LETTERS TO THE EDITOR

Monozygotic twins with chromosome 22q11 deletion and discordant phenotype

I believe that the recent description by Goodship *et al*¹ of discordance in monozygotic twins with a 22q11 deletion raises some interesting issues. The likelihood of a heart defect in a person with a constitutional 22q11 deletion cannot currently be estimated, as ascertainment of such patients is usually on the basis of the presence of a heart lesion. In addition, the true prevalence of 22q11 deletions is not known, although a lower limit of 1:4000 has been quoted on the basis of children presenting with congenital heart defects.² Thus, until prospective studies are carried out that allow complete ascertainment of subjects with a 22q11 deletion, irrespective of phenotype, this question cannot be answered. Variability of phenotypic expression among people with presumably identical deletions (that is, familial cases) strengthens the concept that a 22q11 deletion merely increases the likelihood of certain anomalies being present but does not guarantee them.³ It is clear, therefore, that the association of heart disease with this deletion is far from deterministic.

The discovery of monozygotic twins with a 22q11 deletion who are discordant for heart disease should, therefore, come as no surprise, although until this report, the role of "genetic background" could never be ignored.¹ While it is clearly true that the phenotype in these twins could not have been predicted on the basis of their identical (germline) genotypes, it is not necessarily true that the discordance cannot be attributed to genetic differences between the two. If, in the above paper, the words "chromosome 22q11 deletion" had been replaced by "germline mutation in the retinoblastoma gene", little surprise would have been expressed, and the discussion would have focused on the chance element involved in the "second hit" genetic mutations now known to be necessary for phenotypic expression in retinoblastoma.⁴ The concept of a "second hit" in congenital malformations, however, is not widely accepted. The disorganisation gene in the mouse is an example of a dominant mutation that increases the risk of a wide variety of congenital malformations, from cleft palate to accessory limbs.⁵ Analysis of the likelihood of one, two, or three malformations in this mouse is consistent with the Knudson "second hit" hypothesis, although little can be said about the nature of these secondary somatic events, only that they occur independently of each other.⁵ They may be genetic or epigenetic and are likely, in any event, not to occur at the Ds locus itself. Thus, it is likely that the disorganisation gene is important in development of many organ systems but that abnormalities of these systems result from random somatic events, as yet unidentified.⁵ Of note is the fact that the majority of

mice with the Ds mutation are phenotypically normal, having presumably escaped secondary somatic events occurring at an early stage of development.⁵ With this mechanism in mind, it is possible that a secondary somatic event is required for manifestation of some features known to be associated with a 22q11 deletion.

The velocardiofacial syndrome is remarkable for the wide variety of phenotypic manifestations and is best thought of as comprising features that are relatively constant (facial features, developmental delay), features that are common but not universal (conotruncal heart defects, thymic abnormalities), and features that are only found rarely (upper limb malformations,⁶ meningomyelocele,⁷ cerebellar atrophy⁸), although this analysis is of necessity biased as the number of relatively normal subjects who harbour 22q11 deletions is not known. While it is likely that haploinsufficiency alone may be sufficient to explain the dysmorphic facies and developmental delay, it is not clear that this is true for heart disease and other common but not universal features. While somatic events may play a role, it is possible that haploinsufficiency may simply increase the statistical likelihood of certain malformations in a way that is unpredictable, maybe even in principle. The association of rare anomalies with 22q11 deletions may result from a number of general mechanisms.

(1) Uncovering of recessives. The deletion uncovers a recessive mutation present on the non-deleted chromosome. The frequency of such features in the "22q11 deletion" population should mirror the frequency of heterozygotes in the population for the relevant recessive disorder (for example, Bernard-Soulier phenotype⁹). A careful search for rare associations in "microdeletion syndromes" may be helpful in mapping recessive phenotypes.

(2) A second hit, either on the contralateral 22q11 or elsewhere in the genome, may give rise to the relevant (uncommon) abnormality. This mechanism, as described above for the Ds gene, has been invoked to explain vertical transmission of conditions that give rise to "phenotypic mosaicism" and which had hitherto been thought of as resulting from genetic mosaicism (for example, ILVEN).¹⁰ In this model, the presence of a germline mutation in a large number of subjects does not, of itself, result in the phenotype; in a small proportion, however, a second mutation occurring at an early stage in development will result in the relevant abnormality, which will generally appear mosaically. There will be few such subjects as the chance that the second hit will occur in the right cell type and early enough in development is very low. The majority of such subjects will therefore occur sporadically and be deemed to represent new postzygotic mutations. The discovery, however, of even one example of a parent-child combination, both displaying "phenotypic mosaicism" must force a re-evaluation of the original hypothesis. The report of unilateral preaxial polydactyly in a child with a 22q11 deletion, for example, would be consistent with a somatic event occurring early and affecting a cell line about to be involved in formation of the distal limb bud.⁶

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Large inv dup(15) chromosome in two generations

Inv dup(15) is a relatively common chromosomal abnormality, which alone may account for approximately half of all small supernumerary marker chromosomes detected.^{1,2} The phenotype is highly variable ranging from apparently unaffected persons to those who are severely retarded. According to several authors the severity of the phenotype roughly correlates with the size of the marker. Webb,³ in her review article, suggested cytogenetic division of inv dup(15) markers into three groups according to size. The majority of patients with a marker equal to or larger than a G group sized chromosome were found to be mentally retarded. Robinson *et al*⁴ argued that the number of copies of the Prader-Willi syndrome/Angelman syndrome (PWS/AS) region present in the marker may be directly related to the severity of the retardation. To our knowledge, no non-mosaic inv dup(15) carriers without mental retardation have been described, who appeared to have extra copies of the PWS/AS region, as shown by molecular techniques.^{4,8}

We report a family with inv dup(15) in two generations. The proband, a girl ascertained shortly after birth, was the first child of healthy, non-consanguineous parents. She was born at term but was small for gestational age with borderline microcephaly, bilateral epicanthus, and frontal bossing. Feeding was poor owing to hypotonia. Apart from an unusual head retraction reflex when tapped on the nose, no other abnormalities were noted at birth. At the age of 16 months she was not able to sit without support, did not attempt to speak, and had developed seizures. A G group sized inv dup(15) supernumerary marker was found in all 20 lymphocytes analysed. Subsequent fluorescent in situ hybridisation (FISH) analysis (centromeric D15Z1 probe⁹ and B25E9,¹⁰ as well as

A156E1¹¹ probes in the PWS/AS region) showed the PWS/AS region to be present twice in the marker (data not shown).

The parents of the index patient are illiterate, went to a school for children with learning difficulties, and have a simple job in a sheltered environment. They have had no seizures.

In 18 out of 32 lymphocytes analysed in the mother, the same marker was present. The karyotype of the father was normal. The marker was not found in 100 lymphocytes from the maternal grandparents. Sibs of the mother were not available for further study and we have no information on their mental status.

Numerous cases of small familial inv dup(15) supernumerary markers have been reported.^{2,3,5,7} Apparently, most of these markers have no adverse phenotypic effects. Many of these familial cases have been ascertained accidentally by prenatal screening. To our knowledge, however, only three familial cases of inv dup(15) where mental retardation was a feature have been reported.¹²⁻¹⁴ All retarded probands in these three families inherited the marker from their mother. One of these mothers was not retarded herself, but she was a proven mosaic. Another two families, ascertained through mentally retarded probands, have been reported. However, in these cases it was not likely that the mental retardation could be attributed to the inv dup(15), since other carriers of the familial marker were not retarded.^{5,13} In both families the inv dup(15) was small and unlikely to contain the PWS/AS region. Finally, one report concerned a prenatal diagnosis of an inv dup(15) in a carrier mother, who was described as "mentally slow". No information on the development of the child was presented.¹⁵

In the present report another family is described, in which an inherited inv dup(15) is associated with mental retardation. To our knowledge this is the second familial case where the presence of additional copies of the PWS/AS region was shown by molecular techniques,¹⁴ and the first familial inv dup(15) in which two extra copies of this region were identified. This case confirms that an inv dup(15) may be inherited, even when two copies of the PWS/AS region are present in the marker. The phenotype caused by such a large marker, however, does not always have to be severe, but may be milder owing to a mosaic state, as illustrated by the mother of our index case. The use of FISH probes for the PWS/AS region appears to be of importance in counselling parents, who may be mosaics for an inv dup(15) supernumerary marker, with respect to the expected phenotype of their non-mosaic offspring.

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Simple tests for rhodopsin involvement in retinitis pigmentosa

Retinitis pigmentosa (RP) is an inherited retinal degeneration affecting approximately 1 in 5000 people.¹ The genetic basis of RP is complex, with X linked, autosomal dominant, and autosomal recessive inheritance, and multiple loci for each form. This makes it difficult for diagnostic laboratories to provide useful information to RP patients and their families, especially in dominant RP, which maps to at least eight different loci. However, our work on dominant RP over the last five years indicates that there are three simple tasks which a clinical genetics diagnostic laboratory could carry out as a starting point for DNA analysis, each of which have a reasonable chance of providing useful information.

Published estimates for the frequency of rhodopsin mutations as a proportion of dominant RP range from 20 to 31%,²⁻⁴ but our own recent analyses in large families suggest a figure as high as 50% (Inglehearn et al, manuscript in preparation). Rhodopsin is

therefore a good candidate gene for patients with a dominant family history, and it has also been implicated in several cases of recessive RP.^{5,6} The markers which have been used in the past to exclude rhodopsin as a candidate RP gene are C17 (D3S47), the RFLP marker first linked to ADRP at 3q21,⁷ and a microsatellite in intron 1 of the gene itself.⁸ However, C17 is now estimated to be some 18 cM from rhodopsin⁹ while the intragenic microsatellite has a heterozygosity of only 33%. We have therefore placed the rhodopsin gene on the microsatellite map of Gyapay et al¹⁰ by linkage analysis in rhodopsin RP families. Haplotype analysis (data not shown) locates the rhodopsin gene in a 5 cM gap between markers D3S1589 (heterozygosity 0.68) and D3S1292 (heterozygosity 0.85). By pooling data in linked families we obtained maximum lod scores of 8.55 at $\theta=0.07$ from marker D3S1589 and 21.75 at $\theta=0.02$ for marker D3S1292. These are therefore highly informative microsatellite markers with which to test for rhodopsin linkage in dominant RP.

Screening for mutations in the rhodopsin gene is also complex, since over 60 have now been reported.¹¹ The Pro-23-His mutation was found to account for 12% of US ADRP.¹² However, this has not been reported in any other populations¹³ and is now thought to represent a founder effect. Two other mutations have been reported in different populations. Pro-347-Leu has been seen in US, UK, German, and Japanese patients⁴ and three other base substitutions have been found at the same site. Similarly, Thr-58-Arg has been reported in both US and UK populations. These are therefore probably mutation hotspots for rhodopsin mutations leading to ADRP and may be worth screening in dominant and sporadic cases of RP. This can be done by a simple assay involving PCR amplification followed by restriction digestion, using *MspI* for codon 347 (destroys a site)¹⁴ and *DdeI* for codon 58 (creates a site).¹⁵ Our own data on screening for these mutations showed five patients with the codon 347 Pro-Leu substitution and two with the codon 58 Thr-Arg substitution. These were identified in a sample of 120 RP patients who attended the Moorfields Eye Hospital genetic clinics and gave a family history indicating dominant RP. It is worth noting that both codon 58 pedigrees have a rare sectorial RP phenotype. Sectorial RP cases should therefore be made a priority in testing for the codon 58 mutations.

In summary ADRP families can quickly be assessed for linkage to rhodopsin, using markers D3S1589 and D3S1292, which span the locus. In addition around 6% of dominant RP cases can be characterised by simple PCR/restriction digestion tests at codon 58 and 347 of the rhodopsin gene.

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