An Assessment of the Apex Microarray Technology in Genotyping Patients with Leber Congenital Amaurosis and Early-Onset Severe Retinal Dystrophy

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PURPOSE. Leber congenital amaurosis (LCA) and early-onset severe retinal dystrophy (EOSRD) are genetically heterogeneous, with 11 genes currently implicated. The LCA chip may be used to interrogate many variants in one hybridization reaction. The purpose of this study was to assess the utility of this technology.

METHODS. One hundred fifty-three patients with LCA and EOSRD were screened using an array (Asper Ophthalmics, Tartu, Estonia) containing 344 published disease-causing variants and polymorphisms in eight genes: AIPL1, GUCY2D, CRB1, CRX, RPGRIP1, RPE65, MERTK, and LRAT. One hundred thirty-six probands underwent bidirectional sequencing of the full coding region of the RPE65 gene. The same technique was also used to confirm CRB1 and AIPL1 mutations initially identified with the Apex chip (Asper Ophthalmics). Single nucleotide polymorphism (SNP) analysis within control populations was performed for two variants, P701S and W21R, on the chip for GUCY2D.

RESULTS. Of the possible 109,392 interrogations, 3,346 (3.06%) failed on one strand whereas 259 (0.47%) failed on both. The chip reported mutations in 68 (44%) patients; 26 patients had two alleles identified (17%). Direct sequencing of RPE65 showed no discrepancies, whereas sequencing of AIPL1 and CRB1 revealed seven samples called erroneously. The SNP analysis of both GUCY2D variants revealed equal prevalence in the EOSRD panel and the normal population. Subsequent re-analysis, after excluding these polymorphisms, revealed one (18.3%) or two (11.7%) mutations identified in 46 patients. When evaluated by diagnosis, 46% of patients with LCA had one or two mutations identified, compared with 24% of patients with EOSRD.

CONCLUSIONS. This approach is a rapid and reasonably low-cost technique for identifying both previously identified mutations and common polymorphisms. The addition of further genes and mutations to the chip will improve its utility, though it is advised that all results be checked by direct sequencing. (Invest Ophthalmol Vis Sci. 2007;48:5684–5689) DOI:10.1167/iovs.07-0207

Leber’s congenital amaurosis (LCA) is a severe rod-cone dystrophy that is symptomatic from early infancy; it is clinically and genetically heterogeneous.1,2 LCA is usually inherited as an autosomal recessive trait, but rare autosomal dominant forms have been reported.3 To date, 11 genes have been implicated in LCA and it is evident that mutations in some of them give rise to a different clinical phenotype with onset of symptoms in early childhood and better initial visual function; this group of disorders may be better termed early-onset severe retinal dystrophy (EOSRD).

Several different genes, including GUCY2D,4 AIPL1,5 RPE65,6,7 RPGRIP1,8 CRX,9,10 TULIP1,11,12 CRB1,13,14 RDH12,15,16 MERTK,17 LRC,18 and, most recently, CEP290,19 have been implicated in LCA and EOSRD but these account for only approximately 55% to 65% of patients,1,2,19-20 and more genes remain to be discovered. Molecular diagnosis in EOSRD and LCA is problematic, given the large number of genes; screening of 142 exons in 11 genes is labor intensive and expensive. The development of parallel genotyping technologies (the Affymetrix array; Affymetrix, Santa, Clara, CA; Illumina platform; Illumina, San Diego, CA; or the Apex chip; Asper Ophthalmics, Ltd., Tartu, Estonia) provide an opportunity for simplifying molecular diagnosis in such disorders.

One such technology, the Apex chip (Arrayed Primer Extension; Asper Ophthalmics, Ltd.), was first described in 1996 as a novel method for DNA analysis.21,22 It was designed for LCA23 as a microarray which, at the time of this study, contained 344 disease-associated SNPs and several common variant polymorphisms in six LCA- and two EOSRD-associated genes (GUCY2D, CRX, RPE65, CRB1, RPGR, AIPL1, LRAT, and MERTK). More recently, mutations in two additional genes, RDH12 and CEP290, have been added to the chip. The PCR products for each amplicon are combined and, in a single hybridization reaction, are annealed to oligonucleotide primers arrayed on the chip. A template-dependent single-nucleotide extension reaction follows, with DNA polymerase and four fluorescently labeled dye terminator nucleotides. These detect variation at the site in question for both sense and antisense strand, with Genorama genotyping software (Asper Ophthalmics, Ltd.).

In the present study, the efficacy of the LCA Apex chip (Asper Ophthalmics, Ltd.) was investigated in 153 probands with LCA/EOSRD and, in the case of AIPL1, CRB1, and RPE65, the results were compared with direct sequencing, the accepted and historic gold standard.
METHODS

Study Subjects

One hundred fifty-three unrelated individuals with diagnosed LCA/EROSRD took part in the study. Patients were eligible for the study if they had a clinical diagnosis of LCA or if they had a severe retinal dystrophy that was symptomatic before the age of 5 years with an absent or severely attenuated electroretinogram at diagnosis. Patients with associated systemic disease or family history suggestive of autosomal dominant or X-linked recessive disease were excluded.

Research procedures were in accordance with institutional guidelines and the Declaration of Helsinki. Ethics committee approval was obtained. All patients and parents were provided with information sheets before informed consent was obtained.

More than 90% of the patients were examined by two ophthalmologists (RHII, ATM) at Moorfields Eye Hospital. For the remaining patients, the clinical details were retrieved from the medical records. All patients had diagnoses based on history, clinical appearance, and results of electroretinography. DNA was extracted from whole blood obtained from each affected individual.

In six very young individuals, repeated buccal samples and whole genome amplification (Geneservice Ltd., Cambridge, UK) did not yield DNA of sufficient quality for reliable genotyping. In those cases, parental DNA was used across the chip.

In addition, an SNP assay for two particular variants—the c.2101C>T p.P701S and c.61T>C p.W21R changes in GUCY2D—was performed in unaffected control DNA samples. The P701S change has been discussed in the literature and described by some as disease-causing in certain populations. It is listed on the HGMD (Human Gene Mutation Database; http://www.hgmd.cf.ac.uk/ provided to registered users only) database as a disease associated missense mutation despite evidence that the variant is present within the normal population. The W21R change is listed on the chip as a disease-associated mutation, though it has also been found in normal populations previously.

No mention of the P701S GUCY2D variant is made by Yzer et al. Whether this is because it had been discounted as a polymorphism or it was not found in the 58 northern European patients with LCA is not known.

Molecular Methods

Apex Chip. Concentrated DNA (3.5 μg), from 147 unrelated affected patients and 12 unaffected parents of probands, was sent to Asper Ophthalmics, Ltd. The technology and methods used have been described elsewhere.

RPE65, CRB1, and AIPL1 Sequencing. Each of the 14 exons of RPE65, 18 amplifiers for 12 exons of CRB1, and 6 exons of AIPL1 were amplified (Extensor Hi-Fidelity PCR Master Mix; ABgene, Warrington, UK) according to the manufacturer’s instructions. The amplified products were sequenced with dye termination chemistry, Cambridge, MA and ordered from Sigma-Genosys (Poole, UK). The commonest mutation identified was the C948Y change in CRB1 (nine patients, two homozygous). The two GUCY2D variants P701S and W21R, labeled as disease associated by Asper Ophthalmics, but possibly polymorphisms, were highly prevalent in our cohort (16 and 14 alleles identified in 16 and 11 patients, respectively). Seven of the 10 patients with the W21R change were from the Indian subcontinent. Based on results given below the P701S or W21R variants were not included in the analysis and, after direct sequencing, the AIPL1 R38fs allele was also eliminated from our analysis. This reduced the detection rate significantly with one mutation identified in 28 (18.3%) of 153 patients, and two mutations identified in 18 (11.7%) of 153, giving an indication of genotype in a total of 30%.

RESULTS

Patient Cohort

One hundred fifty-three patients were recruited (90 male, 63 female; one-tailed Fisher exact test; P = 0.06). Of the entire cohort, 58% were white British subjects; 16% were of other white backgrounds, mostly European; and 20% were of Asian extraction, mostly Pakistani (12%) or Indian (5%). Twenty-one percent of the patients were products of consanguineous marriages. The mean age at diagnosis was 3.5 ± 4 years (SD), and the mean age at onset of symptoms was 1.8 ± 3 years. Fifty-nine patients had a diagnosis of LCA, whereas the remaining 94 patients had a diagnosis of either early-onset rod-cone or cone–rod dystrophy (Table 1).

LCA Chip Results

Disease-associated variants, called by Asper Ophthalmics as mutations, were identified in 68 individuals (44%). Homozygous (n = 9) or compound heterozygous mutations were found in 25 (17%) patients, and a further 43 (28%) patients had a single mutation identified (Table 2).

Two heterozygous mutations within two separate genes were seen in seven samples, though four of these were subsequently discounted as polymorphisms. Of the 12 parental DNA samples that were used, changes were identified in 5 (Table 3). One of these was the AIPL1 R38fs change, which has now been discounted.

The commonest mutation identified was the C948Y change in CRB1 (nine patients, two homozygous). The two GUCY2D variants P701S and W21R, labeled as disease associated by Asper Ophthalmics, but possibly polymorphisms, were highly prevalent in our cohort (16 and 14 alleles identified in 16 and 11 patients, respectively). Seven of the 10 patients with the W21R change were from the Indian subcontinent. Based on results given below the P701S or W21R variants were not included in the analysis and, after direct sequencing, the AIPL1 R38fs allele was also eliminated from our analysis. This reduced the detection rate significantly with one mutation identified in 28 (18.3%) of 153 patients, and two mutations identified in 18 (11.7%) of 153, giving an indication of genotype in a total of 30%.

Microarray Genotyping of Patients with LCA

The c.61T>C p.W21R change in GUCY2D was assessed using a restriction digest (HinP11) in two control populations: 96 ECACC control and 60 Asian control subjects. Exon 2 of GUCY2D was amplified in the control DNA samples by using primers designed in Primer 3. A 20-μL reaction volume composed of PCR master mix (Hi-Fidelity; ABgene) was run at 57°C. Ten microliters of the PCR product was incubated with 2 units of HinP11 overnight at 37°C. Eight microliters of the digest was then run on a 3% agarose gel with a PhiX ladder (Fig. 1).

FIGURE 1. HinP11 restriction digest showing the PCR product, homozygous and heterozygous for the W21R variant in GUCY2D against a PhiX ladder.
Chip Results by Diagnosis

The chip results, when analyzed by diagnosis, with the polymorphisms and false-positive AIPL1 R38fs calls discounted, showed that in the LCA patients, one or two mutations were found in 27 (46%) of 59 of patients, while in the patients with EOSRD, changes were found in 24% (Table 4).

Apex Chip Reliability. There were 3346 unidirectional call failures (3.06%) and 259 bidirectional call failures (0.47% i.e., 518 unidirectional calls) of the possible 109,392 allele calls made, producing a combined value of 3.5%. A call failure is defined as the nondetection of a signal by the chip for a particular allele. A unidirectional failure is one strand missed, and in a bidirectional failure neither the forward nor the reverse strand is detected. Bidirectional failures are the most significant since they represent a no-call at the SNP in question. Insertions, deletions, and splice site variants represent a total of 28% (n/H11005 96) of the 344 variants arrayed on the chip: as a proportion of call failures; however, they account for 35%. Five variants were associated with particularly low combined unidirectional and bidirectional call failures (Table 5). This is, however, skewed by a high unidirectional failure rate. A fairer assessment of failure was obtained by assessing the total number of bidirectional failures, shown in Table 6.

RPE65 Sequencing. One hundred thirty-four (84%) of the 159 individuals analyzed using the chip, were also sequenced for mutations in RPE65. There were 83 disease-associated mutations in RPE65 arrayed on the chip. Direct sequencing of RPE65 confirmed each of the 22,244 (83/134 2) calls made.

We identified two novel homozygous missense mutations (L60P, Y249C); two heterozygous mutations (889delA, E6X), of which one mutation had been identified from the chip; and two further heterozygous variants (IVS2+10 A>G, D110V) where a second change could not be found. The homozygous and compound heterozygous sequence variants segregated in the parents and were not found in normal control subjects. All novel changes were assayed in panels of ethnically matched.

Table 2. The Number of Disease-Associated Variants in Each Gene Detected by the Asper Ophthalmics LCA Chip

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>Single Mutation</th>
<th>Two Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>APL1</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>CRB1</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>CRX</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>GUCY2D</td>
<td>27</td>
<td>17</td>
</tr>
<tr>
<td>RPE65</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>RPGRIP1</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>LRAT</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MERTK</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

This version of the Asper Ophthalmics chip did not include mutations in RDH12 or CEP290, which are included in the present version of the chip.
control subjects and were not identified. All mutations occurred at positions that were conserved across several species.

**CRB1 Sequencing.** We confirmed each of the mutations found on the LCA chip by direct sequencing. In one patient, however, a C1181R homozygous mutation called by the chip was determined on sequencing as C1181InsG, leading to a frame shift and termination 12 codons downstream. In one patient, a C1181R homozygous mutation called by the chip was confirmed on one allele, a second mutation, G1103R (a previously reported change that is found on the chip), was noted. This finding represents a false-negative report from the chip. It was reported to Asper Ophthalmics who have subsequently redesigned primers to remedy the problem.

**AIPL1 Sequencing.** Sixteen patients were identified as having mutations within the *AIPL1* gene using the LCA chip. Of these individuals, 14 had only a single allele identified. Five patients who were identified on the chip as having a c.111CdelC, p.R38fs mutation, were in fact discovered on sequencing to have a synonymous c.111C, p.R38fs mutation, were in fact discovered on sequencing as C1181InsG, leading to a frame shift and termination 12 codons downstream. In one patient, a homozygous G850S mutation was confirmed on one allele, a second mutation, G1103R (a previously reported change that is found on the chip), was noted. This finding represents a false-negative report from the chip. It was reported to Asper Ophthalmics who have subsequently redesigned primers to remedy the problem.

**SNP Assay Results**

One hundred ninety-two unrelated control DNA samples as taken from the ECACC control panel were assayed for changes in *GUCY2D* at position 2101C>T which codes for the P701S change. The homozygous C>T change was found in 12/189 samples (6.3%). No homozygous T/T calls were found. The same heterozygous change was found in 10% of the LCA/EOSRD panel as identified by the LCA chip where, again, there were no homozygotes identified. There is no significant difference in the frequency of the P701S allele (one-tailed Fisher exact test; \( P = 0.1 \)) between the LCA/EOSRD populations and the normal population.

### Table 4. Number of Mutations Identified by the Apex Chip for Each Diagnosis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cone-Rod (n = 29)</th>
<th>Rod-Cone (n = 65)</th>
<th>LCA (n = 59)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 Mutations</td>
<td>1 Mutation</td>
<td>2 Mutations</td>
</tr>
<tr>
<td>AIPL1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>CRB1</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>CRX</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RPE65</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>GUCY2D</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RPGRIP1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>4 (14%)</td>
<td>0 (0%)</td>
<td>5 (8%)</td>
</tr>
</tbody>
</table>

See Supplementary Table S1 for full results (online at http://www.iovs.org/cgi/content/full/48/12/5684/DC1).

**DISCUSSION**

Previous validations of the assay have been performed by the designer and manufacturer of the chip and subsequently in a smaller cohort by Yzer et al. Our study presents an independent analysis of the chip in a large cohort of patients with LCA and EOSRD, in whom 90% of the phenotyping has been performed by two individuals. Both disease-causing alleles were identified in 11.7% of patients, and one disease-associated allele in a further 19.6%. Overall, the likely causative gene was identified in a third of patients. The chip was originally designed specifically for LCA patients and, of the 59 LCA patients, one mutation was identified in 31% and two mutations in 15%, giving a combined value of 46%. We validated the array for *RPE65* by direct sequencing of the entire coding region and found the chip to have a sensitivity of 100% for variants arrayed on the chip. Four novel mutations were identified by direct sequencing of the entire coding region and found the chip to have a sensitivity of 100% for variants arrayed on the chip. Four novel mutations were identified by direct sequencing. These mutations were not found in 192 normal Caucasian and 48 Asian control subjects. We identified two missense mutations, p.L60P and p.Y249C; one single-base deletion, c.889delA; and a nonsense mutation, p.E6X. Leucine at amino acid position 60 expect the frequency of LCA/EOSRD to be an order of magnitude greater than is actually seen within the normal population. It was found, by *Hin*II restriction digest assessing the prevalence of the W21R change, that 10 (16.6%) of 60 Asian control subjects had a heterozygous change at this position, whereas in the British ECACC panel, the heterozygous change was identified in 8 (8.3%) of 96, thus excluding it as a disease-associated variant in either population compared to 14 of 159 seen in the EOSRD panel (one-tailed Fisher exact test; \( P = 0.27 \)). Our hypothesis that the W21R change is more prevalent within patients from the Indian subcontinent, based on the finding that 7 of 10 patients identified with this change on the chip were either from Pakistan, India, or Bangladesh, is thus rejected (one-tailed Fisher exact test; \( P = 0.09 \)).

### Table 5. The Five Variants on the LCA Chip with the Greatest Number of Call Failures

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exon</th>
<th>Nucleotide</th>
<th>Protein</th>
<th>Call Failures (n)</th>
<th>% of Total Calls*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPE65</td>
<td>12</td>
<td>1307G&gt;T</td>
<td>G436V</td>
<td>155</td>
<td>49</td>
</tr>
<tr>
<td>RPGRIP1</td>
<td>IVS6-17</td>
<td>907-17delTAA</td>
<td>SPLICE</td>
<td>164</td>
<td>52</td>
</tr>
<tr>
<td>GUCY2D</td>
<td>2</td>
<td>48 bp</td>
<td>AA</td>
<td>123</td>
<td>39</td>
</tr>
<tr>
<td>GUCY2D</td>
<td>2</td>
<td>2&gt;T&gt;A</td>
<td>MIK</td>
<td>113</td>
<td>36</td>
</tr>
<tr>
<td>CRB1</td>
<td>11</td>
<td>-ins16</td>
<td>FS</td>
<td>107</td>
<td>34</td>
</tr>
</tbody>
</table>

* \( n = 318 \).
and tyrosine at position 249 are highly conserved, suggesting that they form part of a functional domain of the protein. The other two mutations, p.E6X and c.889delA, result in premature termination of RPE65 and are likely to be pathogenic.

While identifying a second CRB1 disease-associated allele in those patients in whom a single mutation was found on the chip, discrepancies were noted: one false-negative result (G1103R) on the chip, that was confirmed on repeat testing by Asper Ophthalmics and one sequence variant (C1181InsG) at an SNP (C1181R) found on the chip. This latter finding is interesting, as it demonstrates that the chip has a limited capacity to identify novel variants at previously identified SNP sites. The result reported by Asper Ophthalmics was a homozygous C1181R change, though they did indicate that the reverse strand had failed. This finding has been reported recently when using this technique for detecting mutations in patients with Usher syndrome.28

In addition, our sequencing results of AIPL1 identified that one particular call made in five patients—c.111delC, p.R38fs—was in fact not detecting the correct SNP. Asper Ophthalmics have subsequently redesigned their primers. A single mutation highlighted by the chip allowed us to find a second change in only 4 of 11 possible patients when screening AIPL1 and CRB1. Potential reasons for this include the commonly cited difficulties that PCR-based techniques have in detecting deletions or indeed that other SNPs called as mutations are possibly rare polymorphisms, and hence a second change would not be expected. Yzer et al.25 demonstrated that the AIPL1 p.V96I was present in the heterozygous form in 11 of 186 control subjects and was therefore not included in their analysis (this would remove a further two single alleles from our analysis).

Two previous studies have reported the results of using the Asper Biotech chip in screening for mutations in LCA. Zernant et al.23 identified mutations in 20.3% to 25.8% of the total number of alleles in their cohort. Yzer et al.25 reported that 24% (14/58) of their subjects had homozygous or compound heterozygous changes and in a further six subjects, a single mutant allele was identified. In the present study, investigating both LCA and EOSRD patients, we identified a mutation in both alleles in only 11.7% of subjects but in a further 18.3% of subjects identified, a single heterozygous disease-associated allele.

The results from these studies are not easily compared as other studies23,25 have confined their analysis to LCA and have used earlier versions of the chip which included fewer sequence variants. Furthermore, Zernant et al.23 reported only the number of alleles identified and not the proportion of subjects in whom both disease-causing alleles were identified. Nonetheless, some comparisons can be made: It appears that if one discounts the presence of two alleles in two different genes, then 22 (10%) patients in their cohort were identified by the chip as being homozygotes or compound heterozygotes. Our findings suggest a higher number (9.5% of EOSRD and 15% of LCA patients) which is likely to be consistent with a greater number of mutations arrayed on the newer chip. The smaller study of LCA Yzer et al.25, however, detected a greater number (24%) of subjects with homozygous or compound heterozygous changes.

A like-for-like comparison using the 147 probands in our study and including the GUCY2D P701S and W21R alleles (though not the AIPL1 R38fs allele) as was done by Zernant et al.,25 reveals a screening efficiency of 94 (31.9%) of 294 possible alleles compared with the 20% to 24% efficiency identified by Zernant et al. A more accurate comparison using only the LCA patients in our cohort and including both P701S and W21R polymorphisms revealed a screening efficiency of 39%.

In conclusion, it appears that the screening efficiency, with the updated version of the chip was greater in our cohort (despite our using a less stringently selected group of patients than that used by Zernant et al.,25) but less than that which Yzer et al.25 reported, even when only the LCA patients are analyzed.

An important difference between our study and that of Zernant et al.23 is the inclusion of the GUCY2D P701S change. They refer to this variant as a causative mutation in their analysis, together with a compound heterozygous change, p.H945R, which they identified in an Iranian family and which segregated with disease. It has been demonstrated that the P701S change is present in 6.5% of the normal British blood donor population and in a similar proportion of the EOSRD cohort. This finding, and that the encoded amino acid change

### Table 6. The SNPs with the Greatest Number of Bidirectional Call Failures

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exon</th>
<th>Nucleotide</th>
<th>Protein</th>
<th>Number of Bidirectional Failures</th>
<th>% as a Proportion of 159 Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>GUCY2D</td>
<td>2</td>
<td>del 14bp 226–239</td>
<td>FS</td>
<td>12</td>
<td>7.5</td>
</tr>
<tr>
<td>GUCY2D</td>
<td>3</td>
<td>974T&gt;C</td>
<td>L325P</td>
<td>11</td>
<td>7.0</td>
</tr>
<tr>
<td>GUCY2D</td>
<td>15</td>
<td>2899 del C</td>
<td>FS</td>
<td>10</td>
<td>6.0</td>
</tr>
<tr>
<td>GUCY2D</td>
<td>15</td>
<td>2927G&gt;T</td>
<td>R976L</td>
<td>9</td>
<td>5.6</td>
</tr>
</tbody>
</table>

**Figure 2.** Sequence file for 111C>T SNP in AIPL1, miscalled as 111delC by the Apex chip.
(H945R) is likely to represent a conservative change (both histidine and arginine are basic amino acids), means that it is unlikely that this sequence variant is pathogenic. The argument that was used contended that the P701S change causes disease in some populations but not in others. This notion seems unlikely, especially when considering our unaffected parent who had no signs of retinal disease but was carrying heterozygous variants in AIPL1 (Y134F), RPGRIP1 (R812Q), and GUCY2D (P701S and W21R). Although no unaffected homozygotes for the P701S change were identified in our cohort, we assayed only 192 control subjects, and the likelihood of identifying a homozygote in this number is low.

Zernant et al.25 reported that a third allele was present in 22 (7.5%) of 300 of their cohort and suggested that some of these variants represented modifier alleles. If the P701S allele is included in the analysis of our study, then three alleles in five subjects (3%) were identified, two of whom were parents of probands and the genotype of the affected child was unknown. The reasons for the differences between the two studies are unclear.

We have demonstrated that that the chip is a sensitive technology with a low call failure rate. For example, we have shown that the chip has 100% sensitivity for detection of known mutations in RPE65 when compared with the direct sequencing. It provides an excellent first-pass screening in molecular genetic diagnosis in LCA. In our study, the use of the LCA chip in a population of patients with LCA or EOSRD resulted in the detection of multiple disease-causing alleles in 12% of patients and one allele in 20%. Several anomalies between direct sequencing and the chip results were demonstrated. These included false-positive results in the case of the 11 cited AIPL1 c.111delC, p.R368fs; changes; a false-negative result in the instance of the nondetected heterozygous CRB1 G1103R mutation; and a sequence variant, C1181InsG, at the CRB1 C1181R site.

We have demonstrated that P701S and W21R in GUCY2D are as prevalent in the normal population as they are in the LCA/EOSRD population, making the case that it is unlikely that they are pathogenic sequence variants.

Overall, the Apex chip technology provides a quick and cost-effective means of obtaining a genotype in patients with EOSRD or LCA, for whom it was originally designed. Although false-positive and -negative results occurred and certain SNPs had a relatively high call failure rate, our experience suggests that the problems are no worse than the difficulties that can be experienced with direct sequencing. Improvements in technology and the inclusion of new mutations and additional genes on the chip will increase the proportion of patients in whom a molecular diagnosis can be made by using this technique. These advances will improve genetic counseling in affected families and help identify patients who may benefit from future clinical trials for LCA and early-onset retinal dystrophies.

References