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HotSpots

Two-hit wonder: a novel genetic model to explain variable expressivity in severe pediatric phenotypes

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A recurrent 16p12.1 microdeletion supports a two-hit model for severe developmental delay
Girirajan et al. (2010)
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Genomic disorders involving microdeletions and microduplications have been reported in many individuals with neuropsychiatric disorders such as autism and mental retardation. The recurrent nature of these disorders is often explained by non-allelic homologous recombination (NAHR) mediated by large blocks of highly identical segmental duplications or low copy repeats (LCR). The rapidly growing list of genomic disorders is partly attributable to methodological advances in DNA microarray technologies that have enabled the identification of microscopic DNA losses and gains previously undetectable by standard cytogenetic approaches. Knowledge of the prevalence and characteristics of recurrent microdeletions and microduplications is clinically invaluable; however, molecular diagnostics using copy number variant (CNV) data is frequently hampered by the phenomenon of 'variable expressivity' – when a phenotype is expressed to a different degree among individuals with the same genotype (or underlying mutation/CNV). For example, 16p11.2 microdeletions and duplications have been reported in autism, schizophrenia, mental retardation, and even in apparently healthy individuals (1–5). Explaining the genetic basis of variable expressivity is of great medical and scientific importance. In the current study, Girirajan and colleagues used CNV data from one of the largest collections of individuals with intellectual disability and developmental delay and identified a recurrent pathogenic 16p12.1 microdeletion with an incidence of ~1/15,000 live births. The authors propose a novel genetic basis of variable expressivity in pediatric phenotypes that involves a 'two-hit' hypothesis.

The authors previously performed a genome-wide meta-analysis study of microdeletions and microduplications in individuals with intellectual disability, autism, and schizophrenia, and identified a potentially pathogenic microdeletion on chromosome 16p12.1 in five of 6860 individuals compared with zero of 5674 control individuals. To further characterize this recurrent microdeletion, the authors investigated a discovery cohort comprising 11,873 children with intellectual disability/developmental delay and congenital malformations (cases) and 8540 controls. The 16p12.1 microdeletion was significantly enriched in cases ($n = 20$) *vs* controls ($n = 2$) (Fisher's exact test, $p = 0.0009$, odds ratio (OR) = 7.2). Next, the authors investigated a replication cohort comprising 9254 cases and 6299 controls and again found a significant enrichment of the microdeletion in cases ($n = 22$) *vs* controls ($n = 6$) (Fisher's exact test, $p = 0.028$, OR = 2.5). Combining the two cohorts showed a highly significant association of the 16p12.1 microdeletion in cases *vs* controls (Fisher's exact test, $p = 1.18 \times 10^{-4}$, OR = 3.7).

The authors also examined a schizophrenia cohort comprising 3061 individuals and identified three sporadic cases with 16p12.1 microdeletions; however, this association was not significant when compared to the frequency of the microdeletion in controls ($n = 14,839$) (Fisher's exact test, $p = 0.29$, OR = 1.8). The authors considered the possibility that the lack of association may be due to a lack of statistical power in the schizophrenia cohort ($n = 3061$) compared to the intellectual disability/developmental delay cohort ($n = 21,127$). Interestingly, in one family (LD1205) segregating for both schizophrenia and mental retardation, the 16p11.2 microdeletion was only found in individuals diagnosed with both psychosis and intellectual disability.

To characterize the phenotypic features associated with the 16p12.1 microdeletion, the authors evaluated available medical records and identified multiple features associated with the deletion (note: not all patients could be ascertained for all clinical features, hence the total number of individuals characterized for each phenotypic feature varies). The phenotypic features included developmental delay and learning disability (18 of

18 patients), speech delay (15/15), craniofacial/skeletal features (22/23), growth retardation (9/22), microcephaly (7/20), congenital cardiac defect (7/21), hypoplastic heart (4/21), seizures (8/22), psychiatric/behavioral features (9/16), hearing loss (3/17), hypotonia (10/21), and sacral dimple or tethered cord (4/21). The presence of variable clinical presentations including nontypical facial gestalt indicated that the 16p12.1 microdeletion is nonsyndromic.

Girirajan and colleagues examined whether other chromosomal abnormalities or large (>500 kb) CNVs were present in individuals with the 16p11.2 microdeletion. They identified 6 of 20 individuals from the discovery cohort who harbored an additional anomaly (i.e. a 'double hit'). The frequency of double-hits (30%) was 7.5-fold greater in the individuals with 16p12.1 microdeletion (Fisher's exact test, $p = 0.0005$, OR = 9.7) than in controls who were conditioned for a large deletion/duplication CNV first hit (9 of 217 or 4.1%). In the replication cohort, the double-hit frequency was also enriched in cases (4 of 22, 18.2%) compared to controls (12 of 254, 4.7%) (Fisher's exact test, $p = 0.029$, OR = 4.5). Combining the two cohorts indicated a significant enrichment of double-hits among the individuals with 16p12.1 microdeletion (10 of 42, 24%) compared to controls (21 of 471, 4.4%) (Fisher's exact test, $p = 5.7 \times 10^{-5}$, OR = 6.6). Interestingly, but perhaps not surprisingly, the phenotypes of two-hit carrier patients where the second hit is associated with a described syndrome was more severe or distinct than the typical features of the syndrome.

The authors tested the inheritance of the 16p12.1 microdeletion by obtaining DNA from 23 sets of parents and showed that the microdeletion was inherited in 22 of 23 cases (17 maternal, 5 paternal) and in one case the microdeletion was *de novo*. Of the seven second-hit cases where inheritance could be assessed, 6 of 7 second hits were inherited and one was *de novo*. In 5 of 7 cases, either the second hit was inherited from the parent who did not carry a 16p12.1 microdeletion or it arose *de novo*. The authors examined available parental phenotypic information and showed that 16p12.1 carrier parents were more likely to present with learning disability, depression, bipolar disorder or seizures than the noncarrier parents (Fisher's exact test, $p = 0.037$, OR = 6). In only three cases was a microdeletion inherited from an apparently unaffected parent. Probands presented with more severe, clinically recognizable features (e.g. severe speech delay and motor disability, recognizable facial dysmorphology and systemic organ abnormalities) than the carrier parents.

In summary, the data presented by Girirajan and colleagues show that 16p12.1 microdeletions represent a significant risk factor for intellectual disability and developmental delay. Furthermore, they show that the phenotypes associated with 16p12.1 microdeletions show variable expressivity that depends on the genetic background. Toward this end, the authors propose a 'two-hit' model in which a first hit (e.g. 16p12.1 microdeletions) in concert with a secondary hit (e.g. genetic, epigenetic or environmental insult) results in a more severe pediatric phenotype (Fig. 1). Variable expressivity under this model could arise under

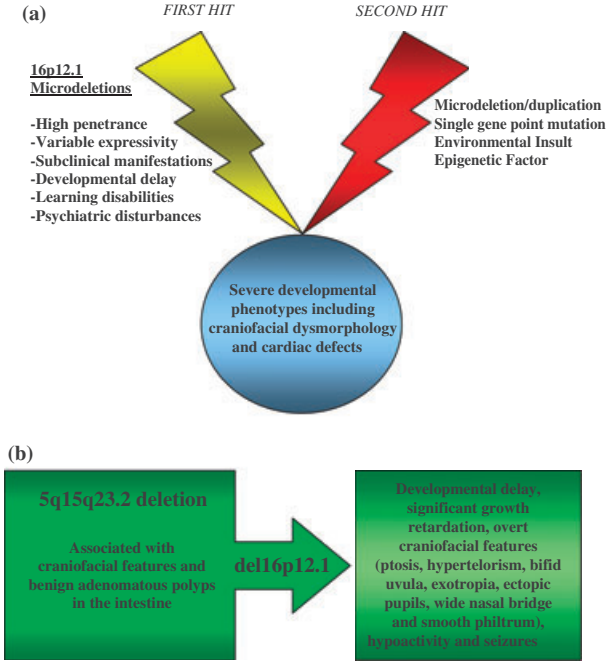

Fig. 1. A two-hit model for severe developmental delay involving chromosome 16p12.1. (**a**) Microdeletions involving 16p12.1 are highly penetrant and independent risk factors for intellectual disability and developmental delay. The presence of a 'second hit' such as a large microdeletion/duplication, a mutation in a single gene, an environmental insult, or an epigenetic factor results in a more severe pediatric phenotype that may include craniofacial dysmorphology and cardiac defects. (**b**) In patients where the second-hit CNV is already associated with a known syndrome, the phenotype of two-hit 16p12.1 carriers is more severe or distinct that the typical features of the known syndrome. For example, patient SG10 in the current study harbors a 35-Mb deletion on chromosome 5q15q23.2 in addition to a 16p12.1 microdeletion. Previously, it has been reported that individuals with 5q15q23.2 deletions present with craniofacial features and benign adenomatous polyps in the intestine. However, the phenotype of the 'two-hit' patient is far more severe and includes developmental delay, significant growth retardation, overt craniofacial features (ptosis, hypertelorism, bifid uvula, exotropia, ectopic pupils, wide nasal bridge and smooth philtrum), hypoactivity and seizures. Thus, the presence of the second hit exacerbates the pediatric phenotype.

several circumstances. For one, the two 'hits' could act independently of each other – the presence of a double hit could therefore act in an additive manner resulting in a phenotype that differs from either hit alone. An alternative hypothesis is that the second hit (e.g. a CNV) might involve a gene(s) that participates in the same or similar biochemical pathway as a gene(s) found on 16p12.1. Modification of the phenotype in this case could therefore result from an epistatic mechanism.

The two-hit model could be integrated to explain variable expressivity in other genomic disorders. The authors proposed that their model might explain the significant comorbidity that exists among neuropsychiatric phenotypes (e.g. cognitive impairment and schizophrenia). To test this hypothesis, the authors interrogated other genomic disorders for the occurrence of two hits, beginning with the recently reported recurrent 1q21.1 microdeletions that are associated with highly variable phenotypes. Girirajan and colleagues showed a 40-fold enrichment for two hits among 1q21.1 cases when compared to controls. They subsequently analyzed an additional seven genomic disorders that included Smith-Magenis syndrome, 17q21.31 deletion, Williams syndrome, DiGeorge syndrome, 15q13.3 deletion, 16p11.2 deletion, and 22q11.2 duplication and found that the 16p12.1 microdeletion ranks as the top recurrent CNV that is most enriched for the occurrence of two hits. The authors also show an inverse correlation between the proportion of cases that are *de novo* and the prevalence of the second hit. They show that genomic disorders that typically arise *de novo* (e.g. Williams syndrome) have far fewer second hits than genomic disorders where the underlying CNV is usually inherited and associated with reduced penetrance and variable expressivity. The elegant work reported by Girirajan and colleagues thus highlights the complex genotype–phenotype relationships that underlie variable expressivity in neurodevelopmental disorders.

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