ELECTRONIC LETTER

Absence of 22q11 deletions in 211 patients with developmental delay analysed using PCR

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J Med Genet 2002;**39**:e18 (<http://www.jmgjnl.com/cgi/content/full/39/4/e18>)

The 22q11 deletion syndrome has been described by the acronym "CATCH 22" (Cardiac defects, Abnormal facies, Thymic hypoplasia, Cleft palate, and Hypocalcaemia resulting from 22q11 deletions).¹ Previous studies have indicated that other features such as growth retardation, developmental delay, renal abnormalities, psychiatric problems, and neurological abnormalities may also be associated with the deletion.² There have been several estimates of the prevalence of the 22q11 deletion, ranging from 1 in 3000 to 1 in 10 000 live births.³⁻⁵ It is possible, however, that the frequency of the deletion has been underestimated because of the large phenotypic variability, or the failure to identify mildly affected subjects in the absence of more severely affected relatives.⁶ Ninety percent of patients have a common 3 Mb deletion and 7% have a nested 1.5 Mb deletion. Both require fluorescence in situ hybridisation (FISH) for reliable detection.⁷⁻⁹ FISH is not, however, routinely carried out in this laboratory unless there are two or more cardinal features of the syndrome or screening for a 22q11 microdeletion is specifically requested. If, therefore, a patient is referred with relatively mild expression of the 22q11 deletion spectrum, a deletion would not be detected using conventional cytogenetics and FISH analysis would not be undertaken. To determine whether it is worth testing patients with less obvious clinical features of a 22q11 deletion, we have used the fluorescent polymerase chain reaction (PCR) to screen a population of 291 subjects with just one of four cardinal features characteristic of this syndrome.

MATERIAL AND METHODS

A computerised search selected a total of 334 consecutive patients with clinical features which included one of (1) developmental delay, (2) cleft palate, (3) hypothyroid, hypoparathyroid, or absent thymus, or (4) psychosis or schizophrenia (table 1). Forty-three patients were excluded as FISH had already been carried out. All patients screened with PCR were karyotypically normal and those with developmental delay had repeats at the FRAXA and FRAXE loci within the normal range.

All of the primer sequences used in this study were obtained from the Genome Database.¹⁰ The marker order is shown in fig 1. DNA was extracted from venous blood samples by a salt precipitation technique,¹¹ diluted to a working concentration

of 50 ng/μl volume, and amplified in an MJ Tetrad thermocycler using 32 cycles and an annealing temperature of 55°C. One primer from each pair was fluorescently labelled to allow analysis on an ABI 377 automated DNA sequencer. Four highly polymorphic microsatellites were used for initial testing (D22S264, D22S311, D22S944, and D22S1638) after validation in known deleted patients and normal subjects. Hemizygosity for loci D22S1638 and D22S944 would be consistent with a 1.5 Mb nested deletion and hemizygosity for all four loci would be consistent with a common 3 Mb deletion. Any cases apparently homozygous for D22S1638 and D22S944 or for any three of the four loci were screened with three additional markers (D22S941, D22S1623, and D22S1709). If the additional markers were also apparently homozygous, copy number was determined using quantitative PCR (QPCR). This approach was streamlined for the large developmental delay group by initially screening with microsatellites D22S1638, D22S944, D22S941, and D22S1623 before moving directly to QPCR if D22S1638 and D22S944 or all four loci were homozygous. DNA for QPCR was purified using the WIZARD™ system (Promega) and three primer pairs at the D22S1572, D22S1575, and D22S1669 loci were used. One primer from each pair was again labelled with a fluorescent dye. PCR was performed in a 20 μl volume in an MJ Tetrad with optimal PCR conditions of 19 cycles and an annealing temperature of 55°C. The potentially deleted target sequence at these loci was coamplified with a control disomic sequence from within the *DP5.2* gene on chromosome 5. The value of the ratio (target PCR product:control PCR product) was compared with the average ratios of two control groups (known deleted patients and normal subjects). The average ratios were used to convert the individual ratios from each test sample into a copy number for each locus. Cases with single alleles at all loci and a copy number of 1 by QPCR were analysed using FISH with cosmid H748 from the TUPLE 1 region to confirm or exclude a deletion.

RESULTS

A total of 291 patients from all four groups were screened with PCR (table 1). No evidence of a deletion was found among the 211 patients from the developmental delay group, the 15 patients from the thymic group, or the 50 patients from the cleft palate group screened with PCR.

Table 1 Referral groups of 334 patients tested for 22q11 deletions by FISH or PCR

Group	No of patients	Initially analysed by FISH	Deleted by FISH	Analysed by PCR	Deleted by PCR	Total deleted
Developmental delay	212	1	0	211	0	0
Psychosis	16	1	1	15	1	2
Thymic hypoplasia	21	6	0	15	0	0
Cleft palate	85	35	1	50	0	1
Total	334	43	2	291	1	3

Figure 1 Marker order on chromosome 22q11.1.

A previously unidentified 22q11 microdeletion was found in one of 15 patients in the psychosis group and confirmed using FISH. The newly detected molecular deletion had a distal breakpoint between D22S1623 and D22S264 and extended proximally to D22S1638, consistent with deletion of the proximal half of the 22q11 deletion critical region rather than loss of the entire 3 Mb region.

DISCUSSION

The absence of any undetected microdeletions among 211 patients referred with developmental delay suggests that mildly affected probands are not frequently being missed by using a minimum of two cardinal features to select patients for FISH analysis. With 95% confidence, these results indicate that the true frequency of 22q11 deletions in a population of patients referred with developmental delay as the sole cardinal feature of 22q11 deletion syndrome is between 0% and 1.4%.¹² On initial testing, only eight patients were homozygous at all four loci and only 25 at the two loci within the 1.5 kb deletion (D22S1638 and D22S944); this suggests that PCR screening could be streamlined by using just two loci followed by QPCR on the remaining subset of patients. Future investigations ought, however, to include the new flanking region implicated in 22q11 deletion syndrome.¹³

The finding of a nested microdeletion by PCR in one adult patient out of 15 with schizophrenia or psychosis is consistent with the relatively high number of 22q11.2 microdeletions found in adult patients with schizophrenia, psychosis, and other features of 22q11.2 microdeletion syndrome.¹⁴ The patient was a 54 year old institutionalised male referred with mental handicap, schizophrenia, chest pains, and a very pale complexion. This phenotype is at the milder end of 22q11 deletion spectrum, although it is possible that the chest pains were secondary to an undiagnosed congenital heart defect. Other psychotic adults have also had fewer palatal and conotruncal anomalies than paediatric patients but do share a high level of learning difficulties.¹⁴ A much larger series of patients would be needed to establish whether the smaller 1.5 Mb deletions are more common in this group.

The utility of a genome wide microsatellite scan for the detection of submicroscopic anomalies has recently been reported¹⁵ and microarrays may ultimately provide the best way of scanning the whole genome for cytogenetic and submicroscopic abnormalities. In the mean time, our results suggest that selection of patients for 22q11.2 microdeletion testing on the basis of at least two cardinal features of the syndrome is a more cost effective means of targeting genetic testing.

ACKNOWLEDGEMENTS

NST was supported and the ABI sequencer provided by the Wellcome Trust. We should like to thank James Macpherson for FRAXA and FRAXE analyses, Paul Strike of the Salisbury Research and Development Support Unit for statistical advice, and Judy Gladding for preparation of the manuscript.

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