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

EDITOR—Use of buccal smears for the detection of numerical sex chromosome abnormalities has long been known. The facultative heterochromatin of the inactive X chromosome in the normal female is visible in Giemsa stained interphase nuclei as a condensed body, the Barr body. The active X is not visible. The constitutive heterochromatin of the long arm of the human Y chromosome, which varies greatly in size between people, is visible as a fluorescent body in interphase nuclei stained with quinacrine.

More recently, FISH using centromere probes has been applied to interphase nuclei of buccal mucosa for the study of oral cancers, for detection of supernumerary i(12p) and i(18p) chromosomes, and for the detection of chromosomal sex.

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.

Pretreatment of the slides was by the method of Arnoldus et al with some modifications. Fixation of the cells was performed by immersing the slides for 10 minutes in fixative (1:1 methanol/acetic acid, according to the Oncor protocol, The Ultimate FISHing Guide, Oncor Inc, 1996, page c-2). After RNase and pepsin treatment the slides were washed in PBS twice for five minutes and incubated for 10 minutes in 3.7% formaldehyde in PBS at room temperature. Following two washes of five minutes each in PBS the slides were denatured by incubation in hybridisation mixture (70% formamide, 2 × SSC, 40 mmol/l phosphate buffer, pH 7.0) at 80°C. This was followed by a dehydration series with ethanol before applying the probes to the slides.

The application of the FISH probes, overnight hybridisation and immunocytochemical detection were carried out according to the probe manufacturer’s instructions. The slides were coded and 50 cells were scored by two independent observers (A and B) using a Zeiss fluorescent microscope with a DAPI, a FITC, and a TRITC filter. As in some cases a limited number of cells was available, the two observers scored from two ends of the same slide. Some overlap between the two results is therefore likely. Only cells showing two green signals were included in the analysis. To estimate the probe efficiency, the mean percentage of cells with the correct number of signals as scored by A and B was calculated for the WS probe and the 22q11 probe.

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 I.

The average probe efficiency in detecting the correct number of signals with the WS probe was 94.9% (±1.7) 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 chromosomal abnormalities 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.
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.

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