External quality assessment for mutation detection in the
BRCA1 and BRCA2 genes: EMQN’s experience of 3 years

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Background: The European Molecular Genetics Quality Network (EMQN) was formed in order to improve external quality assessment for molecular genetic testing in Europe. From 1999 to 2002 it received funding from the European Union under the Standards, Measurement and Testing programme (contract no. SMT4-CT98-7515). Since then, its maintenance has been supported through subscription of the participants, and it has been coordinated by the National Genetic Reference Laboratory at Manchester, UK (Rob Elles and Simon Patton; www.emqn.org).

Materials and methods: Among other external quality assessment (EQA) schemes, EMQN has provided an EQA scheme for mutation detection in the breast cancer genes, BRCA1 and BRCA2, designed to cover the two important aspects of genetic testing: (i) genotyping and (ii) interpretation and reporting of results. The fourth full scheme was completed in 2003, with data evaluation pending for the 47 participants.

Results: Analysis of genotyping data has pinpointed two main types of errors: (i) missing a mutation (in nine of the 17 false results a normal sequence was reported); and (ii) description of the observed sequence change by an incorrect nomenclature. Compared with the more technical process of genotyping, the writing of reports displayed a much wider variation between laboratories.

Conclusions: From the reported data it is clear that external quality control should become an integral part of quality assessment in the laboratory, thus contributing to maintaining confidence in the reliability of genetic testing among patients and health professionals.

Key words: BRCA, genetic testing, quality control

Introduction

The European Molecular Genetics Quality Network (EMQN) was formed in 1996 by a group of clinical molecular geneticists in order to improve and harmonise external quality assessment for molecular genetic testing in Europe. From 1999 to 2002 it received funding from the European Union under the Standards, Measurement and Testing programme (contract no. SMT4-CT98-7515). Since then, its maintenance has been supported through subscription of the participants, and it has been coordinated by the National Genetic Reference Laboratory at Manchester, UK (Rob Elles and Simon Patton; www.emqn.org).

Design of the external quality assessment scheme

Among other external quality assessment (EQA) schemes, EMQN has provided an EQA scheme for mutation detection in the breast cancer genes, BRCA1 and BRCA2. EMQN’s EQA schemes are generally designed to cover the two important aspects of genetic testing: (i) genotyping and (ii) interpretation and reporting of results. Genotyping is the term used for the technical part of mutation detection. In contrast to some other areas of laboratory medicine, an individual, fully interpreted report is considered essential in genetic testing. Molecular genetics in general and BRCA genetics in particular are rapidly evolving fields, where novel scientific insights are being gained at a weekly rate. It cannot, therefore, be expected that practising clinicians are able to keep track of all new developments. Therefore, an interpretation based on expert knowledge is an added value service to the clients. A comprehensive report is also desirable in view of the fact that medical records start to have a life of their own once they have been issued. This may be particularly true in oncogenetics, where diagnosis and treatment is a multidisciplinary task.

Starting with a pilot scheme for BRCA gene testing in 1999, the fourth full EMQN EQA scheme was completed in 2003, and awaits evaluation. Numbers of participants are steadily increasing. A breakdown by country is given in Table 1. It should be noted that national EQA schemes are offered in Germany and the UK (with participation from Dutch
laboratories), which explains the low representation of laboratories from these countries.

The samples distributed for analysis have been selected to represent typical mutations of the two genes, including heterozygous nucleotide replacements as well as small deletions and insertions. Prior to distribution, the mutations are verified by re-sequencing in two independent laboratories. Each year, three genomic DNA samples prepared from lymphoblastoid cell lines are sent out, together with mock clinical patient details and family histories. In order to limit the work-load imposed by the EQA scheme, participants are asked to restrict their mutation analysis to one exon, which is indicated for each sample. The choice of methodology used is left to the laboratories, but it is generally expected that the final analysis is done by DNA sequencing. The use of pre-screening methods is optional. Laboratories are asked to return a written report on their results to the scheme organiser in their usual reporting format. Schemes are strictly anonymous, and the identity of the laboratories is only known to the scheme organiser.

Table 2 shows a list of the samples distributed for analysis in past schemes. The mutations were selected to reflect the true diagnostic spectrum as closely as possible. The BRCA2 gene is underrepresented, as it emerged that a significant number of laboratories are not yet set up to screen the entire coding region of this gene.

### Genotyping

In a diagnostic setting, mutation detection in the BRCA genes usually requires analysis of the entire coding region of either gene. Ultimately, this is done by DNA sequencing. In order to reduce work-loads and costs, however, most laboratories apply one or more pre-screening techniques.

Following a Best Practice Meeting on BRCA testing held by EMQN in Amsterdam in 2000, there was not considered to be adequate evidence to make any specific recommendation as to the use of pre-screening techniques in BRCA analysis. In fact, most published techniques are actually in use (e.g. dHPLC, PTT for the large exons, DGGE and variations thereof), so for the evaluation of the genotyping, only the final results (as stated in the report) were considered, irrespective of the use of any pre-screening technique. Thus, the evaluation covers the whole analytical process and does not allow for a breakdown by technique.

Reports were evaluated for genotyping under the following criteria, and marks were assigned for successful performance.

(i) Identification of the correct sequence (1.0 mark).

(ii) Description of the mutation with the correct nomenclature both at the DNA and amino acid level (e.g. C61G could be a nucleotide or protein replacement alike; for recommendations see den Dunnen and Antonarakis [1, 2]). A reference sequence was given in order to eliminate referral to divergent sequences in the databases (GenBank; BRCA1: U14680; BRCA2: NM_000059; http://www.ncbi.nlm.nih.gov/entrez/) (0.5 marks).

(iii) A biological interpretation, i.e. a description in words of the (known or likely) effect of the gene mutation on the protein’s function. Given the high number of ‘unclassified variants’ in both genes and the continuing efforts to characterise the function of these genes, it is essential to give an interpretation of the raw molecular data (0.5 marks).

Thus, for each case, a maximum score of 2.0 marks can be obtained for genotyping. Failure in minor points leads to deduction of marks in decrements of 0.2.

Table 3 provides an overview of the genotyping performance in the three schemes. A ‘diagnostic error’ is defined here as a report that gives the wrong genotype. Three main types of errors have been observed. (i) Missing a mutation. In nine of the 17 false results a normal sequence was reported instead of the expected sequence aberration (false-negative error). These errors are most likely due to technical failures in mutation detection. (ii) Description of the observed sequence change by an incorrect nomenclature. Mistakes in nomenclature were only recorded as ‘diagnostic errors’ when the mutation description given was so different from the ‘true mutation’ as to mislead any future diagnoses of relatives. As the assessment had to rely on the printed word of the report, it cannot be ruled out that some of these errors were due to laboratories), which explains the low representation of laboratories from these countries.

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trivial typing mistakes. On the other hand, an EQA scheme is designed to reflect the true diagnostic situation as closely as possible, and if this type of error occurs in an EQA scheme there is nothing to guarantee that it would not occur in real cases. (iii) Misinterpretation of an observed sequence change. In a few cases, amino acid replacements were interpreted as ‘unclassified variants’, where in fact published experimental data on the functional relevance of the mutation were available.

The mean score for genotyping has increased from the year 2000 scheme to 2002 from 1.76 to 1.89 (out of 2.00).

**Interpretation and reporting**

Compared with the more technical process of genotyping, the writing of reports displayed a much wider variation between laboratories. The main factor contributing to this diversity appears to be the laboratory setting. In some countries, genetic testing is exclusively carried out in genetic centres that integrate a clinical genetics unit, counselling experts and the molecular genetic laboratory. In such a situation, the laboratory usually writes a technical report to the in-house expert clinicians who have seen and counselled the patient and who in turn write out the final report. Other laboratories operate independently, taking referrals from many different sources. These laboratories tend to write more comprehensive reports, including some clinical advice. Moreover, there are divergent national traditions and/or regulations on how to write medical reports.

For the sake of EQA, laboratories are being asked to handle the samples as routinely as possible and to report ‘in their usual format’. Given the above differences, this makes it very difficult to compare reports. For reasons described above in the paragraph ‘Design of the external quality assessment scheme’, EMQN has adopted the view that the more comprehensive a report is, the better it can stand alone.

For the written report, two aspects were evaluated: (i) clinical interpretation, i.e. the immediate consequence of the observed mutation for the patient and her family; and (ii) reporting format and style.

Major points (leading to the subtraction of 0.2 marks when missing) were: (i) an exact reproduction of the personal data of the patient (double identification by name and date of birth or name and laboratory number); (ii) a brief recapitulation of the patient’s personal and family history; (iii) a clear presentation of the final results (highlighted, bold print, etc.); (iv) an offer of presymptomatic testing to relatives (if applicable); and (v) a statement concerning the tumour risk of the patient and (if applicable) a suggestion to join a clinical surveillance program for early cancer detection—we consider this as the major personal benefit that an index patient can draw from genetic testing (which is otherwise mainly beneficial for the relatives).

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Minor points (not leading to subtraction of marks) were: (i) arrival date of the samples and reporting date; (ii) signature on the report of two authorised persons.

A full score for clinical interpretation and reporting was 2.00 marks.

For the reasons stated above it did not seem fair to compare diagnostic reports by a numerical score. The variation observed

### Table 2. Diagnostic cases and mutations

<table>
<thead>
<tr>
<th>Cases</th>
<th>Gene</th>
<th>Exon</th>
<th>Mutation</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000–1</td>
<td>BRCA1</td>
<td>2</td>
<td>185delAG</td>
<td>Frameshift Truncating, causal mutation; asymptomatic case identified as mutation carrier</td>
</tr>
<tr>
<td>2000–2</td>
<td>BRCA1</td>
<td>11</td>
<td>1259delG</td>
<td>Frameshift Truncating, causal mutation; affection status confirmed</td>
</tr>
<tr>
<td>2000–3</td>
<td>BRCA2</td>
<td>27</td>
<td>A10462G</td>
<td>Ile3412Val UV; polymorphism; risk not changed</td>
</tr>
<tr>
<td>2001–1</td>
<td>BRCA1</td>
<td>11</td>
<td>3600del11</td>
<td>Frameshift Truncating, causal mutation; predictive testing possible</td>
</tr>
<tr>
<td>2001–2</td>
<td>BRCA1</td>
<td>14</td>
<td>G4603A</td>
<td>Arg1495Lys UV; affects last base of exon 14, splice problem (?); life-time risk not changed</td>
</tr>
<tr>
<td>2001–3</td>
<td>BRCA1</td>
<td>16</td>
<td>G5075A</td>
<td>Met1652Ile UV; polymorphism; risk not changed</td>
</tr>
<tr>
<td>2002–1</td>
<td>BRCA1</td>
<td>24</td>
<td>5677insA</td>
<td>Frameshift Truncating, causal mutation; cancer detection programme recommended; predictive testing possible</td>
</tr>
<tr>
<td>2002–2</td>
<td>BRCA1</td>
<td>5</td>
<td>300T → G</td>
<td>Cys61Gly Affects a RING finger domain, mutated BRCA1 protein is non-functional; causative mutation, cancer detection programme recommended; predictive testing possible</td>
</tr>
<tr>
<td>2002–3</td>
<td>BRCA1</td>
<td>11</td>
<td>3875del11</td>
<td>Frameshift Truncating, causal mutation; cancer detection programme recommended; predictive testing possible</td>
</tr>
</tbody>
</table>

### Table 3. Genotyping results and error rates

<table>
<thead>
<tr>
<th>Year</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reports returned</td>
<td>25</td>
<td>41</td>
<td>37</td>
<td>103</td>
</tr>
<tr>
<td>Cases analysed&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68</td>
<td>121</td>
<td>108</td>
<td>297</td>
</tr>
<tr>
<td>Diagnostic errors</td>
<td>4</td>
<td>9</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>Error rate (%)</td>
<td>5.8</td>
<td>7.4</td>
<td>3.7</td>
<td>5.6</td>
</tr>
</tbody>
</table>

<sup>a</sup>Not all laboratories were set up to analyse all exons requested; therefore, the number of cases analysed is smaller than the number of cases times the number of laboratories.

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A full score for clinical interpretation and reporting was 2.00 marks.

For the reasons stated above it did not seem fair to compare diagnostic reports by a numerical score. The variation observed
was much broader than for the genotype score, yet the mean scores increased from 1.46 to 1.78 over the three consecutive schemes.

Conclusions and prospects

As in other areas of laboratory medicine, EQA is one of the few opportunities to obtain feedback on laboratory results, thus ‘objectively’ assessing performance. Furthermore, a horizontal comparison between laboratories allows for a better definition of the ‘state-of-the-art’, and, sometimes, for the detection of systematic technical failures (e.g. in a primer sequence). In general, technical standards in molecular genetic analysis appear to be high. Not surprisingly, however, mutation detection in DNA, like any other analytical technique, has an intrinsic error rate. As far as is apparent from the present experience with the BRCA schemes, this error rate is composed of at least three factors: (i) technical failure of DNA mutation scanning or sequencing techniques; (ii) incomplete knowledge of the relevant literature; and (iii) clerical mistakes in report writing. While factors (ii) and (iii) can easily be minimised by improving the internal quality assurance measures in the laboratory (e.g. by following the rules set for laboratory accreditation), errors of type (i) present a greater challenge, as they may have a technical and a biological component. It is known that the sensitivity of DNA scanning and sequencing techniques depends to some extent on the primary sequence. Inevitably, most genes are composed of sequences with varying complexity; thus, no single technique can detect mutations with equal efficacy in all sequences. The challenge is to apply a suitable combination of methods to balance each’s shortcomings.

EQA can alert laboratories to problems and shortcomings and help to improve, where necessary, their laboratory procedures. It should therefore become an integral part of quality assessment in the laboratory, thus contributing to maintaining confidence in the reliability of genetic testing among patients and health professionals.

References