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Microdeletion syndromes

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The recent explosion in the implementation of genome-wide microarray technology to discover rare, pathogenic genomic rearrangements in a variety of diseases has led to the discovery of numerous microdeletion syndromes. It is now clear that these microdeletions are associated with extensive phenotypic heterogeneity and incomplete penetrance. A subset of recurrent microdeletions underpin diverse phenotypes, including intellectual disability, autism, epilepsy and neuropsychiatric disorders. Recent studies highlight a role for additional low frequency variants, or 'second hits' to account for this variability. The implementation of massively parallel sequencing and epigenetic models may provide a powerful prospective approach to the delineation of microdeletion syndrome phenotypes.

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Introduction

Rare, recurrent microdeletions with pathogenic effects were first identified in clinically distinct syndromes including, amongst numerous others, Smith-Magenis (17p11.2 deletions) [1], DiGeorge/velocardiofacial (22q11.2 deletions) [2•] and Williams-Beurens (7q11.23 deletions) [3]. Each of these disorders was first described based on a series of patients who shared a recognizable collection of clinical features. The clinical description of each syndrome was followed later by the discovery of the molecular basis for the syndrome — a 'phenotype first' discovery, and one that hinted at the important role of copy number variants (CNVs) in disease. Importantly, each of the deletions described above is a recurrent rearrangement that arises due to non-allelic homologous recombination (NAHR) at meiosis [4], resulting in similar or identical breakpoints in each patient.

Over the past 10 years, advances in technology, most notably the development of array comparative genomic hybridization (CGH) and single nucleotide polymorphism (SNP) genotyping arrays, have facilitated a 'genotype first' approach to syndrome identification. These technologies allow genome-wide assessment of copy number variation, and are now routinely used to identify CNVs in both the research and clinical setting. The use of these technologies has had a significant impact on the field of human genetics, with an explosion in the number of disorders attributed to microdeletions and has led to some unexpected findings.

The first impact of these technologies — referred to collectively as chromosome microarrays — was the ability to rapidly screen the entire genome for CNVs in large numbers of patients. Therefore, rather than using a locus-based approach such as fluorescent in situ hybridization (FISH) to probe one genomic location at a time, the entire genome can be interrogated, facilitating rapid discovery of disease-associated CNVs. Remarkably, since 2006 more than 20 recurrent microdeletions and microduplications associated with disease have been described [5,6]. Secondly, large cohorts of patients and controls could be screened fairly quickly. Interestingly, as independent studies to discover disease-associated CNVs were carried out in cohorts of patients with seemingly different disorders, it has become clear that some microdeletions are associated with various phenotypes ranging from mild to severe.

Here, we will focus on the most recently described recurrent microdeletion and duplication syndromes and their disease associations. We will address the phenotypic variability associated with some CNVs and approaches to understanding the genetic and molecular basis of that variability, providing recent examples from the literature. Finally, we will discuss the potential role of epigenetics and massively parallel sequencing in the future delineation of microdeletion syndromes.

Microdeletion discovery in neurodevelopmental disorders

The first large cohorts of patients to be investigated for CNVs were patients with intellectual disability (ID) [7•,8–10,11••,12], autism spectrum disorder (ASD) [13–15,16•,17,18] and congenital anomalies [19–22]. Each of these classes of disorders had previously been associated with chromosomal aberrations, making it likely that submicroscopic chromosome rearrangements would also be important. The first new microdeletion syndromes reported from these studies were characterized by syndromic, or recognizable, features. These include microdeletions at 17q21 [11••,12,23] and 15q24 [24–27].

Table 1

Selected microdeletion syndromes associated with phenotypic heterogeneity

Genomic location	Coordinates (hg19) for critical region (Mb)	Associated phenotypes	Selected references
1q21.1	Chr1: 146.5–147.5	ID, SCZ, MCA	[32,33,34•,35,36]
3q29	Chr3: 195.8–197.4	ID, SCZ	[62,63]
10q22q23	Chr10: 81.5–89.0	ID	[64,65]
15q11.2	Chr15: 22.8–23.1	ID, SCZ, EPI, ASD	[66–70]
15q13.3	Chr15: 31.3–32.5	EPI, ID, SCZ, ASD	[37–39,71]
15q24	Chr15: 74.4–75.5	ID, ASD	[25,27,72]
16p11.2 (proximal)	Chr16: 29.6–30.2	ID, ASD, obesity	[28,30]
16p11.2 (distal)	Chr16: 28.8–29.1	ID, obesity	[29]
16p12	Chr16: 21.9–22.5	ID	[54]
16p13.11	Chr16: 15.0–16.3	ID, EPI, ASD, SCZ	[53,69,73,74]
17q12	Chr17: 34.8–36.3	ID, ASD, SCZ	[75,76]
17q21.3	Chr17: 43.7–44.3	ID	[8]
22q11.2 distal	Chr22: 21.8–23.7	ID, MCA	[77]

Subsequent discoveries, however, were more complicated. Strikingly, a number of microdeletions and duplications were identified as risk factors for disease in large studies of different neurodevelopmental disorders (Table 1). Deletions and duplications of 16p11.2 were first described in patients with autism, but are clearly associated with ID (deletions and duplications; [28–30]) and schizophrenia (duplications; [31]). Similarly, deletions of the 1q21.1 region have been described in probands with various pediatric phenotypes including mild-moderate ID, microcephaly, cataracts and cardiac abnormalities [32,33,34•] and associated with schizophrenia [35,36].

Since 2010, several studies including our own have focused on CNV discovery in epilepsy, and three recurrent deletions have been firmly associated with epilepsy risk. Deletions of 15q13.3 were first described in patients with ID, epilepsy and facial dysmorphism [37]. Subsequently it has been shown that the microdeletion accounts for 1% of idiopathic generalized epilepsy [38,39] and is associated with ASD, speech delay, bipolar disorder and attention deficit hyperactivity disorder (ADHD) [40]. Interestingly, homozygous 15q13.3 deletions result in a more severe neurodevelopmental phenotype including severe ID, epileptic encephalopathy and hypotonia [41,42].

In addition to the clinical heterogeneity associated with these disorders, microdeletions are also often inherited from an unaffected parent, thus demonstrating reduced penetrance. While sufficient evidence now exists to ascribe causality for these microdeletions, a limited number of studies have addressed the factors that underpin variable expressivity and reduced penetrance, these studies and future avenues of research are discussed in the sections that follow and are summarized in Figure 1.

Why such variable expressivity?

Microdeletion architecture

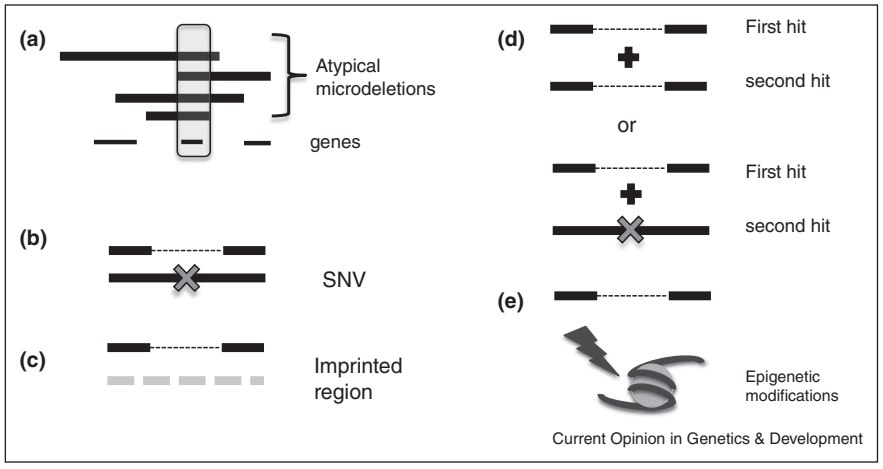
Firstly, breakpoints of microdeletions may vary and the number of haploinsufficient genes in the deleted region may directly correlate with phenotypic severity and breadth of clinical features. For instance, the cognitive features of 15q24.3 microdeletion are attributed to a 1.1 Mb critical region, while distal regions play a role in characteristic facial features and cognition [27]. Additional examples include Smith-Magenis [43], Williams [44] and Potocki-Lupski [45] syndromes.

Mapping of overlapping but distinct microdeletions has led to the delineation of critical intervals, and in some cases, to the discovery of a single causative gene. Examples include *RAI1* (Smith-Magenis syndrome), *EHMT1* (Kleefstra syndrome) [46], *MEF2C* (5q14.3q15 deletions) [47] and *MBD5* (2q23.1 deletions) [48]. Furthermore, recent reports of *CHRNA7* as the only gene encompassed by atypical 15q13.3 deletions (in heterozygous and homozygous states), suggest that this gene may be causative for the clinical features associated with this deletion [49,50]. These instances represent a single causative gene for microdeletions that are recurrent owing to the surrounding genomic architecture. However, it is important to note that mapping of non-recurrent rearrangements will also be important in the future, particularly as chromosomal microarrays have become so widely implemented. The overlap between specific rearrangements published in large disease cohorts, as well as smaller case reports, are likely to yield important causative genes.

Additional genomic variants

An attractive hypothesis to explain phenotypic heterogeneity of microdeletion syndromes is the contributory role of one or more inherited or *de novo* rare variants that modify clinical presentation. For instance variable expressivity may be explained by the 'unmasking' of recessive single nucleotide variants (SNVs) or insertion/deletions (indels) on the second allele. An example comes from the recurrent 22q11 microdeletion, which is associated with variable phenotypes, including schizophrenia. Exploration of genetic variants in the *COMT* gene identified a functional polymorphism in the gene that may

Figure 1



Possible mechanisms underlying phenotypic variability. **(a)** Microdeletions of varying size give rise to different phenotypes, as is the case for 15q24 microdeletion [27]. Mapping of atypical microdeletions can define a critical interval, and in certain cases, ascribe causality to a single gene, for example, MBD5 (2q23.1 deletions) [48]. **(b)** Unmasking of recessive alleles on the hemizygous allele can give rise to specific phenotypes. **(c)** Parent of origin effects or imprinting of the hemizygous allele, for instance in Prader-Willi and Angelman syndrome. **(d)** A two-hit model, where a microdeletion in combination with a rare CNV [54] or a low frequency SNV [55•] accounts for variable clinical presentation. **(e)** Environmental determinants cause epigenetic modifications, that act in combination with haploinsufficiency at key genes to give rise to variability [57].

affect the development of schizophrenia in deletion carriers [51]. Similarly, mutations in *SNAP29*, also encompassed by the 22q11 microdeletion were recently shown to account for certain atypical features, including polymicrogyria and skin abnormalities [52]. Interestingly, mutations in *SNAP29* also cause the autosomal recessive disorder, CEDNIK syndrome, that shares many clinical features of patients with 22q11 microdeletion and hemizygous *SNAP29* variants. Thus, future studies that 'unmask' recessive alleles in microdeletion disorders provide a new avenue for gene discovery in autosomal recessive conditions. However, similar efforts to identify recessive alleles in genes encompassed by the recurrent microdeletion syndromes, 1q21.1 [34•] and 16p13.11 [53] did not identify damaging variants, suggesting a simple recessive model may not account for variability for all microdeletion syndromes.

The unmasking of rare recessive alleles may account for the clinical variability of mutations. Similarly, additional rare variants may also account for the reduced penetrance associated with microdeletion syndromes. For instance, a 'compound inheritance model' was recently shown to cause Thrombocytopenia with Absent Radii (TAR) syndrome [56••]. Microdeletions of the 1q21.1 region, either inherited or *de novo*, have been identified in most cases of TAR syndrome but reduced penetrance is common [20]. To identify additional causative variants, Albers and colleagues [56••] sequenced the exomes of five 1q21.1 deletion positive TAR patients, but detected no common gene with rare coding variants across the five patients. However, four probands carried a low frequency 5′UTR variant, and an additional proband carried a SNV in intron 1 of *RBM8A* (encompassed by the 1q21.1 deletion). Collectively, these two variants were shown to occur in 96% of additional 1q21.1 deletion positive TAR syndrome patients. Both variants reduced promoter activity and reductions in transcript abundance were observed in TAR probands as compared to unaffected parent carriers and controls (though this effect was not seen in healthy individuals with the 5′UTR variant). Also, two additional 1q21.1 deletion negative TAR probands were found to have one of two truncating variants in combination with the 5′UTR variant. A 'dose–effect phenomenon' was thus proposed to cause TAR syndrome, involving a null allele and a low frequency regulatory variant. This represents the first case of a 'compound inheritance model', distinct from autosomal recessive inheritance in that the low frequency regulatory variants, in a compound heterozygous, or homozygous, state, are unlikely to reduce *RBM8A* transcript levels below the critical levels required to develop TAR syndrome.

Small rare variants such as SNVs and indels contribute to the clinical variability and reduced penetrance of microdeletions. However, recent studies have shown that larger genomic regions, more specifically, rare CNVs, act in a similar manner to modify the presentation of probands with microdeletion syndromes. In 2010, Girirajan and colleagues described a series of patients with deletions of 16p12 [54]. This microdeletion was found to be enriched in probands with developmental delay, but was found to be inherited in 95% of cases, though parents were six-times more likely to exhibit neuropsychiatric

features. Of interest, about a quarter of 16p11.2 microdeletion probands carried a second large CNV that likely contributes to a more severe phenotype. Similar observations were made for probands with microdeletions involving 1q21.1, 11.2% of probands had a second large CNV. This 'second hit' model was further tested in a much larger cohort of 2312 probands with rare CNVs at one of 72 loci [55•]. Of these individuals, ~10% were found to carry a second large CNV, conferring an eight fold risk for presentation of developmental delay. These studies present a new paradigm for the pathogenesis of microdeletion disorders, with rare disruptive events acting in a synergistic way to cause distinct phenotypic outcomes.

These three pathogenic models, 'unmasking of recessive alleles', 'compound heterozygous inheritance' and the 'two hit' hypothesis, parallel existing models for complex neurodevelopmental disorders. Digenic or oligogenic models require mutations in two or more interacting genes to produce a specific phenotype. However, these models generally assume equal pathogenic effects for each genetic lesion. In contrast, the models highlighted here generally consider the microdeletion to be the primary causative genetic insult, and the variability in clinical features, or penetrance of these lesions, are modified by the presence of additional rare variants.

Environmental and epigenetic effects

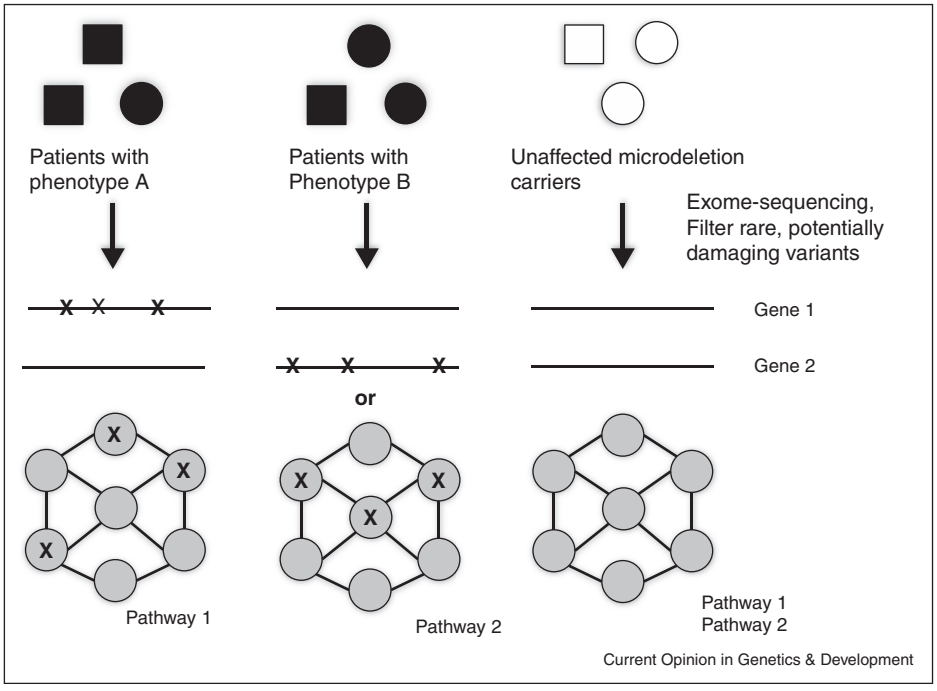
A common hypothesis for phenotypic variability in genetic disease is the effect of variable environmental factors. Though almost every large genomic rearrangement study alludes to these effects, studies directly addressing these factors are limited, mainly due to difficulties in experimental design. However, epigenetic modifications, that is, DNA methylation and chromatin remodeling, can serve as a proxy for the interaction between environment and genotype. Recent technological advances and an enhanced understanding of functional elements through projects such as ENCODE [57], now permit large scale interrogation of these epigenetic modifications. This interaction was eloquently demonstrated in a recent study by Voss and colleagues (2012), aimed at exploring the integrative role of environmental influences and epigenetic factors in the phenotypic variability associated with Velocardiofacial/DiGeorge syndrome (DGS) [58•]. DGS is characterized by cardiac defects, craniofacial abnormalities, thymus dysplasia and cleft palate and is caused by either 22q11 microdeletion or *TBX1* mutations. The severity of clinical presentation is highly variable, even between monozygotic twins, suggesting non-genetic influences [59]. Voss and colleagues demonstrated that lack of the chromatin remodeler, MOZ, a regulator of the *Tbx1* locus, generated a phenocopy of DGS in a homozygous state. More significantly, heterozygous MOZ mutant mice recapitulated the DGS phenotype in conjunction with either first, Tbx1 haploinsufficiency or second, with oversupply of vitamin A. This study demonstrates the interaction between an environmental factor (vitamin A) and an epigenetic modification (MOZ haploinsufficiency) to modulate clinical presentation in DGS. While scarce at present, similar future studies are likely to be crucial to our understanding of phenotypic variability.

Conclusions: moving forward in a massively parallel sequencing era

The extent to which phenotypic variability of some microdeletion syndromes can be explained by a 'second hit' model has, to date, only taken into account the combined effects of large CNVs. The compound inheritance model seen in TAR syndrome has provided the first evidence for the *cis*-interaction between a low frequency regulatory SNV and a microdeletion. Similarly, an interaction in *trans* between a microdeletion and low frequency variants that affect the function of genes in appropriate functional pathways is an attractive hypothesis to explain phenotypic variability. Current technological advances in molecular genetics, and their widespread use, will allow future research to address this hypothesis. The reasons for this are threefold. Firstly, the widespread use of array-CGH has defined large cohorts of microdeletion patients with diverse phenotypes. Secondly, projects such as NHLBI GO Exome Sequencing (ESP) and the 1000 Genomes collaborations have generated large catalogues of human genome variation (SNVs and indels). This information provides not only variant allele frequencies in certain populations, but also enables predictions of variant tolerability in genes. Finally, numerous Mendelian disorders have now been solved by whole-exome sequencing, resulting in an explosion in the numbers of genes that are associated with disease, and an enhanced understanding of aberrant pathways. Used in an integrative fashion, whole genome/exome sequencing in cohorts of microdeletion carriers can be conducted to identify rare variants in overlapping genes that form part of relevant functional pathways. Subscribing to the idea that groups of probands with a specific set of features will have a higher burden of variants in one gene (or group of genes), and that these will differ to gene/genes disrupted in patients with other features of the microdeletion syndrome and unaffected carriers (Figure 2).

The power of massively parallel sequencing, either at an exome, or in the future, the genome level, lies in the wealth of information that can be obtained from a single assay. Both *cis*-acting and *trans*-acting modulators of microdeletions can be investigated simultaneously. This includes not only SNVs, but also numerous algorithms have been developed to detect CNVs from sequencing data [60,61]. While, at present, primarily large events are efficiently detected, further developments will likely lead to the detection of smaller aberrations (5–50 kb), allowing identification of potential modifiers or even

Figure 2



Strategy for modeling phenotypic variability using massively parallel sequencing. Patients with variable phenotypes are divided into discrete groups according to clinical presentation. Individuals with one set of features will have a higher burden of rare, potentially damaging variants in common gene/genes (or pathways) that differ when compared to other phenotypes, and unaffected carriers.

novel recurrent microdeletions. An integrative approach to understanding phenotypic variability, at both the genetic and epigenetic level, will result in a more comprehensive interpretation of microdeletion syndromes, enhancing genetic counseling and prognosis for individuals afflicted with these disorders and their families.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Smith AC, McGavran L, Robinson J, Waldstein G, Macfarlane J, Zonona J, Reiss J, Lahr M, Allen L, Magenis E: **Interstitial deletion of (17)(p11.2p11.2) in nine patients**. *Am J Med Genet* 1986, **24**:393-414.

2. • Edelmann L, Pandita RK, Spiteri E, Funke B, Goldberg R, Palanisamy N, Chaganti RS, Magenis E, Shprintzen RJ, Morrow BE: **A common molecular basis for rearrangement disorders on chromosome 22q11**. *Hum Mol Genet* 1999, **8**:1157-1167.

One of the first studies to associate genomic architecture (low copy repeats) with recurrent rearrangements at 22q.

3. Perez Jurado LA, Peoples R, Kaplan P, Hamel BC, Francke U: **Molecular definition of the chromosome 7 deletion in Williams syndrome and parent-of-origin effects on growth**. *Am J Hum Genet* 1996, **59**:781-792.

4. Bailey JA, Gu Z, Clark RA, Reinert K, Samonte RV, Schwartz S, Adams MD, Myers EW, Li PW, Eichler EE: **Recent segmental duplications in the human genome**. *Science* 2002, **297**:1003-1007.

5. Mefford HC, Eichler EE: **Duplication hotspots, rare genomic disorders, and common disease**. *Curr Opin Genet Dev* 2009, **19**:196-204.

6. Girirajan S, Eichler EE: **Phenotypic variability and genetic susceptibility to genomic disorders**. *Hum Mol Genet* 2010, **19**:R176-R187.

7. • de Vries BB, Pfundt R, Leisink M, Koolen DA, Vissers LE, Janssen IM, Reijmersdal S, Nillesen WM, Huys EH, Leeuw N *et al.*: **Diagnostic genome profiling in mental retardation**. *Am J Hum Genet* 2005, **77**:606-616.

One of the first genome-wide studies of ID using array-CGH, the authors showed that 10% of cases can be attributed to de novo CNVs.

8. Koolen DA, Vissers LE, Pfundt R, de Leeuw N, Knight SJ, Regan R, Kooy RF, Reyniers E, Romano C, Fichera M *et al.*: **A new chromosome 17q21.31 microdeletion syndrome associated with a common inversion polymorphism**. *Nat Genet* 2006, **38**:999-1001.

9. Sagoo G, Butterworth A, Sanderson S, Shaw-Smith C, Higgins J, Burton H: **Array CGH in patients with learning disability (mental retardation) and congenital anomalies: updated systematic review and meta-analysis of 19 studies and 13,926 subjects**. *Genet Med* 2009, **11**:139-146 139.

10. Shaffer LG, Kashork CD, Saleki R, Rorem E, Sundin K, Ballif BC, Bejjani BA: **Targeted genomic microarray analysis for identification of chromosome abnormalities in 1500 consecutive clinical cases**. *J Pediatr* 2006, **149**:98-102.

11. •• Sharp AJ, Hansen S, Selzer RR, Cheng Z, Regan R, Hurst JA, Stewart H, Price SM, Blair E, Hennekam RC *et al.*: **Discovery of previously unidentified genomic disorders from the duplication architecture of the human genome**. *Nat Genet* 2006, **38**:1038-1042.

The authors targeted genomic regions harboring segmental duplication to identify chromosomal aberrations in ID. Five *de novo* events were detected, including aberrations at 17q21.21, 15q13.3, 15q24, 1q21.1 and 17q12, all have since been shown to be recurrent and contribute to a myriad of neurodevelopmental disorders.

12. Shaw-Smith C, Pittman AM, Willatt L, Martin H, Rickman L, Gribble S, Curley R, Cumming S, Dunn C, Kalaitzopoulos D, Porter K, Prigmore E *et al.*: **Microdeletion encompassing MAPT at chromosome 17q21.3 is associated with developmental delay and learning disability**. *Nat Genet* 2006, **38**:1032-1037.

13. Christian SL, Brune CW, Sudi J, Kumar RA, Liu S, Karamohamed S, Badner JA, Matsui S, Conroy J, McQuaid D *et al.*: **Novel submicroscopic chromosomal abnormalities detected in autism spectrum disorder**. *Biol Psychiatry* 2008, **63**:1111-1117.

14. Kumar RA, KaraMohamed S, Sudi J, Conrad DF, Brune C, Badner JA, Gilliam TC, Nowak NJH Jr *et al.*: **Recurrent 16p11.2 microdeletions in autism**. *Hum Mol Genet* 2008, **17**:628-638.

15. Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J, Shago M, Moessner R, Pinto D, Ren Y, Thiruvahindrapduram B *et al.*: **Structural variation of chromosomes in autism spectrum disorder**. *Am J Hum Genet* 2008, **82**:477-488.

16. • Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, Yamrom B, Yoon S, Krasnitz A, Kendall J *et al.*: **Strong association of de novo copy number mutations with autism**. *Science* 2007, **316**:445-449.
The authors demonstrate that up 10% of sporadic autism may be due to large, *de novo* CNVs.

17. Szatmari P, Paterson AD, Zwaigenbaum L, Roberts W, Brian J, Liu XQ, Vincent JB, Skaug JL, Thompson AP, Senman L *et al.*: **Mapping autism risk loci using genetic linkage and chromosomal rearrangements**. *Nat Genet* 2007, **39**:319-328.

18. Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R, Saemundsen E, Stefansson H, Ferreira MA, Green T *et al.*: **Association between microdeletion and microduplication at 16p11.2 and autism**. *N Engl J Med* 2008, **358**:667-675.

19. Erdogan F, Larsen LA, Zhang L, Tumer Z, Tommerup N, Chen W, Jacobsen JR, Schubert M, Jurkatis J, Tzschach A *et al.*: **High frequency of submicroscopic genomic aberrations detected by tiling path array comparative genome hybridisation in patients with isolated congenital heart disease**. *J Med Genet* 2008, **45**:704-709.

20. Klopocki E, Schulze H, Strauss G, Ott CE, Hall J, Trotier F, Fleischhauer S, Greenhalgh L, Newbury-Ecob RA, Neumann LM *et al.*: **Complex inheritance pattern resembling autosomal recessive inheritance involving a microdeletion in thrombocytopenia-absent radius syndrome**. *Am J Hum Genet* 2007, **80**:232-240.

21. Mefford HC, Clauin S, Sharp AJ, Moller RS, Ullmann R, Kapur R, Pinkel D, Cooper GM, Ventura M, Ropers HH *et al.*: **Recurrent reciprocal genomic rearrangements of 17q12 are associated with renal disease, diabetes, and epilepsy**. *Am J Hum Genet* 2007, **81**:1057-1069.

22. Richards AA, Santos LJ, Nichols HA, Crider BP, Elder FF, Hauser NS, Zinn AR, Garg V: **Cryptic chromosomal abnormalities identified in children with congenital heart disease**. *Pediatr Res* 2008, **64**:358-363.

23. Koolen DA, Sharp AJ, Hurst JA, Firth HV, Knight SJ, Goldenberg A, Saugier-Veber P, Pfundt R, Vissers LE, Destree A, Grisart B, Rooms L *et al.*: **Clinical and molecular delineation of the 17q21.31 microdeletion syndrome**. *J Med Genet* 2008, **45**:710-720.

24. Andrieux J, Dubourg C, Rio M, Attie-Bitach T, Delaby E, Mathieu M, Journel H, Copin H, Blondeel E, Doco-Fenzy M *et al.*: **Genotype–phenotype correlation in four 15q24 deleted patients identified by array-CGH**. *Am J Med Genet A* 2009, **149A**:2813-2819.

25. Sharp AJ, Selzer RR, Veltman JA, Gimelli S, Gimelli G, Striano P, Coppola A, Regan R, Price SM, Knoers NV *et al.*: **Characterization of a recurrent 15q24 microdeletion syndrome**. *Hum Mol Genet* 2007, **16**:567-572.

26. El-Hattab AW, Smolarek TA, Walker ME, Schorry EK, Immken LL, Patel G, Abbott MA, Lanpher BC, Ou Z, Kang SH *et al.*: **Redefined genomic architecture in 15q24 directed by patient deletion/duplication breakpoint mapping**. *Hum Genet* 2009, **126**:589-602.

27. Mefford HC, Rosenfeld JA, Shur N, Slavotinek AM, Cox VA, Hennekam RC, Firth HV, Willatt L, Wheeler P, Morrow EM *et al.*: **Further clinical and molecular delineation of the 15q24 microdeletion syndrome**. *J Med Genet* 2012, **49**:110-118.

28. Shinawi M, Liu P, Kang SH, Shen J, Belmont JW, Scott DA, Probst FJ, Craigen WJ, Graham BH, Pursley A *et al.*: **Recurrent reciprocal 16p11.2 rearrangements associated with global developmental delay, behavioural problems, dysmorphism, epilepsy, and abnormal head size**. *J Med Genet* 2010, **47**:332-341.

29. Bachmann-Gagescu R, Mefford HC, Cowan C, Glew GM, Hing AV, Wallace S, Bader PI, Hamati A, Reitnauer PJ, Smith R *et al.*: **Recurrent 200-kb deletions of 16p11.2 that include the SH2B1 gene are associated with developmental delay and obesity**. *Genet Med* 2010, **10**:641-647.

30. Bijlsma EK, Gijsbers AC, Schuurs-Hoeijmakers JH, van Haeringen A, Fransen van de Putte DE, Anderlid BM, Lundin J, Lapunzina P, Perez Jurado LA, Delle Chiaie B *et al.*: **Extending the phenotype of recurrent rearrangements of 16p11.2: deletions in mentally retarded patients without autism and in normal individuals**. *Eur J Med Genet* 2009, **52**:77-87.

31. McCarthy SE, Makarov V, Kirov G, Addington AM, McClellan J, Yoon S, Perkins DO, Dickel DE, Kusenda M, Krastoshevsky O *et al.*: **Microduplications of 16p11.2 are associated with schizophrenia**. *Nat Genet* 2009, **41**:1223-1227.

32. Brunetti-Pierri N, Berg JS, Scaglia F, Belmont J, Bacino CA, Sahoo T, Lalani SR, Graham B, Lee B, Shinawi M, Shen J, Kang SH *et al.*: **Recurrent reciprocal 1q21.1 deletions and duplications associated with microcephaly or macrocephaly and developmental and behavioral abnormalities**. *Nat Genet* 2008, **40**:1466-1471 http://dx.doi.org/10.1038/ng.279.

33. Christiansen J, Dyck JD, Elyas BG, Lilley M, Bamforth JS, Hicks M, Sprysak KA, Tomaszewski R, Haase SM, Vicen-Wyhony LM *et al.*: **Chromosome 1q21.1 contiguous gene deletion is associated with congenital heart disease**. *Circ Res* 2004, **94**:1429-1435.

34. • Mefford HC, Sharp AJ, Baker C, Itsara A, Jiang Z, Buysse K, Huang S, Maloney VK, Crolla JA, Baralle D *et al.*: **Recurrent rearrangements of chromosome 1q21.1 and variable pediatric phenotypes**. *N Engl J Med* 2008, **359**:1685-1699.
Detailed phenotypic examination of patients with 1q21.1 microdeletions reveal a wide spectrum of features, illustrate the first support for a genotype-first approach to molecular diagnosis of disease.

35. Stefansson H, Rujescu D, Cichon S, Pietilainen OP, Ingason A, Steinberg S, Fossdal R, Sigurdsson E, Sigmundsson T, Buizer-Voskamp JE *et al.*: **Large recurrent microdeletions associated with schizophrenia**. *Nature* 2008, **455**:232-236.

36. Van Den Bossche MJ, Johnstone M, Strazisar M, Pickard BS, Goossens D, Lenaerts AS, De Zutter S, Nordin A, Norrback KF, Mendlewicz J *et al.*: **Rare copy number variants in neuropsychiatric disorders: specific phenotype or not?** *Am J Med Genet B: Neuropsychiatr Genet* 2012, **159B**:812-822.

37. Sharp AJ, Mefford HC, Li K, Baker C, Skinner C, Stevenson RE, Schroer RJ, Novara F, De Gregori M, Ciccone R *et al.*: **A recurrent 15q13.3 microdeletion syndrome associated with mental retardation and seizures**. *Nat Genet* 2008, **40**:322-328.

38. Dibbens LM, Mullen S, Helbig I, Mefford HC, Bayly MA, Bellows S, Leu C, Trucks H, Obermeier T, Wittig M *et al.*: **Familial and sporadic 15q13.3 microdeletions in idiopathic generalized epilepsy: precedent for disorders with complex inheritance**. *Hum Mol Genet* 2009, **18**:3626-3631.

39. Helbig I, Mefford HC, Sharp AJ, Guipponi M, Fichera M, Franke A, Muhle H, de Kovel C, Baker C, von Spiczak S *et al.*: **15q13.3 microdeletions increase risk of idiopathic generalized epilepsy**. *Nat Genet* 2009, **41**:160-162.

40. Ben-Shachar S, Lanpher B, German JR, Qasaymeh M, Potocki L, Nagamani SC, Franco LM, Malphrus A, Bottenfield GW, Spence JE *et al.*: **Microdeletion 15q13.3: a locus with incomplete penetrance for autism, mental retardation, and psychiatric disorders**. *J Med Genet* 2009, **46**:382-388.

41. Spielmann M, Reichelt G, Hertzberg C, Trimborn M, Mundlos S, Horn D, Klopocki E: **Homozygous deletion of chromosome 15q13.3 including CHRNA7 causes severe mental retardation,**

seizures, muscular hypotonia, and the loss of KLF13 and TRPM1 potentially cause macrocytosis and congenital retinal dysfunction in siblings. *Eur J Med Genet* 2011, **54**:e441-e445 http://dx.doi.org/10.1016/j.ejmg.2011.04.004.

42. Endris V, Hackmann K, Neuhann TM, Grasshoff U, Bonin M, Haug U, Hahn G, Schallner JC, Schrock E, Tinschert S *et al.*: **Homozygous loss of CHRNA7 on chromosome 15q13.3 causes severe encephalopathy with seizures and hypotonia**. *Am J Med Genet A* 2010, **152A**:2908-2911.

43. Girirajan S, Vlangos CN, Szomju BB, Edelman E, Trevors CD, Dupuis L, Nezarati M, Bunyan DJ, Elsea SH: **Genotype–phenotype correlation in Smith-Magenis syndrome: evidence that multiple genes in 17p11.2 contribute to the clinical spectrum**. *Genet Med* 2006, **8**:417-427.

44. Tassabehji M, Hammond P, Karmiloff-Smith A, Thompson P, Thorgeirsson SS, Durkin ME, Popescu NC, Hutton T, Metcalfe K, Rucka A *et al.*: **GTF2IRD1 in craniofacial development of humans and mice**. *Science* 2005, **310**:1184-1187.

45. Potocki L, Bi W, Treadwell-Deering D, Carvalho CM, Eifert A, Friedman EM, Glaze D, Krull K, Lee JA, Lewis RA, Mendoza-Londono R *et al.*: **Characterization of Potocki-Lupski syndrome (dup(17)(p11.2p11.2)) and delineation of a dosage-sensitive critical interval that can convey an autism phenotype**. *Am J Hum Genet* 2007, **80**:633-649.

46. Kleefstra T, Smidt M, Banning MJ, Oudakker AR, Van Esch H, de Brouwer AP, Nillesen W, Sistermans EA, Hamel BC, de Bruijn D *et al.*: **Disruption of the gene Euchromatin Histone Methyl Transferase1 (Eu-HMTase1) is associated with the 9q34 subtelomeric deletion syndrome**. *J Med Genet* 2005, **42**:299-306.

47. Zweier M, Gregor A, Zweier C, Engels H, Sticht H, Wohlleber E, Bijlsma EK, Holder SE, Zenker M, Rossier E *et al.*: **Mutations in MEF2C from the 5q14.3q15 microdeletion syndrome region are a frequent cause of severe mental retardation and diminish MECP2 and CDKL5 expression**. *Hum Mutat* 2010, **31**:722-733.

48. Talkowski ME, Mullegama SV, Rosenfeld JA, van Bon BW, Shen Y, Repnikova EA, Gastier-Foster J, Thrush DL, Kathiresan S, Ruderfer DM *et al.*: **Assessment of 2q23.1 microdeletion syndrome implicates MBD5 as a single causal locus of intellectual disability, epilepsy, and autism spectrum disorder**. *Am J Hum Genet* 2011, **89**:551-563.

49. Hoppman-Chaney N, Wain K, Seger P, Superneau D, Hodge J: **Identification of single gene deletions at 15q13.3: further evidence that CHRNA7 causes the 15q13.3 microdeletion syndrome phenotype**. *Clin Genet* 2012, **83**:345-351.

50. Liao J, DeWard SJ, Madan-Khetarpal S, Surti U, Hu J: **A small homozygous microdeletion of 15q13.3 including the CHRNA7 gene in a girl with a spectrum of severe neurodevelopmental features**. *Am J Med Genet A* 2011, **155A**:2795-2800.

51. Bearden CE, Jawad AF, Lynch DR, Sokol S, Kanes SJ, McDonald-McGinn DM, Saitta SC, Harris SE, Moss E, Wang PP *et al.*: **Effects of a functional COMT polymorphism on prefrontal cognitive function in patients with 22q11.2 deletion syndrome**. *Am J Psychiatry* 2004, **161**:1700-1702.

52. McDonald-McGinn DM, Fahiminiya S, Revil T, Nowakowska BA, Suhl J, Bailey A, Mlynarski E, Lynch DR, Yan AC, Bilaniuk LT *et al.*: **Hemizygous mutations in SNAP29 unmask autosomal recessive conditions and contribute to atypical findings in patients with 22q11.2DS**. *J Med Genet* 2013, **50**:80-90.

53. Hannes FD, Sharp AJ, Mefford HC, de Ravel T, Ruivenkamp CA, Breuning MH, Fryns JP, Devriendt K, Van Buggenhout G, Vogels A *et al.*: **Recurrent reciprocal deletions and duplications of 16p13.11: the deletion is a risk factor for MR/MCA while the duplication may be a rare benign variant**. *J Med Genet* 2009, **46**:223-232.

54. Girirajan S, Rosenfeld JA, Cooper GM, Antonacci F, Siswara P, Itsara A, Vives L, Walsh T, McCarthy SE, Baker C *et al.*: **A recurrent 16p12.1 microdeletion supports a two-hit model for severe developmental delay**. *Nat Genet* 2010, **42**:203-209.

55. • Girirajan S, Rosenfeld JA, Coe BP, Parikh S, Friedman N, Goldstein A, Filipink RA, McConnell JS, Angle B, Meschino WS *et al.*: **Phenotypic heterogeneity of genomic disorders and rare copy-number variants**. *N Engl J Med* 2012, **367**:1321-1331.
The authors describe a two-hit model for phenotypic severity, where in a series of over 2000 probands with rare CNVs, 10% were found to carry a second large CNV, conferring an eight-fold risk for developmental delay.

56. •• Albers CA, Paul DS, Schulze H, Freson K, Stephens JC, Smethurst PA, Jolley JD, Cvejic A, Kostadima M, Bertone P *et al.*: **Compound inheritance of a low-frequency regulatory SNP and a rare null mutation in exon-junction complex subunit RBM8A causes TAR syndrome**. *Nat Genet* 2012, **44**:435-439 S1–2.
Illustrate one the first cases of 'compound inheritance', demonstrate the combination of low frequency regulatory variants and a microdeletion cause TAR syndrome.

57. ENCODE Project Consortium: Birney E, Stamatoyannopoulos JA, Dutta A, Guigo R, Gingeras TR, Margulies EH, Weng Z, Snyder M, Dermitzakis ET, Thurman RE *et al.*: **Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project**. *Nature* 2007, **447**:799-816.

58. • Voss AK, Vanyai HK, Collin C, Dixon MP, McLennan TJ, Sheikh BN, Scambler P, Thomas T: **MOZ regulates the Tbx1 locus, and Moz mutation partially phenocopies DiGeorge syndrome**. *Dev Cell* 2012, **23**:652-663.
The authors demonstrate a molecular mechanism for the interaction between a chromatin remodeler and an environmental factor, and present a model for phenotypic variability in DiGeorge syndrome.

59. Goodship J, Cross I, Scambler P, Burn J: **Monozygotic twins with chromosome 22q11 deletion and discordant phenotype**. *J Med Genet* 1995, **32**:746-748.

60. Krumm N, Sudmant PH, Ko A, O'Roak BJ, Malig M, Coe BP, NHLBI Exome Sequencing Project, Quinlan AR, Nickerson DA, Eichler EE: **Copy number variation detection and genotyping from exome sequence data**. *Genome Res* 2012, **22**:1525-1532.

61. Fromer M, Moran JL, Chambert K, Banks E, Bergen SE, Ruderfer DM, Handsaker RE, McCarroll SA, O'Donovan MC, Owen MJ *et al.*: **Discovery and statistical genotyping of copy-number variation from whole-exome sequencing depth**. *Am J Hum Genet* 2012, **91**:597-607.

62. Ballif BC, Theisen A, Coppinger J, Gowans GC, Hersh JH, Madan-Khetarpal S, Schmidt KR, Tervo R, Escobar LF, Friedrich CA *et al.*: **Expanding the clinical phenotype of the 3q29 microdeletion syndrome and characterization of the reciprocal microduplication**. *Mol Cytogenet* 2008, **1**:8.

63. Willatt L, Cox J, Barber J, Cabanas ED, Collins A, Donnai D, FitzPatrick DR, Maher E, Martin H, Parnau J *et al.*: **3q29 microdeletion syndrome: clinical and molecular characterization of a new syndrome**. *Am J Hum Genet* 2005, **77**:154-160.

64. Balciuniene J, Feng N, Iyadurai K, Hirsch B, Charnas L, Bill BR, Easterday MC, Staaf J, Oseth L, Czapansky-Beilman D *et al.*: **Recurrent 10q22–q23 deletions: a genomic disorder on 10q associated with cognitive and behavioral abnormalities**. *Am J Hum Genet* 2007, **80**:938-947.

65. van Bon BW, Balciuniene J, Fruhman G, Nagamani SC, Broome DL, Cameron E, Martinet D, Roulet E, Jacquemont S, Beckmann JS *et al.*: **The phenotype of recurrent 10q22q23 deletions and duplications**. *Eur J Hum Genet* 2011, **19**:400-408.

66. Burnside RD, Pasion R, Mikhail FM, Carroll AJ, Robin NH, Youngs EL, Gadi IK, Keitges E, Jaswaney VL, Papenhausen PR *et al.*: **Microdeletion/microduplication of proximal 15q11.2 between BP1 and BP2: a susceptibility region for neurological dysfunction including developmental and language delay**. *Hum Genet* 2011, **130**:517-528.

67. von der Lippe C, Rustad C, Heimdal K, Rodningen OK: **15q11.2 microdeletion — seven new patients with delayed development and/or behavioural problems**. *Eur J Med Genet* 2011, **54**:357-360.

68. de Kovel CG, Trucks H, Helbig I, Mefford HC, Baker C, Leu C, Kluck C, Muhle H, von Spiczak S, Ostertag P *et al.*: **Recurrent microdeletions at 15q11.2 and 16p13.11 predispose to idiopathic generalized epilepsies**. *Brain* 2010, **133**:23-32.

69. Mefford HC, Muhle H, Ostertag P, von Spiczak S, Buysse K, Baker C, Franke A, Malafosse A, Genton P, Thomas P *et al.*:

Genome-wide copy number variation in epilepsy: novel susceptibility loci in idiopathic generalized and focal epilepsies. *PLoS Genet* 2010, **6**:e1000962.

70. Abdelmoity AT, LePichon JB, Nyp SS, Soden SE, Daniel CA, Yu S: **15q11.2 proximal imbalances associated with a diverse array of neuropsychiatric disorders and mild dysmorphic features**. *J Dev Behav Pediatr* 2012, **33**:570-576.

71. van Bon BW, Mefford HC, Menten B, Koolen DA, Sharp AJ, Nillesen WM, Innis JW, de Ravel TJ, Mercer CL, Fichera M *et al.*: **Further delineation of the 15q13 microdeletion and duplication syndromes: a clinical spectrum varying from non-pathogenic to a severe outcome**. *J Med Genet* 2009, **46**:511-523.

72. McInnes LA, Nakamine A, Pilorge M, Brandt T, Jimenez Gonzalez P, Fallas M, Manghi ER, Edelmann L, Glessner J, Hakonarson H *et al.*: **A large-scale survey of the novel 15q24 microdeletion syndrome in autism spectrum disorders identifies an atypical deletion that narrows the critical region**. *Mol Autism* 2010, **1**:5.

73. Ullmann R, Turner G, Kirchhoff M, Chen W, Tonge B, Rosenberg C, Field M, Vianna-Morgante AM, Christie L, Krepischi-Santos AC *et al.*: **Array CGH identifies reciprocal 16p13.1 duplications and deletions that predispose to autism and/or mental retardation**. *Hum Mutat* 2007, **28**:674-682.

74. Heinzen EL, Radtke RA, Urban TJ, Cavalleri GL, Depondt C, Need AC, Walley NM, Nicoletti P, Ge D, Catarino CB *et al.*: **Rare deletions at 16p13.11 predispose to a diverse spectrum of sporadic epilepsy syndromes**. *Am J Hum Genet* 2010, **86**:707-718.

75. Nagamani SC, Erez A, Shen J, Li C, Roeder E, Cox S, Karaviti L, Pearson M, Kang SH, Sahoo T, Lalani SR, Stankiewicz P, Sutton VR, Cheung SW: **Clinical spectrum associated with recurrent genomic rearrangements in chromosome 17q12**. *Eur J Hum Genet* 2010, **18**:278-284.

76. George AM, Love DR, Hayes I, Tsang B: **Recurrent transmission of a 17q12 microdeletion and a variable clinical spectrum**. *Mol Syndromol* 2012, **2**:72-75.

77. Ben-Shachar S, Ou Z, Shaw CA, Belmont JW, Patel MS, Hummel M, Amato S, Tartaglia N, Berg J, Sutton VR *et al.*: **22q11.2 distal deletion: a recurrent genomic disorder distinct from DiGeorge syndrome and velocardiofacial syndrome**. *Am J Hum Genet* 2008, **82**:214-221.