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

S M Singh, B Murphy, R O’Reilly

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 (p>0.99%) 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. Mononzygosity 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, thyrcic 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...
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 of interest 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 uterine environment between the twins. These will include differences in environments 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.

Hatchwell has discussed two possibilities in any genetic explanation for discordance of monozygotic twins, uncovering recessive alleles and involvement of a second hit (mutation). Further, the results on mouse suggest a role for modifier genes in causing discordance among subjects with del 22q11. If one assumes identical deletion in the monozygotic twins, uncovering recessives, gene haploinsufficiency, and differences in modifier genes as explanations for their discordance may not be logical. The twins are expected to carry an identical normal chromosome 22 and therefore the same sets of alleles in a hemizygous condition and the genetic background of the twins is expected to be identical. As a result although the two mechanisms may explain phenotypic differences among unrelated subjects, they may not account for commonly reported phenotypic discord for cardiovascular monozygotic twins. It is possible that the reported cases of discordance for del 22q11 MZ twins represent a reporting bias. Such an argument, if real, does not rule out the fact the discordant twin pairs exist and such observations are not compatible with the hypothesis involving uncovering of recessive alleles or differences in the background genotype. The second hit (mutation) hypothesis, however, is logical and may entail a variety of mutational mechanisms including replication errors, base changes, and additional deletions involving LCR and Alu repeats of this region. Will such mutational mechanisms explain the extensive variability that is seen in the five/six sets of reported cases of discordance of monozygotic twins with 22q11 deletions? It will require a very high rate of mutation as the second hit, which may be unrealistic...
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. This epigenetic hypothesis, 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. Secondly, 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 an individual's 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 twins which would alter the expression of some/most genes of this region. The involvement of methylation with genes in this region is also predicted by the presence of Alu repeats and Sp1 binding sites. These features may define the boundaries between methylated and unmethylated regions of the genome as the unmethylated CpG islands are usually flanked by methylated Alu sequences. Many of the genes of this region have sequence features implicated in the involvement of methylation in their regulation. More importantly, methylation could function as the second hit, which may differ between twins. It could affect monozygotic twins differently depending on the stage of twinning including differential implantation and in utero environment, without altering their DNA sequence. If, however, involvement of methylation in differential regulation of hemizygous genes between monozygotic twins could explain their developmental differences leading to cardiac and neurodevelopmental abnormalities. Although the epigenetic hypothesis could explain phenotypic discordance between monozygotic twins discordant for a 22q11 deletion, there are no methylation data on any of the genes localised to this region. Modern developments in methylation technology using genome wide profiling should facilitate testing of such a hypothesis, which has the potential to explain a variety of unexplained inheritance and expression patterns and profiles.

In summary, we propose that the sequence features of the 22q11 region, with extensive inter- and intrachromosomal repeats involving LCR, Alu, etc. are not only involved in the recurrence of del 22q11, they also subject the genes of this region to epigenetic modifications, particularly DNA methylation, affecting their expression. Further, epimutations such as the second hit contribute to extensive phenotypic heterogeneity of the del 22q11 syndrome in the general population. The proposed explanation is particularly relevant to monozygotic twins with del 22q11 discordant for a variety of developmental abnormalities.

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