Comparison of DNA Array Platform vs DNA Sequencing as Genetic Diagnosis Tools for Familial Hypercholesterolemia

To the Editor:
The report by Blesa et al. (1) compares 2 methods, DNA sequencing and DNA arrays [as previously reported by us (2)], for the genetic diagnosis of familial hypercholesterolemia (FH).

Lipochip® (Lacer SA), the first DNA array-based commercial platform for the genetic diagnosis of FH, is now available and is funded by the Spanish Health Service. The procedure is as follows: blood samples are shipped to a central laboratory, where DNA is analyzed with the first CE-marked (approved for sale in the European Community) DNA array for in vitro diagnosis in Europe. Samples with a negative result undergo large rearrangement analysis by quantitative fluorescence-based multiplex PCR (3). If this analysis is also negative, DNA sequencing is carried out to identify new disease-causing variations. The results are compiled in a full report that is sent to the patient’s physician.

Most of the comments by Blesa et al. on our DNA array refer to an earlier version developed for research only (v1.0) (2). The most recent version (v4.0) detects 204 LDL-receptor (LDLR) gene variations and 3 alterations in the apolipoprotein B gene (APOB) that cause FH or familial defective apolipoprotein B. Our DNA array is updated yearly with all new variations identified by DNA sequencing. We are working on a new version that can detect 230 variations, including those most frequent in Spain.

Patient selection criteria differed in the 2 studies; those of Blesa et al. (1) were based on MedPed’s clinical criteria, and ours (2) were based on Dutch MedPed’s score criteria. With our criteria, 129 FH probands from

Table 1. Demographical data between patients with ESRD and control participants.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ESRD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>50</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Male, %</td>
<td>27 (54)</td>
<td>27 (54)</td>
<td>0.508</td>
</tr>
<tr>
<td>Age, year</td>
<td>61.8 (9.4)</td>
<td>60.5 (10.7)</td>
<td>0.385</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>135.2 (21.3)</td>
<td>139.5 (29.3)</td>
<td>0.031</td>
</tr>
<tr>
<td>ABI</td>
<td>1.03 (0.13)</td>
<td>0.96 (0.18)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PEDF, µg/mL</td>
<td>14.5 (2.3)</td>
<td>36.3 (5.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>202.9 (33.4)</td>
<td>148.4 (32.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-c, mg/dL</td>
<td>114.7 (29.0)</td>
<td>74.8 (26.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-c</td>
<td>62.7 (16.8)</td>
<td>53.1 (16.0)</td>
<td>0.006</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>90.6 (17.1)</td>
<td>92.1 (17.4)</td>
<td>0.888</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>95.9 (11.3)</td>
<td>102.8 (11.2)</td>
<td>0.053</td>
</tr>
<tr>
<td>BUN, mg/dL</td>
<td>18.5 (4.9)</td>
<td>83.2 (17.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>0.7 (0.2)</td>
<td>12.1 (3.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Uric acid, mg/dL</td>
<td>5.1 (1.3)</td>
<td>7.1 (1.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total protein, g/dL</td>
<td>7.3 (0.4)</td>
<td>6.5 (0.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td>4.5 (0.2)</td>
<td>3.9 (0.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST, unit/L</td>
<td>25.5 (9.3)</td>
<td>14.6 (6.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT, unit/L</td>
<td>24.3 (18.4)</td>
<td>11.1 (8.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intima-media thickness, mm</td>
<td>0.7 (0.2)</td>
<td>0.8 (0.1)</td>
<td>0.284</td>
</tr>
</tbody>
</table>

Values are mean ± (SD) or percentage, unless indicated otherwise.

* ABI, ankle-brachial pressure index; BP, blood pressure; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

b Log-transformed values were used for the calculation of means and SD.

References
the Blesa study present a Dutch MedPed score ≥8, with a lower rate of variation detection than our study (69% vs 72%). With our DNA array commercial platform, the variation detection rate in samples with a Dutch MedPed score ≥8 is ~73% with more than 1000 analyses (data not shown).

The community of Valencia studied by the Blesa group is only 1 of 17 Spanish regions. Therefore, it is to be expected that our DNA array, designed for the entire Spanish population, identifies the most frequent Spanish variations. The latest version of our DNA array (v4.0) should detect 65% of the variations detected by Blesa et al., 10% more than v1.0. Of the undetected 35% of variations, most are very low frequency variations that have been described only by Blesa et al. (1), which could explain why they were not detected by a DNA array focused on the most frequent variations in Spain. Because the last step of our DNA array platform is DNA sequencing, however, the variations described by Blesa et al. most likely will be detected by our DNA array platform and will be introduced in new versions of the DNA array.

Although we do not know if the population studied by Blesa et al. is representative of the community of Valencia, our DNA array detected a causative variation in 80% of the 50 DNA samples we tested from that community. Variation Q12X, the most frequent, is not described in the report by Blesa et al. (1), whereas variation 111insA, the most frequent in the Blesa et al. report, was not found in our subgroup.

During the past 2 years, our commercial DNA array platform has analyzed more than 1000 patients with a clinical history of FH, more than reported for conventional sequencing techniques (4) and more than the 129 cases reported by Blesa et al. The throughput of the platform is estimated to be 5000 analyses per year. The average turnaround time from DNA extraction to physician report is 23 to 65 days, depending on the required procedure steps. According to these data, FH genetic screening by DNA array platform allows higher throughput screening than do other molecular techniques with lower analytical capacity. Our DNA array has analyzed more than 3000 samples, as part of several studies, yielding good rates of sensitivity, and validation has been performed to ensure the quality of the analytical processes, and all the results obtained so far have confirmed their robustness (data not shown).

The WHO has identified FH as one of the most relevant genetic diseases (5). Responding to that concern and seeking methods for early detection and treatment, an expert panel of researchers and physicians presented their recommendations for FH diagnosis and management, including development of a tool for general screening and a method of genetic diagnosis in countries where most of the causative variations are known to occur (6).

In conclusion, our DNA-array platform provides rapid results and offers important health benefits to the genetically heterogeneous Spanish FH population.

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References

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False Reduction in Serum Methadone Concentrations by BD Vacutainer® Serum Separator Tubes (SST™)

To the Editor:

Methadone maintenance is the primary treatment modality in the US for opioid dependence, with doses typically titrated to therapeutic response such as improvement of withdrawal symptoms or abstinence from drug use. Serum methadone concentrations are used to guide dosing in