Rapid detection of microdeletions using fluorescence in situ hybridisation (FISH) on buccal smears

Although patients with Williams syndrome (WS) and 22q11 deletion syndrome have a normal standard karyotype, a submicroscopic deletion can be found in both syndromes using FISH. We report the use of FISH on buccal smears using cosmid probes for 7q11.23 (WS) and 22q11.2 (22q11 deletion syndrome) for the rapid detection of these two microdeletion syndromes.

Six patients known to have a microdeletion (three 7q11 and three 22q11) were compared to eight healthy controls. All the patients had been previously diagnosed by FISH on metaphase chromosomes of peripheral blood samples. Buccal smears were obtained by standard methods. FISH was carried out with Vysis LSI dual colour probes. With the probe set for WS (Vysis LSI Williams/Y Chromosome dual colour probe), a red (spectrum orange) signal is obtained at the site of the chromosomal segment that is usually deleted in WS patients (the elastin gene region in 7q11.23) and a spectrum green fluorescent control signal is obtained at band 7q31 for the identification of the chromosome. With the 22q11 deletion syndrome probe set (Vysis LSI DiGeorge/VCFs region dual colour probe), the red signal is obtained at band 22q11.2 and the spectrum green fluorescent control signal at band 22q13.

The greater specificity of the probes used in FISH techniques has made detection of chromosomes in interphase nuclei, including buccal smears, more reliable. Harris et al. used FISH probes on buccal smears of newborns for rapid diagnosis of autosomal trisomies or chromosomal sex. Schad et al. compared the interphase FISH method with the conventional X and Y chromatin methods on buccal smears of 15 men and 15 women. They found the efficiency of detection of the X improved from 12% ± 3% with the conventional method to 98% ± 0.7% with FISH and for the Y from 51.5% ± 4.9% to 99.8 ± 0.4%.

Table 1 shows the numbers of cells with one or two red signals for either the 7q11 or the 22q11 probe, as scored by observer A and observer B. The remaining cells with either no or ≥3 red signals are not included in table 1, but can be inferred from it. Cases 3, 4, 6, 9, 10, and 12 were judged by the observers to have a deletion. After decoding the slides, all patients and controls appeared to have been correctly predicted. The result was therefore unambiguous for all 14 cases. The last column of table 1 shows the success rate, that is, the mean percentage of cells with the correct number of signals. Representative cells of the patients and the controls are shown in fig 1.

The average probe efficiency in detecting the correct number of signals with the WS probe was 94.9% (±3.7) and with the 22q11 probe 94.1% (±3.7).

Although the buccal smear test for sex chromosomes has its uses, it has remained essentially unreliable. This is because the Barr body is visible in only 30–50% of the cells from buccal smears from normal females. The fluorescent signal of the Y body is difficult to detect because it varies with the size of the Y heterochromatin. It can also be easily confused with other fluorescent signals from autosomal heterochromatic segments which are also variable in size.

The greater specificity of the probes used in FISH techniques has made detection of chromosomes in interphase nuclei, including buccal smears, more reliable. Harris et al. used FISH probes on buccal smears of newborns for rapid diagnosis of autosomal trisomies or chromosomal sex. Schad et al. compared the interphase FISH method with the conventional X and Y chromatin methods on buccal smears of 15 men and 15 women. They found the efficiency of detection of the X improved from 12% ± 3% with the conventional method to 98% ± 0.7% with FISH and for the Y from 51.5% ± 4.9% to 99.8 ± 0.4%.

Table 1 FISH results from observer A and B. Only cells with two green signals were analysed. Cells with either one or two red signals are shown as a proportion of the total number of cells analysed. The remaining number of cells (per observer) with either 0 or ≥3 red signals can be inferred from these figures. The success rate is shown as the percentage of cells with the correct number of signals.

<table>
<thead>
<tr>
<th>Probe and case No</th>
<th>No of cells with 1 red signal</th>
<th>No of cells with 2 red signals</th>
<th>% of cells with the correct number of red signals (mean of A and B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of cells with 1 red signal</td>
<td>No of cells with 2 red signals</td>
<td>% of cells with the correct number of red signals (mean of A and B)</td>
</tr>
<tr>
<td>WS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>45/50 46/49</td>
<td>3/50 1/49</td>
<td>100%</td>
</tr>
<tr>
<td>5</td>
<td>45/50 50/50</td>
<td>4/50 0/50</td>
<td>60%</td>
</tr>
<tr>
<td>6</td>
<td>1 Control 0/46</td>
<td>1/46 0/46</td>
<td>75%</td>
</tr>
<tr>
<td>7</td>
<td>0/49 2/50</td>
<td>49/49 47/50</td>
<td>60%</td>
</tr>
<tr>
<td>8</td>
<td>44/44 44/49</td>
<td>0/46 1/46</td>
<td>60%</td>
</tr>
<tr>
<td>9</td>
<td>45/45 46/46</td>
<td>2/46 1/46</td>
<td>60%</td>
</tr>
<tr>
<td>10</td>
<td>44/46 44/46</td>
<td>0/46 1/46</td>
<td>60%</td>
</tr>
<tr>
<td>11</td>
<td>3/47 2/50</td>
<td>47/50 48/50</td>
<td>60%</td>
</tr>
<tr>
<td>12</td>
<td>48/49 47/49</td>
<td>3/50 4/50</td>
<td>60%</td>
</tr>
<tr>
<td>13</td>
<td>2/49 4/50</td>
<td>47/49 46/50</td>
<td>60%</td>
</tr>
<tr>
<td>14</td>
<td>1/49 0/50</td>
<td>47/50 50/50</td>
<td>60%</td>
</tr>
</tbody>
</table>
Our experiment shows that FISH on buccal smears can now be applied for the detection of microdeletion syndromes. For WS and 22q11 deletion syndrome we have obtained unequivocal results. As mosaicism rarely plays a role in these two syndromes and as correct numbers of signals were seen in >86% of the cells, analysis of 50 cells is sufficient to distinguish a patient from a control.

This method, which is reliable, easy to use, quick, and non-invasive, could be useful in studying these two microdeletion syndromes in large numbers of patients, for instance in institutions for the mentally retarded, or for studying young children.

In those patients in whom a microdeletion has been identified and for whom family studies are indicated, any possible chromosomal rearrangement involving the chromosome with the microdeletion would have to be excluded on metaphases from a lymphocyte culture.

We thank Ina Koning and Joan Werners for their expert technical assistance.

A W M NIEUWINT
J M VAN HAGEN
Y M HEINS
K MADAN
L P TEN KATE

Department of Clinical Genetics and Human Genetics, University Hospital Vrije Universiteit, PO Box 7057, 1007 MB Amsterdam, The Netherlands

Correspondence to: Dr Nieuwint, A.Nieuwint@azvu.nl

1 Barr ML, Bertram EG. A morphological distinction between neurons of the male and female and the behaviour of the nucleolar satellite during accelerated nucleoprotein synthesis. Nature 1949;163:676.
Rapid detection of microdeletions using fluorescence in situ hybridisation (FISH) on buccal smears

A W M NIEUWINT, J M VAN HAGEN, Y M HEINS, K MADAN and L P TEN KATE

J Med Genet 2000 37: e4
doi: 10.1136/jmg.37.6.e4

Updated information and services can be found at:
http://jmg.bmj.com/content/37/6/e4

These include:

References
This article cites 10 articles, 1 of which you can access for free at:
http://jmg.bmj.com/content/37/6/e4#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

- Screening (oncology) (234)
- Valvar diseases (30)
- Head and neck cancer (7)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/