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# Understanding variable expressivity in microdeletion syndromes

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**A new study reports an elevated frequency of second-site genomic alterations among children with severe developmental delay who carry a recurrent microdeletion at chromosome 16p12.1. The work highlights the complex relationship between genotype and phenotype and provides a model to explain the clinical variability associated with this and other common microdeletion syndromes.**

The introduction of genomic microarrays has led to the discovery of new developmental syndromes caused by microdeletions or microduplications, also termed copy number variants (CNVs). Many of these CNVs are fully penetrant in that they are found only in affected individuals and never in their unaffected parents or siblings. Such discoveries have promoted the acceptance of microarrays as a first-line screening test for individuals with mental retardation, replacing karyotyping in diagnostic units around the world<sup>1</sup>. But not all CNV syndromes are easy to interpret in a clinical setting. Another class of CNVs has been found in individuals with a much wider range of phenotypes, ranging from mental retardation to autism, schizophrenia and epilepsy<sup>2</sup>. In addition to showing clinical variability, CNVs falling in this latter class are also present at lower frequencies in healthy individuals, raising questions as to what determines their pathogenicity. On page 203 of this issue, Evan Eichler and colleagues<sup>3</sup> report a striking example of such a CNV syndrome associated with a 520-kb deletion at 16p12.1. They found that this deletion was enriched among children with developmental delay but was often inherited from an unaffected parent and frequently co-occurred with a second large deletion or duplication elsewhere in the genome. These findings provide a model to explain the clinical

variability typical of this and other common microdeletion syndromes.

## One hit or two

Girirajan *et al.*<sup>3</sup> studied the 16p12.1 deletion among children with severe developmental delay as well as in controls. They found that this deletion was inherited from an apparently healthy parent in 22 of 23 instances. By studying several large cohorts comprising 20,000 affected individuals (cases) and 15,000 controls, the authors established that the frequency of the 16p12.1 deletion was about fourfold higher in cases than controls. This suggests that the 16p12.1 deletion should be considered a risk allele rather than a fully penetrant causative mutation. Previous studies have also suggested that some CNVs may act as risk alleles, with a particularly strong example involving the clinically severe TAR syndrome. Individuals with this syndrome harbor a deletion at 1q21 that is also present in a third of their unaffected parents and siblings, suggesting that another event is necessary for the deletion to manifest clinically<sup>4</sup>.

Girirajan *et al.* went on to explore the possibility that the 16p12.1 deletion was associated with milder phenotypes in carrier parents. To this end, the authors collected information on the parents' history of psychological traits as well as neurological, behavioral and psychiatric features. They then compared parents with and without the 16p12.1 deletion and found that those carrying the deletion more often reported features such as learning disability, depression and seizures.

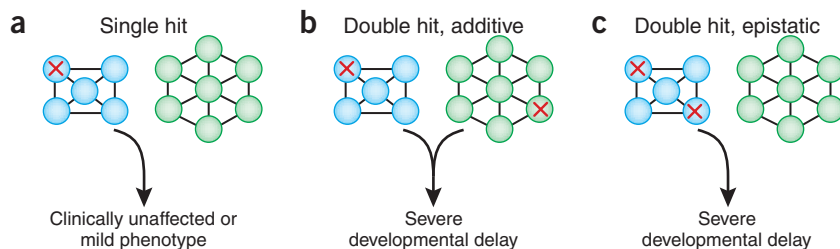
The most striking result from the study was that a quarter of the affected individuals with the 16p12.1 deletion carried a second genomic alteration, in the form of another large CNV,

that likely contributed to their phenotype. Girirajan *et al.* hypothesize that these genomic alterations serve as second hits that convert the 16p12.1 deletion from a risk factor to a determinant or modifier of the developmental phenotype (Fig. 1). If this is the case, then one might expect other clinically variable microdeletion syndromes to show a similar increase in second-site genomic events. Indeed, the authors found an excess of such second large CNVs in individuals with 22q11.2 duplications or 1q21.1 deletions. These CNVs are well known for yielding high clinical variability, and they are also found in healthy individuals. In contrast, CNVs that cause substantial mental retardation in all carriers, such as those underlying Williams, velocardiofacial and Smith-Magenis syndromes, did not show an excess of second-site genomic alterations.

## Phenotypic variability

How do these second hits convert the 16p12.1 deletion phenotype from the healthy development seen in carrier parents into the severe developmental phenotypes found in their affected offspring? One hypothesis is that the two genomic events act independently and that the simple addition of their effects leads to developmental delay (Fig. 1b). An argument in favor of this might be that the second hit was different in each case. For example, one 16p12.1 deletion case had an additional homozygous duplication of the DiGeorge and velocardiofacial syndrome region at 22q11.2. Another individual with severe mental retardation had a *BRAF* mutation consistent with Costello syndrome, as well as a small duplication at 14q32 as the second CNV event. In fact, many of the second-hit CNVs are known to be pathogenic by themselves and may

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**Figure 1** Models to explain the variable expressivity of the 16p12.1 deletion. **(a)** When the 16p12.1 deletion occurs as a single event, it produces mild phenotypes with incomplete penetrance. When the deletion occurs along with a second large CNV, the two events act in concert to produce a more severe phenotype. **(b)** The additive model is depicted as two co-occurring CNVs affecting independent functional modules. **(c)** The epistatic model is depicted as two CNVs affecting the same functional module.

well have had more impact on the resulting phenotype than the 16p12.1 deletion itself. Nonetheless, the contribution of the 16p12.1 deletion was detectable in the phenotypes of cases in this study, and this contribution appeared to be more severe than in previous reports of cases where the second-hit CNV occurred in isolation.

Apart from a simple additive effect, another hypothesis is that the second event may cause a clinical phenotype by affecting the same pathway, possibly with a more severe impact. This would suggest a mechanism whereby the two hits affect the same functional module (Fig. 1c)<sup>5</sup>. Such epistatic effects are frequently observed in studies of other organisms and for that reason may be expected to occur in humans as well<sup>6</sup>. Under this scenario, one

would expect that the genes located in each of the CNVs contributing to the phenotype should be part of the same functional module. A number of disease-associated pathways or modules are already known. For instance, there is converging evidence that mental retardation can be caused by changes in several genes encoding Rho GTPases<sup>7</sup>. Similarly, digenic inheritance has been documented in retinitis pigmentosa, as has the existence of modifiers in Bardet-Biedl syndrome and other ciliary disorders<sup>8–11</sup>. Mutations in unlinked genes that together form a functional module likely explain such interactive effects. This raises the possibility that the systematic study of modifiers or second hits in large numbers of individuals with low-penetrance CNVs, such as the 16p12.1 deletion, may yield

valuable clues to the genes and pathogenetic mechanisms causing mental retardation. This is clearly a large task, given that the majority of second hits are probably not detectable even by very high-resolution arrays. Exome and eventually whole-genome re-sequencing may well reveal a surprising number of additional contributing loci, illuminating key signaling pathways and connections, such as have been documented for human cancers<sup>12</sup>. In that sense, the study of Girirajan *et al.*<sup>3</sup> carries the promise that an understanding of variable expressivity in terms of complex genotype-phenotype correlations may soon become a reality.

#### COMPETING INTEREST STATEMENT

The authors declare no competing financial interests.

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