Reliable Low-Density DNA Array Based on Allele-Specific Probes for Detection of 118 Mutations Causing Familial Hypercholesterolemia

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Background: Patients with familial hypercholesterolemia (FH) have a high risk of premature cardiovascular disease (PCVD). Mutations in the LDL receptor (LDLR) gene and the R3500Q mutation in the apolipoprotein B (APOB) gene are known to cause FH, but lack of high-throughput methods makes routine genetic diagnosis difficult. The objective of this work was to develop a DNA array for large-scale identification of mutant LDLR alleles.

Methods: We developed a low-density oligonucleotide microarray to identify 118 DNA sequence variations (117 for the LDLR gene and 1 for the APOB gene). We verified specificity and sensitivity by analyzing 1180 previously sequenced DNA samples, and conducted a blind study screening 407 Spanish patients with a clinical diagnosis of FH.

Results: The DNA array confirmed the previous genotyping results in almost all cases. In the blind study, the microarray detected at least 1 mutation in 51% of the patients for whom clinical diagnosis was classified as certain according to Dutch FH-MEDPED criteria; it also identified mutations in 37% of those with a diagnosis of probable/possible FH, thus giving a definite diagnosis.

Conclusions: The proposed DNA array allows large-scale population screening and provides molecular information regarding mutation type and its correlation with clinical severity of FH, which can be used to develop therapeutic strategies.

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Familial hypercholesterolemia (FH, MIM 143890) is caused by mutations in the gene encoding the LDL receptor (LDLR) that lower LDL clearance from plasma. Although a limited number of mutations give rise to FH in some ethnic groups, such as the Finns (1) and Afrikaners (2), numerous FH-causing mutations have been found in other populations, e.g., The Netherlands (159 different mutations) (3), United Kingdom (67 mutations) (4), Italy (71 mutations) (5), and Spain (117 mutations) (6–8). As of August 2002, the total number of reported mutations in the LDLR gene was 840 (9). FH is a good example of a monogenic disease with high prevalence, difficult clinical diagnosis, high risk of cardiovascular disease (CVD), and available effective treatment when early diagnosis is made (10). Familial defective apolipoprotein B-100 (FDB), another disease that can cause hypercholesterolemia, is the result of a missense mutation at amino acid position 3500 (R3500Q) in apolipoprotein B-100 (apoB-100) that interferes with the ability of apoB-100 to bind to the
LDLR. Persons with FDB may also have a family history of hypercholesterolemia and clinically significant coronary atherosclerosis. Therefore, FDB can be distinguished from FH only by a genetic diagnosis (11).

In many countries, high-throughput genetic testing techniques such as single-strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis, and sequencing are too expensive and time-consuming to be practical. In contrast, oligonucleotide microarrays allow rapid, cost-effective screening for a large number of different mutations (13). The purposes of the present study, therefore, were to develop a DNA array to identify new FH patients in the Spanish population who carry known LDLR mutations; to evaluate the specificity and sensitivity of the array, using previously genotyped DNA samples; and to determine the performance of the FH array in large-scale population screening studies.

**Materials and Methods**

**STUDY POPULATION**

In 1999, a National Register of Spanish persons with a diagnosis of FH was established by the Spanish Familial Hypercholesterolemia Foundation (14). Since then, ~2400 FH patients have been identified based on the Dutch FH-MEDPED clinical diagnostic criteria (14, 15). A total of 70 clinics (Spanish FH group; see the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol51/issue7/) sent clinical data and fasting blood samples to a central laboratory for lipid and lipoprotein analysis and genetic diagnosis. CVD was defined as any of the following events: myocardial infarction, vascular surgery, coronary angioplasty, significant (>50% stenosis) coronary lesions, unstable angina, ischemic stroke, or lower-extremity ischemic disease. When the event occurred before the age of 55 years in men and 65 years in women, CVD was considered to be premature (PCVD). The ethics committee of each institution approved the study, and the participants gave written informed consent.

Screening for LDLR gene mutations was carried out based on results of SSCP, sequencing, and restriction polymorphism analysis (8). A total of 117 different LDLR gene mutations were found in this FH population, and the APOB R3500Q gene mutation was detected in 1.4% (16). These 117 LDLR gene mutations and the R3500Q mutation were selected for a first version of the DNA array [Table 1 of the online Data Supplement and Refs. (17, 18)]. More than one-half of these mutations have been reported in Western Europe (Holland, France, German, Italy, Greece, and the United Kingdom) and the United States (19, 20).

The specificity and sensitivity of the DNA array were assessed by use of 10 control DNA samples for each mutation (1180 samples), identified from previous screening.

A blind study was performed using samples from 407 nongenotyped, unrelated persons from the Spanish National Register. Among these, 62% had a definite clinical FH diagnosis (Dutch MEDPED score ≥8 points). In the remaining 38%, the clinical diagnosis was probable or possible (4–8 points).

**SAMPLES**

After the individuals had fasted 12 h, venous blood samples were collected into tubes containing disodium EDTA (to obtain genomic DNA) and tubes containing SST clot-activating gel (to obtain serum). Genomic DNA was extracted from peripheral blood cells by use of commercial reagents (Puregene® DNA Isolation Kit; Gentra).

**QUANTIFICATION OF LIPIDS AND LIPOPROTEINS**

Total serum cholesterol and triglyceride concentrations were quantified enzymatically on a Beckman Synchron CX7 analyzer (Boehringer Mannheim). HDL-cholesterol was measured after precipitation of apoB-containing lipoproteins with Mg2+ phosphotungstate (Boehringer Mannheim). LDL-cholesterol was calculated by use of the Friedewald formula (21). Lipoprotein(a) was measured by kinetic immunonephelometry with polyclonal antibodies (Beckman Coulter) (22).

**DNA ARRAY METHODOLOGY**

**Oligonucleotide design.** Two pairs of oligonucleotides were designed for each mutation to ensure the accuracy of mutation detection. Each probe pair consisted of a probe specific for the wild-type allele and a probe specific for the mutant allele. The target base, in the case of point mutations, is defined as the nucleotide at which the DNA sequence variation is found. In the case of insertions, duplications, or deletions, >1 nucleotide position is different. The first nucleotide in the wild-type sequence to differ from the mutant sequence was considered the target position. This target nucleotide was always located in the central position of the oligonucleotides to maximize the specificity of hybridization.

The ability of the allele-specific oligonucleotide to differentiate between the wild-type and the mutant alleles is determined by the hybridization conditions, the nucleotide sequence that flanks the single-nucleotide polymorphism (SNP), and the secondary structure of the target sequence (23). The use of a defined hybridization procedure (as we describe below) allowed us to establish the sense and the appropriate length of the probes for each mutation to maximize specific hybridization. On average, 8 oligonucleotides per mutation were tested experimentally to select both oligonucleotide pairs, starting from probes with 25 nucleotides. For each mutation, the probes designed interrogated both DNA strands, with lengths ranging from 19 to 27 nucleotides, and the melting temperature varied from 75 to 85 °C. The oligonucleotide sequences are described in Table 2 of the online Data Supplement.
Microarray design, fabrication, and quality controls. Several replicates of each oligonucleotide probe were spotted on aminosilane-coated glass slides (Corning) by use of a Microgrid II robotic spotter (BioRobotics) under controlled humidity and temperature conditions. Oligonucleotides were attached to slides by cross-linking with ultraviolet radiation and baking at 80 °C. Positive and negative hybridization controls were also printed. A commercial DNA (k562 DNA High Molecular Weight; Promega) was used to control the quality of the process.

Target DNA preparation and hybridization. Target DNA for hybridization was prepared in 4 independent multiplex PCR reactions, each of which contained 5 separate primer pairs. The multiplex PCRs were performed simultaneously under the same time and temperature conditions, allowing amplification of the promoter and 18 exons of the LDLR gene and amplification of exon 26 of the APOB gene. Amplicon size ranged from 136 to 300 bp. Each 30-μL multiplex PCR contained 160 ng of genomic DNA, 1.5 mM MgCl₂, 50 μM each deoxynucleotide triphosphate, and 3 U of AmpliTaq Gold (Applied Biosystems). Primer concentrations were optimized for each amplification, and concentrations of 0.15–0.6 μM were used. The sequences of the primers used in the multiplex amplification are given in Table 3 of the online Data Supplement. In addition, a biotinylated nucleotide and dUTP were incorporated during the PCR process (Perkin-Elmer). Thermo-cycling was performed on a Gene Amp® PCR System 9700 (Applied Biosystems) with an initial denaturation at 95 °C for 10 min, followed by 43 cycles of denaturation at 94 °C for 30 s, primer annealing at 62 °C for 1 min, and primer extension at 72 °C for 3 min. After 43 cycles, a final extension reaction was carried out at 72 °C for 10 min. The biotinylated PCR products were pooled and fragmented enzymatically with uracyl-DNA-glycosylase (Amersham Biosciences) to improve the hybridization signal. The target DNA was denatured at 95 °C for 5 min and then immediately placed on ice until used for hybridization. Automatic hybridization was carried out at 45 °C for 1 h in a Ventana Discovery station using ChipMap hybridization buffers and the protocol for the Microarray 9.0 Europe station (Ventana Medical Systems). Finally, DNA arrays were automatically stained with Cy3-conjugated streptavidin (Amersham Biosciences) in the automatic hybridization station.

Microarray scanning and quantification. DNA array images were captured by use of a ScanArray 4000 confocal fluorescent scanner (Perkin-Elmer), equipped with a green laser (543 nm for Cy3 excitation; Fig. 1 and Fig. 1 of the online Data Supplement). Absolute values of the Cy3 hybridization signal from each oligonucleotide probe were obtained by use of Quant-Array 3.0 software (Perkin-Elmer).

Data analysis and genotyping software. Absolute values of the Cy3 hybridization signal were processed automatically by MG 1.0 software (PROGENIKA BIOPHARMA), which calculated the mean hybridization signal of the replicates for each oligonucleotide probe. The ratio of the hybridization signal mean of the wild-type allele to the sum of hybridization signal means of the wild-type and mutant alleles was then defined for the 2 pairs of oligonucleotides used for genotyping each mutation. For each mutation, 1170 homozygous wild-type individuals, 10 heterozygous mutant FH patients, and 10 homozygous mutant FH patients (a restricted number of cases) clustered perfectly into 3 groups when the 2 ratios were plotted in an x-y scatter plot in the verification study (Fig. 2). These DNA control samples were used to determine the 2 ratio values corresponding to the 3 clusters. In the subsequent blind study, MG 1.0 software was used to determine to which of the previously defined clusters each of the 407 samples belonged.

In the large-scale analysis, the software gave an output of the parameters necessary for the quality control of each step in the hybridization process. Positive and negative hybridization controls, means of whole array hybridization and background signals, mean of whole array specificity, and the CV of each probe replicate were all verified to be in a previously validated experimental range to assure reliable hybridization results.
The statistical analysis was performed with Statistical Package for the Social Sciences (SPSS), Ver. 11.0. Distributions of all quantitative variables were tested for normality by use of the Kolmogorov–Smirnov test. Data that did not follow a gaussian distribution [body mass index, lipoprotein(a), total cholesterol, and LDL-cholesterol] were log-transformed before analysis. Quantitative variables were compared among groups by 1-factor ANOVA adjusted for age, sex, and body mass index. Categorical variables were compared by the \( \chi^2 \) test. The Kaplan–Meier method was performed to determine PCVD-free survival time. Survival curves were compared by the Breslow test. The independent contribution of each variable to the length of PCVD-free survival was evaluated by multivariate Cox regression, taking into account only those variables that were found to be significantly predictive in univariate analysis carried out between patients with and without PCVD. The independent categorical variables were coded as follows: 1 for male and 0 for female, 1 for presence and 0 for absence of hypertension, 1 for presence and 0 for absence of arcus cornealis, and 1 for presence and 0 for absence of tendinous xanthomas. The type of \( LDLR \) gene mutation was coded by use of 2 dummy categorical variables: null mutations vs missense mutations (1 for null mutations, 0 for splicing mutations, 0 for missense mutations) and splicing mutations vs missense mutations (0 for null mutations, 1 for splicing mutations, 0 for missense mutations). \( P \) values, relative risks, 95% confidence intervals, and partial regression coefficients were calculated. A \( P \) value <0.05 was considered statistically significant for all analyses described above.

**Results**

**Verification**

The specificity and sensitivity of the DNA array were tested with 1180 previously sequenced DNA samples. Both specificity and sensitivity were 100% for 115 of the tested \( LDLR \) mutations; however, for 3 mutations, specificity and sensitivity were between 90% and 100%. Heterozygous mutant and homozygous wild-type clusters in these 3 cases were very close to each other, and as a consequence, a low number of samples were misclassified with respect to these 3 mutations. The overall specificity and sensitivity obtained for all mutations tested were 99.7% and 99.9%, respectively.

These specificity and sensitivity results are supported by the mean signal ratios and power in differentiating among genotypes (Table 1). In addition, mean signal ratios 1 and 2 for each genotyping cluster and for all mutations identified by the DNA array in the verification study are presented in Fig. 2 of the online Data Supplement. Nonoverlapping mean (SD) signal ratios were obtained for each genotype.

Overall the reproducibility rate, calculated as the CV, was as follows:

- The CV of replicates of the same oligonucleotide ranged from 10% to 15% in any given array. CV was defined as the ratio of the hybridization signal SD of the replicates to the hybridization signal mean.
- The CV of the ratios used to identify the 118 mutations in 20 independent DNA array experiments tested with the same commercial DNA (K562 DNA High Molecular Weight) was 4.8%. CV was defined for each mutation as the ratio of the SD ratios to the mean ratios.

**Blind study**

The DNA array detected at least 1 mutation in 51.2% of the persons with a definite clinical diagnosis of FH according to the Dutch MEDPED criteria. It also detected

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Homozygous wild-type cluster</th>
<th>Heterozygous mutant cluster</th>
<th>Homozygous mutant cluster</th>
<th>Mean (SD) power of discrimination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio 1</td>
<td>0.72 (0.10)</td>
<td>0.51 (0.10)</td>
<td>0.24 (0.09)</td>
<td>0.21 (0.08)</td>
</tr>
<tr>
<td>Ratio 2</td>
<td>0.75 (0.11)</td>
<td>0.52 (0.11)</td>
<td>0.27 (0.15)</td>
<td>0.23 (0.08)</td>
</tr>
</tbody>
</table>

*Distance between the homozygous and heterozygous genotype clusters.

*Ratio between the signals from the mutant allele and the sum of the signals from the wild-type and mutant alleles.
mutations in 37.4% of the probable/possible FH cases, thus providing a definite diagnosis.

One or more mutations were identified in 187 of the 407 patients analyzed (44.5%). Among them, 181 patients had an LDLR gene defect, and 6 had the APOB R3500Q gene mutation. The clinical diagnosis was definitive in 129 cases, and in the remaining 58 patients, the clinical diagnosis was probable/possible (15). Only 59 of the 118 mutations tested by the DNA array were found among the patients studied in this study (50%). In 29 patients, 2 or 3 different mutations were detected (Table 1 of the online Data Supplement).

For patients with no identified mutations who had a Dutch MEDPED score ≥8 points, nucleotide sequencing of the LDLR gene was carried out to establish whether unidentified mutations were present that could be introduced in a subsequent version of the DNA array. Among 123 samples sequenced, 28 mutations not detected previously in the Spanish population were identified in 43 patients. In addition to point mutations, large deletions or insertions comprised ~5%–10% of the alterations observed in the LDLR gene (24, 25). Overall, the rate of genetic diagnosis was 72% in 252 patients with a Dutch MEDPED score ≥8.

The characteristics of the 407 persons included in the blind study are shown in Table 2. Body mass index, PCVD prevalence, the presence of tendinous xanthomas and arcus cornealis, total and serum LDL-cholesterol concentrations, and the frequencies of the 118 mutations were significantly higher in patients with a definite clinical diagnosis of FH.

The persons carrying an LDLR gene mutation identified by the DNA array were classified into 3 groups according to the type of mutation: splicing, null, and missense. These sequence variations are predicted to produce a defect in splicing, a truncated protein, and a defective receptor, respectively. To determine the PCVD-free survival time depending on the type of mutation, we formulated Kaplan–Meier curves, using the age limits of 55 years in men and 65 years in women (Fig. 3). The Breslow test showed significant differences between curves for null mutations and missense mutations (P = 0.02). The mean (SD) survival time was 53 (2) years for null mutations and 58 (1) years for missense mutations. Finally, we performed multivariate Cox regression to evaluate the influence of the type of mutation on PCVD-free survival time. This analysis showed that the relative risk of PCVD in patients with a null mutation was ~3.1-fold higher than that in patients with a missense mutation (Table 3).

Discussion
In the present study, we demonstrate that our DNA array can be used as a highly reproducible, sensitive, and specific tool for genotyping the FH-causing mutations in the Spanish population.

Microarrays based on allele-specific oligonucleotides have been reported to fail in distinguishing between heterozygous and homozygous SNP genotypes despite the use of 40–50 probes per SNP (26). The novelty of our procedure is that it has high sensitivity and specificity but

Table 2. Characteristics of the 407 persons included in the blind study and mutation frequencies.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Definite (n = 252)</th>
<th>Probable/Possible (n = 155)</th>
<th>Pp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M/F</td>
<td>126/126</td>
<td>62/93</td>
<td>NSa</td>
</tr>
<tr>
<td>Mean (SD) age, years</td>
<td>47.6 (14.2)</td>
<td>46.0 (18.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean (SD) body mass index, kg/m²</td>
<td>26.4 (5.5)</td>
<td>25.2 (4.3)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Smokers, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>53.1</td>
<td>65.5</td>
<td>NS</td>
</tr>
<tr>
<td>Former</td>
<td>18.8</td>
<td>6.9</td>
<td>NS</td>
</tr>
<tr>
<td>Current</td>
<td>28.1</td>
<td>27.6</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>20.7</td>
<td>12.3</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>3.6</td>
<td>3.7</td>
<td>NS</td>
</tr>
<tr>
<td>PCVD, %</td>
<td>18.7</td>
<td>6.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Tendinous xanthomas, %</td>
<td>33.1</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arcus cornealis, %</td>
<td>50.0</td>
<td>23.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean (SD) total cholesterol, mg/L</td>
<td>3804 (635)</td>
<td>3620 (363)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean (SD) LDL-cholesterol, mg/L</td>
<td>3016 (629)</td>
<td>2836 (326)</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean (SD) HDL-cholesterol, mg/L</td>
<td>546 (155)</td>
<td>567 (170)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean (SD) triglycerides, mg/L</td>
<td>1190 (524)</td>
<td>1068 (410)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean (SD) lipoprotein(a), mg/L</td>
<td>484 (516)</td>
<td>444 (460)</td>
<td>NS</td>
</tr>
<tr>
<td>Frequency of the 118 mutations, %</td>
<td>51.2</td>
<td>37.4</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

a Clinical diagnosis based on the Dutch MEDPED Score (MEDPED Program 1998).

b P value for the difference between definite and probable/possible clinical diagnosis.

NS, not significant.
uses only 2 pairs of allele-specific oligonucleotides per mutation analyzed.

The DNA array described here was evaluated according to US Food and Drug Administration recommendations for the preparation of multiplex tests for heritable mutations. Tight clustering of signal ratios from different samples indicated robust genotyping, and the distance between the clusters supported the power of discrimination between genotypes (Table 1). Primer extension using microarrays is supposed to be more robust and to provide more specific allele distinction than hybridization methods (27). Although the power of discrimination was smaller when we compared our results with primer extension, the clusters were tighter in our FH array and the genotyping more robust (28).

Waldmüller et al. (29) described a low-density microarray to screen for 12 known mutations in 4 different genes that cause hypertrophic cardiomyopathy. However, the FH array described here is the first low-density DNA array based on hybridization to allele-specific oligonucleotide probes that is able to screen for such a large number of known mutations. Another recently reported FH test based on allele-specific hybridization on membrane strips includes the 7 more common mutations in the South African population (30).

The advantage of using only 4 probes per mutation is that a large number of mutations, limited in number only by the number of features that can be introduced on the array, can be analyzed with low-density microarrays. Therefore, mutations occurring in other countries could be included in a new version of the DNA array, ultimately allowing worldwide application. In addition, this approach could be used for the diagnosis of other disorders caused by high numbers of mutations located in 1 or a limited number of genes.

The FH array described here detected a defect in either the LDLR gene or in the APOB gene predominantly in patients with a definite clinical diagnosis of FH; however, a large number of persons clinically misdiagnosed as probable/possible FH patients were given an unequivocal genetic FH diagnosis based on the DNA array.

Once the index case has been identified, early diagnosis of FH in other family members could allow them to take measures to reduce the risk of suffering PCVD. When LDL-cholesterol concentrations are the only clinical phenotype, however, it is not possible to make unequivocal diagnoses among family members of the index case by means of cholesterol measurements (31–34). The use of DNA arrays to identify FH-causing mutations could allow reliable early diagnosis. Because carriers of null mutations have a higher relative risk of PCVD than carriers of missense mutations, the type of sequence variation in patients with definite FH could help in determination of disease prognosis. Gaudet et al. (35) and Bertolini et al. (5) also found that the relative risk of PCVD was increased ~3-fold in patients with mutations leading to the absence of the LDLR. Response to statins is known to be strongly linked to the type of mutation carried (7). Posttreatment LDL-cholesterol concentrations are higher in individuals with severe mutations, such as null mutations; intermediate in those with mild mutations, such as missense mutations; and lowest in the group with no identified mutations (36). Patients identified as having some particular mutations may need more aggressive lipid-lowering treatment to achieve the LDL-cholesterol concentrations recommended to reduce the risk of PCVD (10). In this respect, the proposed DNA array could provide a useful tool for designing individualized treatments related to PCVD risk and for monitoring response to treatment, based on the mutation carried by each patient.

In our blind study of 407 individuals, 187 patients with possible and confirmed FH carried a mutation present in the DNA array (44.5%). In populations having a large heterogeneity in FH clinical inclusion criteria, such as the individuals included in the blind study reported here, LDLR mutation screening methods have detection rates ranging from 30% to 50% (12). However, in studies with very strict clinical inclusion criteria, such as coronary

<table>
<thead>
<tr>
<th>Variable</th>
<th>$\beta$ (SE)</th>
<th>RR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>1.15 (0.50)</td>
<td>3.16 (1.20–8.28)</td>
<td>0.02</td>
</tr>
<tr>
<td>Null mutations vs</td>
<td>1.15 (0.58)</td>
<td>3.14 (1.00–9.87)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>missense mutations</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

$\beta$ is the partial regression coefficient.

$^a$ RR, relative risk for male vs female and null mutations vs missense mutations. Relative risk is defined as $e^\beta$. Values in parentheses are the 95% confidence intervals.
heart disease, decreased LDLR activity, the presence of xanthomas, or a Dutch MEDPED score ≥8, mutations can be detected by SSCP, denaturing gradient gel electrophoresis, sequencing, or RNA analysis in a larger number of patients, and the detection rates range from 60% to 80% (3, 37–39). In our study, after DNA array genotyping, detection of large rearrangements, and sequencing, the rate of genetic FH diagnosis was 72% in patients with a Dutch MEDPED score ≥8. The remaining patients with confirmed FH are predicted to have a genetic defect in other loci associated with FH [e.g., the ARH gene (40), ABCG5 and ABCG8 genes (41), PCSK9 gene (42), and CYP7A1 gene (43)].

In conclusion, we have developed a robust DNA array-based method that uses specific oligonucleotide probes for FH-causing mutations identified in the Spanish population to perform large-scale screening for FH. In the future, with the incorporation of additional oligonucleotide probes designed to identify a wider range of mutations in the LDLR gene, as well as in other genes associated with FH, it could be possible to identify the majority of FH patients by use of a simple and inexpensive DNA array-based assay. In addition, the example described here of an array for diagnosis of FH raises the possibility of developing a similar strategy for the diagnosis of many monogenic diseases.


References


