methyated allele, however, was detected only in tumours that also showed a methyated allele by Southern blot hybridisation.

To show that the *RB1*-MSP is applicable in routine diagnosis, a second set of 20 randomly selected tumour DNA samples with unknown methylation status was tested by the *RB1*-MSP. All tumours had been investigated for LOH at *RB1*2 and *RB1*20. In two of these samples, a methyated *RB1* promoter region was identified by the MSP assay and verified by Southern blot analysis. Although this is a small number of tumours, the finding of methyated *RB1* alleles in 10% of unilateral sporadic retinoblastomas is in agreement with previous estimates on the frequency of hypermethyated *RB1* alleles.⁴ An additional 154 bp PCR product representing an unmethyated allele was also obtained in these two tumours, which was to be expected as they did not show LOH at the intra-genic polymorphic loci *RB1*2 and *RB1*20.

In summary, analysis of 40 samples has shown that our MSP reliably identifies tumours with hypermethyated *RB1* alleles as detected by Southern hybridisation. Compared to Southern blot analysis, however, MSP is faster, can be performed with small amounts of genomic DNA, and does not need radioactive components. Thus, MSP facilitates identification of *RB1* gene hypermethylation in RB and other tumours.¹²

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Frequency and predictive value of 22q11 deletion

EDITOR—Malformations resulting from the adverse effects of 22q11 deletion are now recognised to be of one of the most important diagnostic categories in dysmorphology. The predictive value of this common deletion remains to be fully elucidated since even large reviews¹ are by their nature subject to ascertainment bias. In our original report of familial heart disease associated with submicroscopic 22q11 deletion, which predated the routine availability of fluorescence in situ hybridisation (FISH), the carrier mother was dysmorphic but had a normal heart.² We and others have drawn attention to the marked variability of the phenotype and the need to be alert to the possibility of subtle features in a parent.³⁻⁵ These observations raise the possibility of subclinical deletion being more common than has been recognised. This would have significance in genetic counselling when 22q11 deletion is detected in the first trimester as occurred in the case described below.

Following the recognition of a deletion 22q11 in III.2 (fig 1) diagnosed clinically as DiGeorge syndrome with complex cardiac malformations including pulmonary atresia, double outlets of the right ventricle, subaortic interventricular septal defect, mitral atresia, restrictive intra-atrial communication, hypoplastic left ventricle, patent ductus arteriosus, and right aortic arch leading to neonatal death, the parents were invited to undergo chromosome analysis. The father (II.4) (fig 2) was found to carry a del22q11.1-11.2 karyotype and displayed minor facial features, mild learning difficulties, and was on the 10th centile for height.⁴ The couple went on to have three subsequent pregnancies. In their third pregnancy, early amniocentesis showed a 22q11 deletion but no evidence of

Figure 1 22q11 deletion in two generations with marked variation in phenotype.

structural abnormality was detected on an anomaly scan and fetal echocardiography at the 17th and 18th week. A further examination at 19 weeks showed a major structural heart defect. At necropsy the aborted fetus (III.3) was found to have slightly abnormal facial features, pulmonary atresia, perimembranous ventricular septal defect, secundum atrial septal defect, retro-oesophageal right subclavian artery, a small thymus, and one ectopic parathyroid. The couple's fourth pregnancy resulted in a healthy daughter (III.4) while their fifth (III.5) was a severely affected fetus with 22q11 deletion who died in utero.

There have been eight reports of prenatal diagnosis based on a 22q11 deletion but the growing recognition of this disorder in clinical practice means this will become a more common event. In order to improve the predictive value of detection of 22q11 deletion in pregnancy, we undertook an investigation of 22q11 deletion in an unselected population.

Figure 2 The father found to have 22q11 deletion had minimal clinical features (reproduced from Burn *et al*, 1995, with permission).

The North Cumbria Community Genetics Project (NCCGP) was established in 1996 to provide a consecutive series of samples from all newborn infants in a defined area. With the written consent of parents, samples of cord blood, viable cells, tissue, and DNA are collected and stored.⁶ NCCGP samples were analysed by dual colour FISH using cosmid probes specific to the commonly deleted area. Probe F5429 contains sequences from the TUPLE-1 region,⁷ and probe F9130 encompasses the ADU/VDU breakpoint isolated from an apparently balanced t(2;22) translocation identified from both a severely affected daughter with DiGeorge syndrome and her mildly affected mother.⁸ Both probes detect sequences from the proximal shortest region of overlap (SRO) of 22q11 deletions and, according to Rauch *et al*,⁹ could detect approximately 90% of all potential deletions in the chromosome region of 22q11. Three specimens known to carry a 22q11 deletion mixed with the NCCGP samples on a blind basis were all detected correctly, but no 22q11 deletions were detected among 1731 study samples.

Based on a Poisson distribution, the highest prevalence of 22q11 deletion at birth with which these data are compatible is 1 in 577 ($p > 0.95$) and we conclude, therefore, that the frequency of this deletion in the general population is probably less than 1 in 600. Jacobs *et al*¹⁰ estimated the frequency of all unbalanced chromosome abnormalities to be 1 in 1639. This estimate predated the availability of

routine FISH analysis for 22q11 and other microdeletions. Previous estimates of the prevalence of this deletion have relied on results from cases with clinical abnormalities, and minimum values of about 1 in 4000,¹¹ 1 in 6000,¹² and 1 in 10 000¹³ have been reported. A study in the Northern Region of England of 22q11 deletion associated with significant malformations estimated its prevalence at around 1 in 10 000 births.¹⁴ This was based on referrals with medical complications early in life, particularly to the paediatric cardiology service. Since cases with less obvious congenital defects continue to present later in childhood,¹⁴ it is apparent that the previous reports represent minimum estimates of the prevalence in the whole population. By comparing our unselected population data with the middle estimate for minimum prevalence (1 in 6000) we conclude that when 22q11 deletion is discovered in pregnancy, the chance of major malformation is at least 10% and further investigation such as high resolution ultrasound scan and fetal echocardiography are indicated.

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