

## ELECTRONIC LETTER

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

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*J Med Genet* 2002;39:e71 (<http://www.jmedgenet.com/cgi/content/full/39/11/e71>)

The 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.<sup>1</sup> This high frequency has been attributed to low copy repeats (LCR) on chromosome 22,<sup>2</sup> 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.<sup>3</sup> In fact, the clinical phenotype of the 22q11 deletion syndrome is characterised by extensive variability.<sup>4</sup> 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 ( $p>0.99$ ) with 22q11 deletions have discordant phenotypes. In this discussion we will review all published cases of monozygotic twins concordant 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 region of chromosome 22, and the "second hit" hypothesis involving genetic and/or epigenetic somatic events.

## SUMMARY OF CASE REPORTS

### Twin pair 1

Twin pair 1<sup>5</sup> 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 ( $p>99\%$ ) adult twin pair.<sup>6</sup> They share VCF syndrome related facial appearance with

## 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.

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.<sup>7</sup> 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<sup>8</sup> was delivered by caesarean section at 32 weeks of gestation to a 27 year old mother from a diamniotic,

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<sup>9</sup> 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 mispatterning 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 TULPE1 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.<sup>10</sup> It is attributed to the low copy repeats (LCR) in this region.<sup>2</sup> More than 90% of patients with VCFS and a 22q11 deletion have a comparable 3 Mb hemizygous deletion,<sup>3</sup> 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.<sup>2</sup> 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,<sup>12</sup> which may also account for extensive duplication of the regions on this chromosome in the human genome.<sup>10</sup> 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.<sup>13, 14</sup>

The second observation on 22q11 deletions deals with the remarkable variability in phenotype of the hemizygous subjects, most with very similar deleted regions.<sup>4</sup> Such interpersonal variability among unrelated members 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 con-

trol over phenotypic variability of the del 22q11 syndrome associated phenotype.<sup>15</sup> It indicates that the same deletion shows differential penetrance 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 phenotypically discordant. The exception to this pattern may be Rauch *et al*,<sup>16</sup> 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 only 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 amnions 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<sup>17</sup> 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 mice 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 discordance involving 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 five of the six (>80%) 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.<sup>6</sup>

The second somatic hit 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.<sup>18</sup> The methylation of a CpG has two effects. First it predisposes such sites to a high rate of substitution<sup>19</sup> 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.<sup>20-22</sup> Methylation of such CpG islands is associated with control of gene expression.<sup>23</sup> Thus, the methylation of genomic DNA may affect a variety of processes related to gene expression including imprinting,<sup>24</sup> X chromosomes inactivation,<sup>25</sup> and as an epimutation in a number of cancers.<sup>26</sup> Generally, expressed sequences are associated with an unmethylated CpG island of its promoter, while a methylated promoter causes gene silencing.<sup>27</sup> Methylation of DNA is involved in establishing and maintaining a particular state of gene expression during differentiation including early development.<sup>28</sup> Given the variety of developmental anomalies associated with 22q11 deletions, it is logical to implicate a methylation difference between the twins that 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.<sup>29</sup> 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.<sup>30</sup> 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 operational, 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 concordant 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<sup>31-32</sup> 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.

#### ACKNOWLEDGEMENTS

This research was financially supported by grants from the Schizophrenia Society of Ontario and Ontario Mental Health Foundation.

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#### REFERENCES

- DuMontcel ST, Mendizabal H, Ayme S, Levy A, Philip N. Prevalence of 22q11 microdeletion. *J Med Genet* 1996;**33**:719.
- Edelmans E, Pandita RK, Spiteri E, Funke B, Goldberg R, Palanisamy N, Chaganti RSK, Shprintzen RJ, Morrow BE. A common molecular basis for rearrangement disorders on chromosome 22q11. *Hum Mol Genet* 1999;**8**:1157-67.
- Carlson C, Sirotkin H, Pandita R, Goldberg R, McKie J, Wadey R, Patanjali SR, Weissman SM, Anyane-Yeboah K, Warburton D, Scambler P, Shprintzen R, Kucherlapati R, Morrow BE. Molecular definition of 22q11 deletion in 151 velo-cardio-facial syndrome patients. *Am J Med Genet* 1997;**61**:620-9.
- Swillen A, Vogels A, Devriendt K, Fryns JP. Chromosome 22q11 deletion syndrome: update and review of the clinical features, cognitive-behavioral spectrum, and psychiatric complications. *Am J Med Genet (Semin Med Genet)* 2000;**97**:128-35.
- Goodship J, Cross I, Scambler P, Burn J. Monozygotic twins with chromosome 22q11 deletion and discordant phenotype. *J Med Genet* 1995;**32**:746-8.
- Fryer A. Monozygotic twins with 22q11 deletion and discordant phenotypes. *J Med Genet* 1996;**33**:173-6.
- Yamagishi H, Ishii C, Maeda J, Kajima Y, Matsuoka R, Kimura M, Takao A, Momma K, Matsuo N. Phenotypic discordance in monozygotic twins with 22q11.2 deletion. *Am J Med Genet* 1998;**78**:319-21.
- Vincent MC, Heitz F, Tricoire J, Bourrouillou G, Kuhlein F, Rolland M, Calvas P. 22q11 deletion in DGS/VCFS monozygotic twins with discordant phenotypes. *Genet Couns* 1999;**10**:43-9.
- Lu JH, Chung MY, Hwang B, Chien HP. Monozygotic twins with chromosome 22q11 microdeletion and discordant phenotypes in cardiovascular patterning. *Pediatr Cardiol* 2001;**22**:260-3.
- Dunham I, Shimizu N, Roe BA, Chissoe S, et al. The DNA sequence of human chromosome 22. *Nature* 1999;**402**:489-95. [AQ1]
- Shaikh TH, Kurahashi H, Saitta SC, O'Hare AM, Hu P, Roe BA, Driscoll DA, McDonald-McGinn DM, Zachai EH, Budarf ML, Emanuel BS. Chromosome 22-specific low copy repeats and the 22q11.2 deletion syndrome: genomic organization and deletion endpoint analysis. *Hum Mol Genet* 2000;**9**:489-501.
- Shaikh TH, Kurahashi H, Emanuel BS. Evolutionarily conserved low copy repeats (LCRs) in 22q11 mediate deletions, duplications, translocations, and genomic instability: an update and literature review. *Genet Med* 2001;**3**:6-13.
- Kuroda-Kawaguchi T, Shkaletsky H, Brown LG, Minx PJ, Cordum HS, Waterston RH, Wilson RK, Silber S, Oates R, Rozen S, Page DC. The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. *Nat Genet* 2001;**29**:279-86.
- Osborne LR, Li M, Pober B, Chitayat D, Bodurtha J, Mandel A, Costa T, Grebe T, Cox S, Tsui LS, Scherer SW. A 1.5 million-base pair inversion polymorphism in families with Williams-Beuren syndrome. *Nat Genet* 2001;**29**:321-5.
- Taddei I, Morishima M, Huynh T, Lindsay EA. Genetic factors are major determinants of phenotypic variability in a mouse model of the DiGeorge/del22q11 syndromes. *Proc Natl Acad Sci USA* 2001;**98**:11428-31.
- Rauch A, Hofbeck M, Bahring S, Leipold G, Trautmann U, Singer H, Pfeiffer RA. Monozygotic twins concordant for Cayler syndrome. *Am J Med Genet* 1998;**75**:113-17.
- Hatchwell E. Monozygotic twins with chromosome 22q11 deletion and discordant phenotype. *J Med Genet* 1996;**33**:261-4.
- Grunau C, Hindermann W, Rosenthal A. Large-scale methylation analysis of human genomic DNA reveals tissue-specific differences between the methylation profiles of genes and pseudogenes. *Hum Mol Genet* 2000;**9**:2651-63.
- Jones PA, Rideout WM, Shen J, Spruck CH, Tsai YC. Methylation, mutation and cancer. *BioEssays* 1992;**14**:33-6.
- Ponger L, Duret L, Mouchiroud D. Determinants of CpG islands: expression in early embryo and isochore structure. *Genome Res* 2001;**11**:1854-60.
- Venter JC, Adams MD, et al. The sequencing of the human genome. *Science* 2001;**291**:1304-51.
- Bestor TH, Tycko B. Creation of genomic methylation patterns. *Nat Genet* 1996;**12**:363-7.
- Roberson KD, Wolffe AP. DNA methylation in health and disease. *Nat Rev Genet* 2000;**1**:11-19.
- Reik W, Walter J. Genomic imprinting: parental influence on the genome. *Nat Rev Genet* 2001;**2**:21-32.
- Percac I, Barlolomei MS. Do X chromosomes set boundaries? *Science* 2002;**295**:287-8.
- Plass C, Soloway PD. DNA methylation, imprinting and cancer. *Eur J Hum Genet* 2002;**10**:6-16.
- Cedar H. DNA methylation and gene activity. *Cell* 1988;**53**:3-4.

- 28 **Santos F**, Hendrich B, Reik W, Dean W. Dynamic reprogramming of DNA methylation in the early mouse embryo. *Dev Biol* 2002;**241**:172-82.
- 29 **Tomatsu S**, Orii KO, Islam MR, Shah GN, Grubb JH, Sukegawa K, Suzuki Y, Orii T, Kondo N, Sly WS. Methylation patterns of the human  $\beta$ -glucuronidase gene locus: boundaries of methylation and general implications for frequent point mutations at CpG dinucleotides. *Genomics* 2002;**79**:363-75.
- 30 **Millar DS**, Paul CL, Molloy PL, Clark SJ. A distinct sequence (ATAAA)<sub>n</sub> separates methylated and unmethylated domains at the 5' end of the GSTP1 CpG island. *J Biol Chem* 2000;**275**:24893-9.
- 31 **Tompa R**, McCallum CM, Delrow J, Henikoff JG, van Steensel B, Henikoff S. Genome-wide profiling of DNA methylation reveals transposon targets of CHROMETHYLASE3. *Curr Biol* 2002;**12**:65-8.
- 32 **Gitan RS**, Shi H, Chen C, Yan PS, Huang TH. Methylation-specific oligonucleotide microarray: a new potential for high-throughput methylation analysis. *Genome Res* 2001;**12**:158-64.