Monozygotic twins with chromosome 22q11 deletion and discordant phenotypes: updates with an epigenetic hypothesis

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T he completion of the human genome sequence affords novel approaches to studies on contiguous gene deletion syndromes. These syndromes are caused by a deletion and loss of one copy of a set of contiguous genes on a given chromosome. Here, the syndromic phenotypes are often attributed to haploinsufficiency of a number of deleted genes. One such syndrome deals with deletion of 22q11.2. It is the most common microdeletion syndrome with a frequency of 1:4000 live births. This high frequency has been attributed to low copy repeats (LCR) on chromosome 22 with most cases (85-90%) representing de novo mutations. Also, the critical common region is relatively large (>1.5-3.0 Mb), may involve >30 genes, and there is no evidence of any correlation between the size of the deletion and the observed syndromic phenotypes. In fact, the clinical phenotype of the 22q11 deletion syndrome is characterised by extensive variability. It includes velocardiofacial syndrome (VCFS), DiGeorge syndrome (DGS), and associated physical, developmental, neurological, and neuropsychiatric phenotypes. This phenotypic variability associated with the 22q11 deletion is an exception to all the other contiguous gene syndromes. More puzzling are recent reports that monozygotic twins discordant for del 22q11 have different phenotypes. In this discussion we will review all published cases of monozygotic twins discordant for del 22q11 and assess phenotypic discordance/concordance between them. More important, we will discuss an epigenetic explanation for their discordance that is compatible with modern molecular understanding of the human genome, the sequence features of this genomic region and sensitivity to in vivo effects during development and differentiation.

SUMMARY OF CASE REPORTS

Twin pair 1
Twin pair 1 was born to a 32 year old mother of European ancestry, at a gestational age of 38 weeks, weighing 2200 g (twin 1) and 2800 g (twin 2), apparently from a single placenta. Although the facial features of the male twins were similar and related to DGS, only twin 1 had a heart murmur at week 1 and a diagnosis of tetralogy of Fallot was made at week 8. Twin 1 also had slow development, more pronounced nasal speech, and more marked toe deformity. There is no family history of congenital heart disease or other handicap. High resolution cytogenetic analysis on 100 metaphases on each of the twins was compatible with a single de novo deletion event leading to a 46,XY,del(22)(q11.21q11.23) karyotype in both twins. Four hypervariable DNA polymorphisms and nine red cell antigens established that the twins are 99.998% monozygotic. The authors argue that the discordant phenotype cannot be explained by genotypic differences alone.

Twin pair 2
Twin pair 2 was a female monozygotic (>99%) adult twin pair. They share VCF syndrome related facial appearance with nasal speech, mild learning difficulties, and triphalangeal thumb. Twin 1 had no cardiac defect clinically or on ECG. Twin 2 required a pharyngoplasty and had surgery for an aortic defect during childhood. Interestingly, twin 1 had a daughter with mild learning disabilities, VCFS appearance, and normal heart, while twin 2 had a daughter who died following surgery for tetralogy of Fallot with absent pulmonary valve and hemitruncus. Twin 2 had another child with 22q11 deletion but a normal heart.

Twin pair 3
Twin pair 3 was delivered by vacuum extraction at 37 weeks of gestation to a 30 year old mother of Japanese ancestry as her first pregnancy. Both parents were clinically normal and there is no family history of heart disease. The male twins had a single placenta. Twin 1 weighed 1576 g and was anaemic while twin 2 weighed 2376 g and was plethoric. FISH analysis on 100 cells showed that there was no mosaicism and the twins were similar for deletion of 22q11.2, while neither parent had the deletion. Monozygosity of the twins was established by a total of 18 markers with a probability of 99.9997%. Clinical observations showed that although both twins had an abnormal face, there was little else in common between them. Although twin 1 had cardiac defect, thymic hypoplasia, velopharyngeal insufficiency, mental retardation, short stature, and anal atresia, twin 2 had no such complications. Such differences were suggested to be because of environmental factors, postzygotic events, or the twinning process.

Twin pair 4
Twin pair 4 was delivered by caesarean section at 32 weeks of gestation to a 27 year old mother from a diamniotic,

Key points

- Deletion 22q11 is the most common deletion syndrome in human populations. It is characterised by extensive phenotypic heterogeneity representing velocardiofacial syndrome (VCFS), DiGeorge syndrome (DGS), and associated physical, developmental, neurological, and neuropsychiatric phenotypes.
- This heterogeneity has been attributed to individual specific recessive alleles and haploinsufficiency caused by the deletion. Such explanations, however, are not compatible with common discordance of monozygotic twins with identical del 22q11.
- We propose that discordance of such MZ twins are best explained by differences in epigenetic mechanisms involving DNA methylation, which are compatible with the sequence features of this genomic region and sensitive to in vivo effects during development and differentiation.
monochorionic pregnancy and weighed 1900 g (twin 1) and 2000 g (twin 2). Although infant twin 1 had facial abnormalities and a normal cardiovascular system, her chest x-ray suggested thymus aplasia or hypoplasia. Her plasma calcium levels were normal and she displayed no developmental delay. Twin 2 displayed a dysmorphic face, developmental abnormalities, and a cardiac outflow tract defect. It included an interrupted aortic arch with a ventricular septal defect, a truncus arteriosus, and a large arterial duct. She had a hypoplastic thymus and slight hypocalcaemia and died on day 5 from cardiac respiratory failure. Five DNA markers established the monozygosity of the twins with a probability of monozygosity of 99.99%. The twins were established to have deletion of 22q11 by lack of a paternal allele at locus D22S944. Further FISH analysis on 25 and 30 metaphase spreads of twin 1 and twin 2 suggested a homogeneous 22q11 deletion in the two children that was not found in their parents. The results were interpreted as the result of complex interaction between genetic and environmental systems.

**Twin pair 5**

Twin pair 5 was a caesarean delivery at 38 weeks of gestation to a 24 year old mother of Chinese ancestry, as her first pregnancy. In both twins, the main cardiac diagnosis was tetralogy of Fallot with pulmonary atresia. Twin 1 weighed 2450 g at birth and had dysmorphic facial features, abnormal outflow septation, and mispattering and misalignment of the great vessels. She suffered frequent hypoxia and severe systemic infections and died of sepsis at 11 months of age. Twin 2 had a birth weight of 2100 g and facial dysmorphism with thymic function within the lower limit of the reference ranges. Nine microsatellite markers established monozygosity of the twins. The de novo 22q11 deletion in the two infants was established by quantitative PCR for D22S264 and TULP1 loci. Comparisons were made to internal controls and the parents’ results.

**DISCUSSION AND HYPOTHESIS**

There are two specific observations about 22q11 deletions that need a biological explanation. The first is the common recurrence of this deletion that has formed the focus of research following completion of the sequence of chromosome 22. It is explained by low copy repeat (LCR) elements in this region. More than 90% of patients with VCFS and a 22q11 deletion have a comparable 3 Mb hemizygous deletion, which suggests that LCR sequences at the breakpoints confer susceptibility to this rearrangement. Within these repeats that surround the deletion, there is a 200 kb duplication of sequences, including a tandem repeat of genes/pseudogenes. The repeats are virtually identical in the 200 kb region, suggesting that the deletion could be mediated by homologous recombination. Examination of a three generation family has also shown that meiotic intrachromosomal recombination does mediate the deletion. Further, the common 3 Mb deletion of the DGS/VCFS contains four copies of the LCRs (LCR-A, B, C, and D) and intrachromosomal recombination may lead to a set of specific deletions: (2, 11, 12). The origin of the complex organisation of the LCR region of chromosome 22q11 has been traced to at least 40 million years ago, which may also account for extensive duplication of the regions on this chromosome in the human genome. The LCR based hypothesis for the common occurrence of the 22q11 deletions appears logical and a similar mechanism has recently been proposed for a number of contiguous gene deletion syndromes. The second observation on 22q11 deletions deals with the remarkable variability in phenotype of the hemizygous subjects, most with very similar deleted regions. Such interpersonal variability among unrelated subjects could be explained by different sets of deleted genes if the deleted region is not exactly the same. Further, a mouse model of the del 22q11 suggests an involvement of a complex genetic control over phenotypic variability of the del 22q11 syndrome associated phenotype. It indicates that the same deletion is linked to different phenotypes for cardiovascular, thymic, and parathyroid anomalies under different genetic backgrounds, suggesting involvement of modifiers elsewhere in the genome. Such explanations, although logical for unrelated subjects, do not account for phenotypic differences among members of a family with 22q11 deletions, unless one assumes a difference in genetic backgrounds among affected members of the family. Further, this hypothesis will be highly unlikely for monozygotic twins that carry the deletion but are discordant for syndrome related phenotypes. In fact, most reports on monozygotic twin pairs with 22q11 deletions are reported to be amniotically discordant. The exception to this is the report by Rauch et al, who reported on a pair of diamniotic and dichorionic monozygotic twins with 22q11.2 deletions who were concordant for Cayler syndrome. Even this pair had differences in the severity of the cardiac abnormality; twin 1 had a ventricular septal defect while twin 2 had tetralogy of Fallot. The reported phenotypic discordance in all five reported pairs (see above) involves a number of developmental abnormalities. Does this discordance represent a feature for all/most del 22q11 MZ twin pairs or are the published cases exceptions? It is likely that discordant pairs may be viewed as an exception in any publication. However, the fact remains that five/six del 22q11 MZ twin pairs are reported to be discordant for a variety of features that include cardiac, developmental, mental, and behavioural features. Such results for any group of monozygotic twins are usually explained by assumption of random events, genetic and/or environmental. The genetic events usually call for such somatic events as differential mosaicism, a new or expanded mutation, while the environmental effects are attributed to differences in utero environment between the twins. These will include differences in nutrition and chorions and the relative vascular supply affecting nourishment and exposure during fetal development. At this stage in our understanding of the variability associated with the twinning process, it is not possible to identify all possible in utero factors that may cause the discordance of monozygotic twins. However, the completion of the human genome sequence and advances associated with it now offer novel theories for the discordance of such twins.
even for this region of the genome. Also, it will become germinal, offering the progeny of two twins’ different risks, which is not concordant with published reports.  

The epigenetic modification, however, need not be restricted to genetic changes at the level of the DNA sequence. We propose that the most likely mechanism for the second hit may involve epigenetic changes. These changes are able to influence the expression of the gene(s) without affecting the DNA sequence. Although such changes could be brought about by a variety of means, one of the epigenetic mechanisms is DNA methylation. In humans it operates on the cytosines, primarily localised to CpG dinucleotides. The methylation of a CpG has two effects. First it predisposes such sites to a high rate of substitution leading to TpG, which may alter the coding sequence resulting in an abnormal or truncated protein. Second, most CpG dinucleotides are located in the promoter region of most genes as CpG islands. Methylation of such CpG islands is associated with control of gene expression. Thus, the methylation of genomic DNA may affect a variety of processes related to gene expression including imprinting, X chromosomes inactivation, and as an epimutation in a number of cancers. Generally, expressed sequences are associated with an unmethylated CpG island of its promoter, while a methylated promoter causes gene silencing. Methylation of DNA is involved in establishing and maintaining a particular state of gene expression during differentiation including early development. Given the variety of developmental anomalies associated with 22q11 deletions, it is logical to implicate a methylation difference between the two hematopoietic lineages of monozygotic twins differently depending on the stage of development. More importantly, methylation could function as an epimutation in a number of cancers.


