolished.

For several years, considerable effort has been put into the identification of new FH patients at the Icelandic University Hospital Lipid Clinic by using an approach similar to that recommended by the Med Ped project. Approximately half of the estimated number of FH patients in Iceland have already been identified. When the genealogical information in the present study was viewed, it was apparent that contacting the first-degree relatives of the probands and then the first-degree relatives of all FH individuals identified would have identified 61 FH patients of the total 75 patients found to have FH (excluding 11 probands) if full cooperation of the FH patients had been obtained. The remaining 14 individuals (19%) were from 3 family lineages that could not have been identified by contacting first-degree relatives of known FH patients because they were more distantly related. Two of the lineages were 4 meioses from the next FH relative, and 1 was at a distance of 6 meioses.

Although the screening was limited to extended families in the present study, the number of individuals alive in the families is substantial (2201 individuals). To reduce the number of individuals needed to be tested, the screening was limited to the key individuals only; thereby, the number to be screened was reduced by 83%. The likelihood of identifying an FH patient among offspring of negative key individuals is no more than 1 in 500 and was therefore not carried out. The overall 20% yield (11% yield of FH patients when testing the key individuals and 59% yield when testing first-degree relatives of positively tested key individuals) suggests that a similar approach is feasible for further FH screening in the whole of Iceland.

The main limitation of using this novel approach in other populations is that it is based on access to comprehensive genealogical information, which is rarely available in large multiethnic populations. However, there is an indication, even in large populations (eg, English and Italian), that in certain regions a high proportion of the FH mutation can be traced to few ancestors. Webb et al showed that 11 of 77 FH patients from Manchester had the same mutation and haplotype analysis, which suggests that all the patients had inherited the mutated allele from a common ancestor. A screening of unrelated FH patients in southern Italy similarly showed a high prevalence of a single mutation detected in 20% of the patients. In regions like these, local information, such as church records, could possibly be used to construct local genealogical databases, to find common ancestors of FH families, and to conduct family screening as described in the present study. For example, Bertolini et al identified a novel...
mutation in the LDL receptor gene in 3 apparently unrelated families in northern Italy and traced them back to a common ancestor in the 17th century. In the Netherlands, Sijbrands et al.\(^\text{i}\) have recently shown to be possible not only the tracing to a common ancestor but also the downtracing of all descendants essential for the screening method described in the present study. Whether this family-screening approach turns out to be beneficial in regions other than Iceland remains to be tested. However, with increased accessibility to affordable information technology tools, construction of large pedigrees by conventional methods of genealogical tracing (such as through birth records, and church books) may become more easily achievable. The availability of pedigree software for constructing pedigrees in large genealogies\(^\text{ii}\) might increase the feasibility of systematic screening for any common monogenic disorders where there is evidence of ethnic settlements, such as in the United States.

For most cases, cholesterol measurements and family history are sufficient for diagnosis of FH. The sensitivity and specificity of using the 95th cholesterol percentile as a cutoff value for FH diagnosis in these FH families were 93% and 95%, respectively. These results are similar to those of Koivisto et al.\(^\text{iii}\) (in 1992), who found that 95% of the diagoses were correct when the age- and sex-specific 95th percentiles for LDL cholesterol were used in index patients and first-degree relatives. This indicates that DNA tests are not essential for the application of this familial screening method. In men aged <20 years, FH should not been ruled out if cholesterol is above the 80th percentile (Figure 2). However, application of mutation analysis to ensure that the probands carry the same mutation should increase the likelihood of tracing the probands to an ancestor that surely had FH and should consequently lead to a successful screening.

These results suggest that DNA testing remains the gold standard for identifying FH individuals and should be used to ensure the diagnosis and to identify carriers with cholesterol levels below the 95th percentile when possible. Cholesterol measurements are of importance in identifying those who have high cholesterol for reasons other than FH.

The World Health Organization conference in Paris, 1997, urged for an early diagnosis and treatment of individuals with FH. The main challenge is to prevent premature atherosclerosis in individuals with FH. This necessitates identification of these individuals at an early age and an aggressive treatment of all known risk factors for coronary heart disease. Screening extended families is a feasible approach for achieving that goal and may well be practical in other populations in addition to the Icelandic population.

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References

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