The Genetics of Microdeletion and Microduplication Syndromes: An Update

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Abstract

Chromosomal abnormalities, including microdeletions and microduplications, have long been associated with abnormal developmental outcomes. Early discoveries relied on a common clinical presentation and the ability to detect chromosomal abnormalities by standard karyotype analysis or specific assays such as fluorescence in situ hybridization. Over the past decade, the development of novel genomic technologies has allowed more comprehensive, unbiased discovery of microdeletions and microduplications throughout the human genome. The ability to quickly interrogate large cohorts using chromosome microarrays and, more recently, next-generation sequencing has led to the rapid discovery of novel microdeletions and microduplications associated with disease, including very rare but clinically significant rearrangements. In addition, the observation that some microdeletions are associated with risk for several neurodevelopmental disorders contributes to our understanding of shared genetic susceptibility for such disorders. Here, we review current knowledge of microdeletion/duplication syndromes, with a particular focus on recurrent rearrangement syndromes.

INTRODUCTION

Chromosomal microdeletions and microduplications have been associated with syndromic forms of intellectual disability (ID) and developmental delay (DD) since the 1980s. Classic examples include the 15q11–q13 deletion associated with Prader–Willi and Angelman syndromes (24), the 17p11 deletion associated with Smith–Magenis syndrome (29), the 7q11 deletion associated with Williams–Beuren syndrome (131), and the 22q11 deletions associated with velocardiofacial syndrome (45). 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. Improved cytogenetic techniques, including high-resolution karyotype analysis and fluorescence in situ hybridization (FISH), facilitated diagnostic testing for the deletion associated with each suspected diagnosis. For example, if a physician suspected that a patient had Prader–Willi syndrome, a FISH test could be performed to determine whether the causative 15q11–q13 deletion was present.

Chromosomal microdeletions and microduplications make up a fraction of copy-number variants (CNVs). CNVs are defined as either the gain or loss of a stretch of DNA as compared with the reference human genome; they may range in size from a kilobase to several megabases or even an entire chromosome (trisomies and monosomies). CNVs can involve multiple, one, or no genes, and although some CNVs cause disease, many others remain benign variants within the population (68, 71, 103, 140, 151).

There are two major classes of CNVs: recurrent and nonrecurrent. Recurrent CNVs generally arise by nonallelic homologous recombination (NAHR) during meiosis, with breakpoints in the large duplicated blocks of sequence flanking the CNV event (**Figure 1**). Because the breakpoints cluster within defined regions, the extent of recurrent CNVs is essentially identical even in

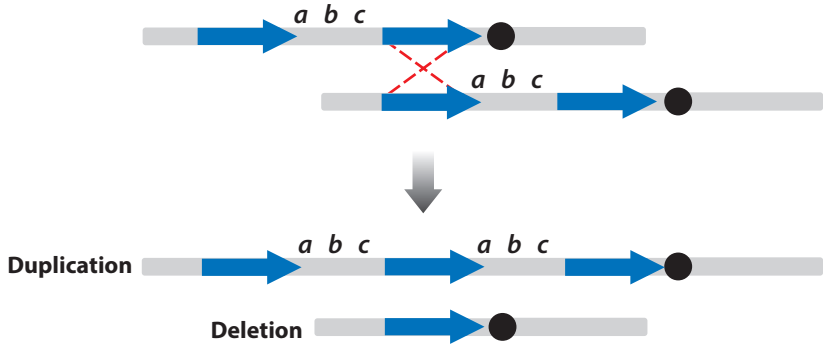


Figure 1

Nonallelic homologous recombination (NAHR), the primary mechanism underlying the generation of recurrent copy-number variants (CNVs). Segmental duplications, also termed low-copy repeats, represent regions of extended sequence identity that can provide a substrate for NAHR-mediated chromosomal rearrangements. In this schematic, two large segmental duplications (*blue arrows*) with high sequence similarity flank a region containing genes *a*, *b*, and *c*. Following misalignment of the homologs, these duplications facilitate NAHR during meiosis by assisting an illegitimate crossing-over event between paralogous, rather than allelic, segmental duplications. This results in two reciprocal products: one chromosome carrying a duplication of the intervening region and an additional copy of genes *a*, *b*, and *c*, and a second chromosome carrying a deletion of this same region. Such rearrangements are common causes of many recurrent genomic disorders characterized by reciprocal rearrangements of specific chromosomal regions (see **Table 1**).

unrelated individuals (101). In contrast, nonrecurrent CNVs have breakpoints that generally lie within unique sequence and do not result from a predisposing genomic architecture. Nonrecurrent CNVs can arise by several different mechanisms, including nonhomologous end joining and fork stalling and template switching (FoSTeS) (25, 64, 194). As a result, although two unrelated individuals may have overlapping nonrecurrent CNVs, they are unlikely to share the same breakpoints.

Over the past 10 years, there have been incredible advances in high-throughput genomic technologies. It is now possible to rapidly interrogate the entire genome for CNVs, providing vastly improved screening methods for the detection of microdeletions and microduplications compared with the technology available a decade ago (183). Array comparative genomic hybridization (aCGH) was introduced in 2003 and was initially used to characterize the many large somatic rearrangements that often occur in cancer (182). aCGH is a comparative assay in which two samples are differentially labeled with fluorescent dyes. Cohybridizing these labeled genomes to an array comprising probes spaced throughout the genome allows for the detection of relative differences in copy number between the two samples. Although not originally designed for detecting CNVs, single-nucleotide polymorphism (SNP) genotyping arrays can also be used to detect copy-number changes by combining measures of fluorescence intensity and allelic ratios produced by the array (34). Throughout this review, we refer to aCGH and SNP genotyping arrays as chromosome microarrays. Although the resolution of these methods is largely dependent on probe density, modern arrays comprising hundreds of thousands or millions of probes can detect CNVs that are several orders of magnitude smaller than those visible by standard karyotype analysis. Chromosome microarrays are now widely used in both research and diagnostic settings.

Soon after their introduction, microarrays were used to profile the CNV patterns of normal individuals. The results of these studies were unexpected, revealing that the genomes of two healthy people could differ in DNA content by many millions of base pairs (140, 151, 156). Shortly thereafter, similar studies were initiated to also identify CNVs in individuals with genetic syndromes. The first large cohorts to be studied were individuals with ID (41, 88, 145, 152, 155, 159), autism spectrum disorder (ASD) (30, 92, 108, 150, 173, 187), or congenital anomalies (54, 86, 111, 141). More recently, studies of CNVs have been extended to more common phenotypes that are not traditionally considered to be chromosomal disorders, including schizophrenia (11, 82, 127, 168, 170, 184), epilepsy (39, 43, 66), amyotrophic lateral sclerosis (161), autoimmune diseases (38), and craniosynostosis (73). Through comparisons of the frequency of a given CNV in cases and controls, disease-related CNVs have been rapidly identified for each of these conditions.

The size of CNV detected by chromosome microarray technologies depends largely on the probe density, which is determined by the spacing of probes on the array. Although probe density continues to increase, the smallest CNVs detected by these methods are generally on the order of ~50 kb or larger. However, recent major advances in sequencing technologies are providing novel insights into CNVs, particularly those that have not been resolvable by chromosome microarray methods. Next-generation sequencing or massively parallel sequencing (MPS) involves highly parallelized sequencing of millions of short DNA fragments from the genome. Indeed, an entire genome can be sequenced using MPS in a matter of days. By taking advantage of these technologies, researchers have developed several approaches that enable the identification of CNVs from MPS data (192, 193, 196); these approaches have facilitated the routine genome-wide discovery of much smaller CNVs, which have not been comprehensively assayed using current technologies. Whole-genome sequencing using MPS has the potential to provide a truly unbiased assay that can identify CNVs ranging in size from a single base pair to entire chromosomes, simultaneously with a complete assessment of single-nucleotide sequence changes.

The use of these technologies has had a significant impact on the field of human genetics. Indeed, as we discuss below, many "syndromes" that are difficult to recognize solely by clinical

features have now been identified and defined purely by the nature of a shared genomic rearrangement; this "genotype-first" approach to clinical diagnosis is now routine. Here, we review the discovery, genetic architecture, and evolution and characterization of novel microdeletion and microduplication syndromes. We also discuss approaches to understanding the clinical variability associated with many novel deletions and duplications and mechanisms of CNV formation. Finally, we address the clinical implications that these and other new technologies have for clinical practice. Although many of the principles of our discussion are relevant to both recurrent and nonrecurrent events, we place particular emphasis on the truly recurrent genomic disorders that are catalyzed by the local genome architecture and arise via NAHR.

HOT SPOTS OF RECURRENT REARRANGEMENTS

The term genomic disorder was first applied to diseases for which the primary underlying cause involved rearrangements of specific chromosomal regions (104). From early studies of such rearrangements, it became clear that these CNVs were recurrent, and this recurrence was strongly related to the local genome architecture. Most cases were de novo and represented reciprocal deletion and duplication of a specific chromosomal interval impacting one or more dosage-sensitive genes (104). Seminal examples are the 17p12 duplications and deletions, which result in the development of Charcot–Marie–Tooth disease type 1A (CMT1A) and hereditary neuropathy with liability to pressure palsies (HNPP), respectively (27, 105), owing to contrasting dosage effects of the *PMP22* gene (26). These recurrent, reciprocal, disease-causing CNVs were two of the first genomic disorders described. More examples soon followed, including Prader–Willi and Angelman syndromes (15q11–q13) (4) and Smith–Magenis syndrome (17p11.2) (29). The recurrent CNVs causing each disorder were shown to involve NAHR-mediated rearrangements in regions of the genome exhibiting local architectures characterized by repetitive DNA features, termed segmental duplications (SDs) or low-copy repeats (LCRs) (104, 166) (**Figure 1**).

Upon noting that recurrent genomic disorders are catalyzed by the presence of pairs of large, highly identical flanking repeats, Eichler and colleagues (9) generated a genome-wide map of more than 8,000 SDs. Analysis of this SD map identified 169 regions of the human genome that were predicted to be potential rearrangement hot spots because of the presence of large blocks of SDs with >95% sequence similarity that were separated by 50 kb–10 Mb of intervening sequence. Interestingly, 24 of these regions had already been linked to recurrent genetic diseases (9). Testing of these hot-spot regions in a population of normal individuals using a targeted array revealed that CNVs were significantly more common within these predicted rearrangement hot spots compared with the genome average (156). However, for many of these regions, CNVs were apparently not observed in this normal population, prompting a strategy to target such regions in human disease patients. Indeed, the initial use of this targeted microarray method in patient cohorts with ID/DD and various congenital anomalies proved fruitful in the discovery of novel pathogenic recurrent rearrangements (111, 155). For example, by screening 290 patients with ID/DD for which underlying genetic causes had not previously been found, Sharp et al. (155) identified 16 individuals carrying large submicroscopic deletions and duplications that were associated with their disease. Localization of the breakpoints in each of these patients to flanking clusters of SDs defined five disease-associated NAHR hot spots, located at 1q21.1, 15q13, 15q24, 17q12, and 17q21.31. Subsequent studies have replicated all of these loci as associated with recurrent genomic disorders in a variety of cohorts of patients with ID/DD, and several of them are now associated with clinically recognizable syndromes (88, 115, 176).

The widespread application of microarray technologies to additional series of patients with ID/DD has led to a renaissance in the understanding of the chromosomal basis of human disease.

Since 2006, more than 20 recurrent microdeletion/duplication syndromes have been identified for ID/DD; these are listed with associated references in **Table 1**. A central conclusion drawn from these newly described syndromes is that, in each case, a series of patients were initially classified based on the characterization of common overlapping genetic lesions rather than on constellations of clinical features, reflecting a transition in the field from the phenotype-first approach to the genotype-first approach (113). Importantly, in such instances, the identification of a group of patients with shared genomic rearrangements can allow for more definitive characterization of clinical symptoms and lead to improved patient diagnoses and management.

An illustrative example of the power of this approach is the 15q24 microdeletion syndrome, which was first described in 2006 and then confirmed as a site of recurrent rearrangement in 2007 (155, 158). Carriers of this syndrome may present with a spectrum of clinical features that include DD, mild to moderate ID, dysmorphic facial features, physical abnormalities, neurological symptoms, and recurrent infections as well as psychiatric traits including behavioral problems, hyperactivity, and attention deficit hyperactivity disorder (106). There have now been some 35 patients characterized with this syndrome, each carrying highly penetrant deletions that range from 0.26 to 3.75 Mb in size (5, 48, 49, 86, 115, 179). The majority of these patients' characterized deletions (24 of 32) have breakpoints that map to five clusters of highly identical SDs within 15q24, implicating NAHR as the underlying mutational mechanism. Assessments of clinical presentations in these patients have revealed that, whereas DD and distinct facial features occur in nearly all cases, presentations of other associated traits tend to be more variable between patients (106, 115). Genotype–phenotype studies comparing the clinical features of patients with overlapping 15q24 deletions provide important insight into potentially disease-relevant genes (115). For example, 29 of the 32 fully characterized 15q24 deletions span a critical 1.2-Mb region (5, 48, 49, 86, 115, 179), strongly suggesting that this interval contains the dosage-sensitive element(s) underlying the cardinal features of the disorder. In contrast, the 3 patients with atypical deletions mapping outside of or only partially overlapping this 1.2-Mb critical region lacked certain core features seen in most other patients, such as cardiac abnormalities and seizures (115).

Genome-wide screens in larger and more comprehensive collections of patients and normal controls have aided in the continued discovery of novel syndromes in ID/DD. This is particularly relevant for very rare disorders, which require large sample sizes to detect recurrence, and for those that show incomplete penetrance and/or variable expressivity (discussed in detail below). Based on an analysis of 15,767 patients with a general diagnosis of ID/DD and 8,329 normal individuals, Cooper et al. (33) recently identified 14 novel loci showing a significant enrichment of CNVs in disease cases compared with controls, with overlapping CNVs of each locus observed in multiple patients. In addition, 3 of these loci had previously been described in single case reports, indicating that these regions are likely associated with genuine syndromes that warrant further investigation and clinical classification. The relatively low frequency of these events also illustrates the increased power of surveys of larger sample sizes in the identification of novel rare pathogenic CNVs. In addition to the 14 putative pathogenic loci, another 1,400 of the 15,767 patients were found to have rearrangements in one of 45 chromosomal regions previously determined to be causative in other known genomic syndromes. Thus, from their data, the authors were able to make comprehensive estimates of prevalence and penetrance for a significant majority of known microdeletion/duplication syndromes, with an estimate that ~14% of ID/DD in their cohort was attributable to pathogenic CNVs greater than 400 kb in size. Notably, partitioning data based on CNV size and phenotypic characteristics revealed that odds ratios increased with the size of pathogenic events, and the burden of larger CNVs was greater in patients with more severe developmental phenotypes (33).

Table 1 Known recurrent microdeletions and microduplications

Chromosomal location	Rearrangement	Associated syndromes and phenotypes	ID[a]	ASD[a]	Epilepsy[a]	Scz[a]	Associated inversion	Reference(s)
1q21.1	Deletion	TAR syndrome						87
1q21.1	Deletion/duplication	Variable ID, cataracts, MCA (deletion); autism (duplication); heart defects (both)	✓	✓		✓	Unknown relationship to deletion/ duplication risk	22, 63, 116, 181
1q22	Deletion	Gaucher disease, lysosomal storage disorder						197
2q11.2[b]	Deletion/duplication	DD, hypotonia, macrocephaly (duplication); ID, congenital heart defect (deletion)						144
2q13	Deletion	Familial juvenile nephronophthisis						148
2q21.1	Deletion	ID/DD, ADHD, epilepsy, behavioral abnormalities	✓	✓	✓	✓		42
3q29	Deletion/duplication	Dysmorphic features, microcephaly (deletion); variable ID (both)	✓			✓	Unknown relationship to deletion/ duplication risk	189
4q35	Deletion	Facioscapulohumeral muscular dystrophy						55, 191
5q35	Deletion/duplication	Sotos syndrome (deletion); 5q35 duplication syndrome (duplication)					Prerequisite for deletion/duplication	174
6p21.32	Deletion	Congenital adrenal hyperplasia III						96
7q11.23	Deletion/duplication	Williams–Beuren syndrome (deletion); 7q11 duplication syndrome (duplication)		✓			Increased risk for deletion/duplication	146
7q11.23 distal	Deletion/duplication	Infantile spasms, ID		✓	✓			139
8p23.1	Deletion/duplication	ID/DD, congenital heart defects, microcephaly, behavior problems					Prerequisite for deletion/duplication	31
8q24.3	Duplication	Glucocorticoid-remediable aldosteronism						100
10q11.21–q11.23	Deletion/duplication	ASD, seizures, MCA, immunodeficiency, encephalopathy, hypotonia (duplication); ID/DD, dysmorphic features (both)	✓		✓			165

10q22–q23	Deletion/duplication	Infantile juvenile polyposis, ID, behavioral abnormalities	✓					10, 118
12q14.2	Deletion	Globozoospermia						51
15q11.2	Deletion/duplication	Variable presentation of ID, behavioral abnormalities, epilepsy	✓	✓	✓	✓		83, 114, 168, 178
15q11–q13	Deletion/duplication	Prader–Willi and Angelman syndromes		✓			Increased risk for deletion/duplication	124
15q13.3	Deletion/duplication	Variable ID, ASD, epilepsy	✓	✓	✓	✓	Unknown relationship to deletion/duplication risk	66, 120, 157, 177
15q24	Deletion/duplication	ID, ASD, dysmorphic features, MCA	✓	✓			Unknown relationship to deletion/duplication risk	115, 155
15q25.2	Deletion	Congenital diaphragmatic hernia, ID, ASD	✓	✓				130, 186
16p11.2	Deletion/duplication	Early-onset obesity/underweight	✓	✓	✓	✓		17, 187
16p12.1	Deletion/duplication	Variable DD, neuropsychiatric disorders	✓				Prerequisite for deletion/duplication	60
16p13.11	Deletion/duplication	Variable ID, ADHD, epilepsy, schizophrenia	✓	✓	✓	✓		39, 65, 69, 114
17p11.2	Deletion/duplication	Smith–Magenis syndrome (deletion); Potocki–Lupski syndrome (duplication)						29
17p11.2–p12	Deletion/duplication	HNPP (deletion); CMT1A (duplication)						26, 27, 105
17q11.2	Deletion/duplication	Neurofibromatosis type 1						44
17q12	Deletion/duplication	Renal cysts and diabetes	✓	✓	✓	✓	Unknown relationship to deletion/duplication risk	111, 123
17q21.31	Deletion/duplication	17q21 microdeletion syndrome (deletion)					Prerequisite for deletion/duplication	88, 155
22q11.2	Deletion/duplication	VCFS, DiGeorge syndrome, schizophrenia (deletion); 22q11 duplication (duplication)	✓			✓		82, 153

(*Continued*)

Table 1 (*Continued*)

Chromosomal location	Rearrangement	Associated syndromes and phenotypes	ID[a]	ASD[a]	Epilepsy[a]	Scz[a]	Associated inversion	Reference(s)
22q11.2 distal	Deletion/duplication	Growth delay, ID, MCA (deletion); variable ID (duplication)	✓					21
22q11.2–q12	Deletion	Cat eye syndrome						110
Xp22.31	Deletion	X-linked ichthyosis						180
Xq28	Deletion	Incontinentia pigmenti						8
Xq28	Deletion	Red-green color blindness						126
Xq28	Deletion/duplication	ID, ASD, dysmorphic features	✓	✓				47
Yq11.23	Deletion	Azoospermia						160
der(8)t(8;12) (p23.1;p13.31)	Translocation	Obesity, DD, ID, seizures, macrocephaly, eczema, dysmorphic features	✓		✓		Unknown relationship to translocation risk	62, 129
der(4)t(4;11) (p16.2;p15.4)	Translocation	Features of Wolf–Hirschhorn syndrome, Beckwith–Wiedemann syndrome, and Russell–Silver syndrome						129
der(X)t(X;Y) (p22.33;p11.2)	Translocation	46,XX maleness					Prerequisite for translocation	125
t(17;22)(q11;q11)	Translocation	Offspring at risk of aneuploidy due to malsegregation of the t(17;22)						94
t(11;22)(q23;q11)	Translocation	Offspring at risk of aneuploidy due to malsegregation of the t(11;22)						46
Xq28	Inversion	Hemophilia A/factor VIII deficiency					Pathogenic inversion	95
Xq28	Inversion	Hunter syndrome/ mucopolysaccharidosis II					Pathogenic inversion	19

Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; CMT1A, Charcot–Marie–Tooth disease type 1A; DD, developmental delay; HNPP, hereditary neuropathy with liability to pressure palsies; ID, intellectual disability; MCA, multiple congenital anomalies; Scz, schizophrenia; TAR, thrombocytopenia absent radius; VCFS, velocardiofacial syndrome.

[a]Nonsyndromic forms.

[b]One patient each reported for deletion and duplication.

Within the past five years, genome-wide CNV studies have also been conducted in patients affected by a variety of other phenotypes. Among these are both traits and disorders that have traditionally been associated with ID/DD, such as congenital anomalies (54, 111), epilepsy (39, 66, 114, 117), and autism (14, 61, 97, 108, 133, 146, 187), as well as other complex neuropsychiatric disorders, such as schizophrenia (70, 83, 123, 124, 170, 184) and attention deficit hyperactivity disorder (50, 190). Not surprisingly, results from these studies have indicated that the impact of large regions of aneuploidy resulting from CNVs extends beyond the scope of ID/DD. In addition, particularly for neurological disorders, strong evidence has emerged in support of more prominent roles for rare and de novo events over more common CNVs.

In autism and schizophrenia, for example, both family-based genome-wide studies and those conducted in case–control cohorts have reported an increased CNV burden in affected individuals. Comparing the rates of CNV formation in 195 simplex and multiplex families with those of 99 control families, Sebat et al. (150) identified a significant enrichment for the occurrence of de novo CNVs in cases, a finding that has since been confirmed by additional studies (97, 108, 133, 146). A recent study in schizophrenia also observed an increase of de novo microdeletions and microduplications in some cases (5.1%) compared with controls (2.2%), and, similar to trends reported in autism (150), the frequency of de novo CNVs was greater in cases with a family history of disease compared with those without such a history (83).

With respect to rare CNVs (population prevalence <0.1%), using a case–control design, Pinto et al. (133) reported a 1.19-fold increase in the number of genic CNVs in autistic cases compared with controls. Notably, a 1.26-fold increase was observed when only deletions were considered, an observation consistent with an idea commonly believed in population studies, namely that deletions are more likely to result in negative phenotypic consequences than are duplications (32, 81). Similar trends have been noted for schizophrenia using case–control cohorts. For example, a survey of >3,000 individuals found that large (size > 100 kb) and rare (frequency < 0.1%) CNVs were enriched 1.15-fold in cases compared with controls. In addition, CNV burden varied depending on event type, size, and frequency and the number of genes included; the greatest difference between cases and controls was observed for deletions greater than 500 kb in size (70).

Enrichments of rare CNVs, both inherited and de novo, have also been observed in epilepsy. For example, Mefford et al. (114) detected rare CNVs in 46 out of 517 epilepsy cases, none of which were identified in a control cohort of 2,493 individuals. In addition, deletions comprised the majority (69%) of the 51 characterized events (114), also consistent with results noted above for autism (133). Thus, CNVs have a strong influence on risk for a wide variety of neurodevelopmental disorders.

The ability to assay large cohorts with different phenotypes has also been important for understanding the diverse risk profile associated with some recurrent CNVs. Collectively, findings from CNV studies conducted in the diseases noted above, including in ID/DD, have revealed that in many instances pathogenic CNVs occurring within the same microdeletion/duplication hot spot are associated with several different disorders. For example, of the five regions initially characterized by Sharp et al. (155), rearrangements in 1q21.1, 15q13, 15q24, and 17q12 have now also been identified in patients exhibiting various congenital defects, autism, schizophrenia, or epilepsy (39, 54, 66, 70, 123, 150, 168), suggesting that the underlying causes of these disorders may in some cases involve common developmental pathways.

DEFINING THE PATHOGENICITY OF COPY-NUMBER VARIANTS

Many criteria can be used to help interpret the clinical relevance of a CNV, including inheritance, size, type, and gene content (119). The inheritance pattern of a CNV, when accompanied by clinical

and family history information, can be useful. De novo CNVs are more likely than inherited CNVs to be pathogenic, especially for severe disorders, though there are certainly exceptions. Conversely, as illustrated by the example of the 1q21.1 syndrome and others, inherited CNVs may cause a range of severity and presentation of neurodevelopmental disorders and can clearly be pathogenic, even when present in a phenotypically normal parent (22, 70, 112).

In the clinical setting, it may at times be difficult to test both parents for financial or social reasons, so other criteria must be considered. CNV size and type (deletion or duplication) are often used as guides to interpret pathogenicity. Large CNVs are more likely than small CNVs to cause disease (41). In part, this is because larger CNVs generally encompass more genes, with a concomitant increase in the probability of altering a dosage-sensitive element. Likewise, deletions result in haploinsufficiency, the consequences of which are known for some genes. Duplications are more difficult to interpret, and in the clinic, a larger minimum size threshold is often employed for duplications than for deletions. Gene content is also a consideration. CNVs that contain many genes or known disease genes are more likely to be pathogenic than those that contain few genes or genes of uncertain function. CNVs within "gene deserts" are particularly difficult to interpret, though as we learn more about regulatory regions within noncoding DNA (52), interpretation will become easier.

All of these criteria are probabilistic in nature, and there are documented instances in which, for example, small noncoding duplications cause genetic syndromes (37). Despite these exceptions, with careful consideration, likely determinations of pathogenicity can be made in most cases.

CLINICAL TESTING FOR COPY-NUMBER VARIANTS

Since the introduction of chromosome microarrays, several large studies have addressed the overall importance of copy-number changes in the diagnostic workups for DD, ID, and autism (119, 145, 152). Today, there is no question that chromosome microarrays have a far higher diagnostic yield than the standard karyotype, indicating that microarray analysis should replace karyotyping as the method of choice for aneuploidy screening. In 2010, a review of 33 published studies involving 21,698 patients with DD, congenital anomalies, or autism who were tested for CNVs with chromosome microarrays found a diagnostic yield of 15–20% across all studies compared with ~3% for the standard G-banded karyotype (119). Other studies of large patient cohorts screened by microarray have similarly concluded that ~14% of DD cases can be explained by a detectable CNV (33).

Importantly, in addition to providing a diagnosis, chromosome microarray testing can lead to changes in medical management recommendations. In a retrospective review of 1,792 patients with ID/DD, ASD, and/or congenital anomalies who had chromosome microarray testing, individuals who had a positive diagnosis had a higher rate of recommendation for clinical action than those who had an uncertain result (54% versus 34%, $p = 0.01$) (35). More recently, Riggs et al. (142) identified at least 146 disorders that can be diagnosed by chromosome microarray testing and that have published literature supporting specific clinical management implications. Approximately 7% of cases that undergo chromosome microarray testing are diagnosed with one of these conditions. The authors concluded that chromosome microarray testing impacts clinical management at a rate similar to that of other genetic tests for which insurance coverage is often more readily approved. In summary, undiagnosed individuals with ID, DD, ASD, or congenital anomalies should be referred for chromosome microarray testing, which may lead to diagnosis and management recommendations.

VARIABLE EXPRESSIVITY OF MICRODELETION AND MICRODUPLICATION SYNDROMES

Comparison of CNV findings across studies reveals several recurrent rearrangements that are associated with a wide range and severity of phenotypes (113) (**Table 1**). Rearrangements that fall into this category include deletions and duplications of 1q21.1, 16p11.2, 16p13.11, and 15q13.3. For most of these regions, recurrent rearrangements have been associated with risk for some or all of several neurodevelopmental disorders, including ID, ASD, epilepsy, attention deficit hyperactivity disorder, and schizophrenia. Not only is each of these disorders distinct in its presentation, but the severity of each phenotype associated with these rearrangements can also vary significantly.

The factors underlying such extreme clinical variability are still poorly understood. Several possible explanations for different clinical presentations in carriers of the same deletion or duplication have been proposed (154). Differences in genetic background—that is, the milieu of sequence and CNVs present within the genomes of specific individuals—could contribute, and there is now evidence showing that both common and rare variants can play a role in modifying phenotypic outcome. Epigenetic differences are another possible factor, and phenomena such as imprinting may also play a role. Finally, environmental or sporadic effects might interact to alter the risk of abnormalities associated with an aneuploidy event.

One way in which the genetic basis of variable phenotypes in patients with the same recurrent rearrangement is explored is by looking for sequence variants in candidate genes within the deleted region. This is a reasonable strategy based on the hypothesis that a "second hit" in a gene that is already reduced to a single copy following a deletion event on one homolog may worsen the phenotype in a carrier. An example comes from the recurrent 22q11 microdeletion, which is associated with several variable phenotypes, including schizophrenia. Exploration of genetic variants within the *COMT* gene identified a functional polymorphism, the presence of which appears to increase the risk of schizophrenia in carriers of the 22q11 deletion (13). *COMT* encodes catechol-*O*-methyltransferase, a reasonable candidate for the source of the schizophrenia phenotype. Similarly, mutations in *SNAP29* are responsible for atypical phenotypes in some patients with 22q11 deletions (109).

Targeted studies have also been performed to explore phenotypic variability in some of the recently described microdeletion syndromes, such as in the 1q21.1 deletion syndrome, which is associated with a wide range of phenotypic severity. To date, there is no evidence that differences in deletion breakpoints, in the methylation of the 1q21 region, or in the coding sequence of at least two genes within the region are responsible for phenotypic differences (116). There is also no evidence for the role of additional pathogenic CNVs in this small set of families (63). Expression studies using lymphoblast cell lines in a subset of 1q21 patients showed that most of the unique genes within the 1q21 region (*PRKAB2*, *CHD1L*, *BCL9*, *ACP6*, *GPR89A*, and *PDIA3P*) correlate with the copy number of the region, but there were no differences among affected individuals to explain the variable phenotypes (63). Similarly, deletions of 16p13.11 are associated with ID, ASD, schizophrenia, and epilepsy. Again, expression studies showed that expression levels were decreased for genes within the region in seven patients with the 16p13.11 deletion and epilepsy, with no significant differences among patients. Sequence analysis of all genes within the 16p13.11 deletion region resulted in no evidence for secondary mutations within that region (65).

Distinct from the more distal 1q21.1 deletions mentioned above, deletions of proximal 1q21.1 were recently identified in patients with thrombocytopenia absent radius (TAR) syndrome (87). Interestingly, individuals with TAR syndrome may carry either an inherited or de novo deletion, and in many cases an unaffected parent also carries the deletion. Because parent-to-child transmission is rare, the disorder was thought to be due to either autosomal recessive or biallelic

inheritance. To look for potential second hits that could act as modifiers of penetrance in 1q21.1 deletion carriers, Albers et al. (3) performed exome sequencing in five affected deletion carriers. In four of the five cases, they identified a low-frequency SNP within the 5′ untranslated region of *RBM8A*, a gene within the TAR deletion region, with an intronic variant identified in the fifth case. Functional assays showed that both variants significantly reduced *RMB8A* promoter activity, indicating that TAR syndrome results from an insufficiency of RMB8A. Investigation of 25 additional patients with TAR syndrome who inherited a 1q21.1 deletion from a normal parent revealed that a second *RBM8A* SNP was inherited from the other parent. This confirmed that compound inheritance of a deletion together with a low-frequency noncoding SNP in *RBM8A* causes TAR syndrome, likely by reducing protein levels below a critical threshold in certain tissues.

Taking advantage of the fact that microarrays provide data on genome-wide CNVs, Girirajan et al. (59) investigated the hypothesis that the co-occurrence of additional genetic variants can contribute to the phenotypic outcome of genomic disorders. In 2,312 children with a disease-associated CNV, 10% were found to carry at least one additional large CNV elsewhere in their genome. Interestingly, these second hits were more likely to occur in patients who harbored a CNV that is associated with a range of neurodevelopmental disorders, including 1q21.1 deletions and duplications. This study therefore suggests that the overall mutational burden, detected here in the form of additional CNVs elsewhere in the genome, likely influences the phenotypic outcome and penetrance of microdeletion/duplication events.

Exome sequencing data taken from patients with ASD also suggest that multiple mutations may affect phenotypic outcome. Moreover, O'Roak et al. (128) observed that patients carrying both a rare or de novo CNV and at least one other de novo damaging point mutation scored significantly lower in nonverbal IQ than did individuals with no events. In previously published series of patients with 1q21 rearrangements, it was already clear that 10–15% of cases have additional CNVs. However, a comprehensive analysis of second hits in a large series of patients has not yet been performed.

Studies of mice with haploinsufficiency for genes with roles in vertebral development showed a strong interaction between the penetrance of the accompanying scoliosis phenotype and maternal environmental factors. Using a mouse model, Sparrow et al. (163) showed that exposure of the developing embryo to hypoxic conditions for even a few hours—a relatively common event that can occur during pregnancy—resulted in a significant increase in the rate of scoliosis in the offspring via a disruption of FGF signaling (163). Although scoliosis is not a common finding in most genomic disorders, this study demonstrates an important principle: The penetrance of genetic mutations can be modified by interaction with common environmental factors, potentially explaining the phenotypic heterogeneity associated with many recurrent microdeletion/duplication syndromes.

MECHANISMS OF RECURRENT COPY-NUMBER VARIANTS

The mechanisms underlying the generation of structural variations in mammalian genomes can be broadly divided into two main categories. In the vast majority of cases, truly recurrent CNVs (i.e., those in which the breakpoints in unrelated individuals recur de novo in local hot-spot regions) are now known to occur as a result of recombination-based mechanisms, specifically NAHR. Here, deletion, duplication, inversion, or translocation events are generally mediated by large blocks of flanking homologous sequences that typically share 95–99% sequence identity of over tens to hundreds of kilobases, allowing illegitimate recombination to occur. NAHR appears to occur overwhelmingly during meiosis, although in some rare cases mitotic NAHR rearrangements are observed, potentially leading to somatic mosaicism (77, 99, 199). In contrast, sporadic or nonrecurrent CNVs (i.e., those in which, even where there may be a common overlapping region

of aneuploidy between two unrelated carriers, different breakpoints are observed in each instance) result from errors during the repair of double-strand chromosomal breaks and are mediated by non-homology-based mechanisms. Several such mechanisms have been proposed, including nonhomologous end joining, FoSTeS, and microhomology-mediated break-induced replication (64). Studies of the rates of de novo rearrangement in sperm at several genomic disorder loci have revealed marked variation in the frequency of NAHR at different loci (175) and have shown that rates of NAHR can vary among different individuals at any one locus (16, 122). For a given microdeletion/duplication syndrome, the rate of formation of de novo rearrangements by NAHR is directly related to both the length and level of sequence identity between the flanking SDs that catalyze the rearrangement, and this rate is inversely related to their separation (102).

In addition to these fundamental mechanisms, studies of commonly identified CNVs have provided additional insights into some of the factors contributing to the generation of structural rearrangements. For example, analysis of the breakpoint regions of thousands of CNVs identified in the normal population has recognized enrichments for secondary structures such as G-quadruplexes and cruciforms, recombination motifs, *Alu* signal recognition particle motifs, and microsatellites (32). These observations indicate that local sequence features contribute toward CNV formation, and the generation of even nonrecurrent CNVs is not an entirely random process. Indeed, a small number of recurrent rearrangement syndromes are thought to be attributable to the presence of breakpoint motifs that form inherently unstable secondary structures. Two recurrent translocations, t(11;22)(q23;q11) and t(17;22)(q11;q11), have been shown to be catalyzed by large AT-rich repeats that are thought to form cruciform structures that mediate double-strand breaks and subsequent translocation (46, 93, 94). Similarly, Béna et al. (15) described a recurrent microdeletion of 14q32.2 that is catalyzed by large $(TGG)_n$ tandem repeats that are predicted to have extremely strong secondary structure (15).

In addition to these sequence-based features, it is now recognized that the relative stage at which a genomic region replicates its DNA during cell division influences the formation of CNVs. By comparing different classes of CNV with genome-wide maps of replication timing, Koren et al. (89) observed that human CNVs generated by NAHR-mediated events were enriched >4-fold in early-replicating regions, whereas events attributable to nonhomologous end joining were enriched ~2-fold in late-replicating regions. Data from the study of recurrent translocations that occur somatically in cancer have shed light on additional genomic features that can influence rates of chromosomal rearrangement. Burrow et al. (23) showed that the majority of such translocations involve regions known to be common fragile sites, representing regions of frequent chromosomal breakage. Furthermore, spatial organization within the nucleus also appears to influence rates of mitotic rearrangement; genomic regions that show stronger interactions undergo elevated rates of translocation (53, 195).

There are several examples of structural variations at genomic disorder loci that act to modify the rate at which subsequent deletions/duplications occur. Cuscó et al. (36) described a deletion variant in the flanking SDs that increases susceptibility to the recurrent 1.5-Mb deletion underlying Williams–Beuren syndrome (36). Many genomic disorder loci are also now known to be sites of common inversion in the general population (**Table 1**). A theme common to most such cases is that, although the inversions themselves are apparently benign variants, one specific orientation predisposes the region to further rearrangement, presumably creating a local architecture that increases the probability of NAHR by aligning SDs into a direct orientation (6, 7, 57, 88). In some cases, such as at 7q11.23 and 15q11–q13, the presence of an inversion alters the frequency of subsequent rearrangement, whereas for other loci the presence of an inversion appears to be a prerequisite for deletion/duplication (154). For example, 17q21.31 microdeletions (88, 155, 159) are strongly associated with a haplotype-specific common inversion polymorphism (88). A detailed

screening of 22 patients with 17q21.31 microdeletions showed that all these deletions arose in a parent who was a carrier of the inversion in either a homozygous or heterozygous form (88). Interestingly, this inversion haplotype occurs at a relatively high frequency in Europeans (~20%) compared with Asians (1%) and Africans (6%) (167), and, not surprisingly, the 17q21.31 deletion syndrome occurs at a much higher frequency in European populations. In fact, an estimated 97% of all reported cases are in individuals of European descent (33). For several other genomic disorder loci, although common inversions have been recognized, whether these have any effect on the rate of subsequent deletion/duplication in carrier individuals is not known. Sharp (154) also previously proposed an alternative mechanism in which the presence of a heterozygous inversion at a locus could be mutagenic by suppressing meiotic synapsis, leading to the formation of an unstable asynaptic bubble. Although this hypothesis has not been formally tested, detailed sequencing studies of several hundred CNVs have since revealed that ~5% of deletions do indeed have inverted sequences around their breakpoints (32). Although this observation is also compatible with some CNVs occurring via more complex underlying rearrangements involving simultaneous deletion and inversion of a region, one interpretation of these data is that these inversions were preexisting and rendered the local regions prone to further rearrangements.

In addition to these local variants, *trans*-acting factors have been shown to influence rates of CNV formation. The best described of these is PRDM9, a zinc finger protein that specifies the location of meiotic crossovers in mammals (12). *PRDM9* contains a zinc finger repeat that codes for its DNA-binding domain, and this repeat is highly polymorphic in humans. Different *PRDM9* alleles are associated with significantly altered patterns of meiotic recombination across the genome, and Berg et al. (16) showed that an individual's *PRDM9* genotype can alter the usage of a pair of recombination hot spots in 17p11.2, leading to interindividual variation in the risk of producing offspring with CMT1A or HNPP. Studies of Williams–Beuren syndrome trios have also shown a significant increase in the frequency of uncommon *PRDM9* alleles in parents who transmit de novo 7q11.23 deletions to their offspring, suggesting that allelic variation in *PRDM9* is associated with alterations in the rate of NAHR at this locus (20).

Even though these known factors can result in modest increases in the frequency of certain genomic disorders in some families, it is generally presumed that all cases of pathogenic de novo microdeletion/duplication result from sporadic NAHR events that are mediated purely by the underlying genomic architecture. However, could it be that some cases of de novo microdeletion are not due simply to chance rearrangement? Specifically, what if some individuals are genetically predisposed to have children with genomic disorders? Relevant to this question are studies showing that, in yeast, there are numerous genes that act to suppress both spontaneous chromosomal rearrangements and recurrent rearrangements attributable to NAHR. Many of these CNV suppressor genes are involved in pathways such as double-strand break repair, DNA replication stress checkpoint signaling, mismatch repair, and chromatin modification. Interestingly, mutation of these genes can lead to massively elevated rates of spontaneous chromosomal rearrangement in the yeast genome that can be up to 1,000-fold higher than background mutation rates (28, 138, 162). It therefore seems plausible that if defects in similar pathways in humans were also associated with increased rates of meiotic rearrangement, then the offspring of any such individuals might have a significantly increased probability of inheriting a de novo aneuploidy event. We suggest that this represents an interesting hypothesis that warrants future exploration.

THE IMPACT OF TECHNOLOGY

Major strides in patient screening and diagnosis have been made over the past decade with the advent of genome-wide technologies (113). Chromosome microarray technologies were rapidly

introduced into the clinical diagnostic laboratory after they appeared in the research arena. Although aCGH arrays were the first chromosome microarrays used in the clinic, within a short period of time, SNP microarrays were also utilized. Both platforms are now routinely used for clinical diagnosis, though there are a wide variety of specific array designs that have evolved over the past several years. Notably, early aCGH testing used targeted arrays with a higher density of probes in genomic regions known to be important for disease (e.g., 15q11–q13 for Prader–Willi and Angelman syndromes and subtelomeric regions). These were generally combined with backbone probes spaced evenly, but at lower density, throughout the genome to facilitate the detection of sporadic large deletions and duplications. As a result, the majority of CNVs that were detected by these platforms were pathogenic, because they were either within clinically relevant regions or large in size. With the evolution of the technology, most chromosome microarrays in use now have a large number of probes (often greater than 1 million) that tile across the human genome, allowing the detection of very small CNVs (size < 10 kb). These dense genome-wide arrays as well as more refined targeted arrays have greatly enhanced the ability to detect CNVs.

For example, Girirajan et al. (58) recently conducted a large CNV association study in a cohort of 2,588 ASD cases using a custom targeted array with a probe density of one probe per 50–1,000 base pairs. Building on a previous strategy that targeted regions of the genome flanked by pairs of large SDs, probes on this array were preferentially placed in 1,367 gene-containing rearrangement hot spots, including 1,247 regions characterized by shorter flanking repetitive sequences, such as *Alu* and other repeats and short SDs with >100 base pairs of identical sequence. Approximately 10% of these regions contained CNVs, 86% of which were inherited. Although none of these variants were found to be significantly enriched in ASD cases compared with controls, several showed evidence of shared breakpoints between unrelated individuals, indicating that there are potentially many novel recurrent microdeletion/duplication regions in the genome that are below the resolution of older microarray platforms.

Adding to the wealth of data generated by array-based methods, the more recent development and application of novel sequencing methods and associated analysis pipelines have continued to pave the way for improvements in the genetic detection and characterization of causative variants underlying microdeletion/duplication syndromes and related disorders. A key prerequisite for building technologies for variant detection is the availability of a reliable reference genome, which can affect both the development and interpretation of genome-wide assays (143); this is particularly true for structurally complex regions of the genome for which complete assemblies are lacking. Indeed, in many instances, pathogenic CNVs map to regions with known assembly gaps (72). This notion has motivated attempts in the field to construct more comprehensive maps of structural variation and improve existing reference assemblies, both of which have contributed to significant advances in CNV detection and characterization. An important contribution toward this aim evolved from the development of methods that enable the discovery of structural variants from the end sequencing and mapping of large-insert clones (78–80, 176). The power of this approach is that, following the identification of clones harboring structural variants, these loci can be fully sequenced and assembled to provide base-pair characterization of novel sequence not yet represented in the genome reference assembly.

Additional improvements to maps of structural variation have come with the increased use of next-generation sequencing technologies (e.g., Illumina, SOLiD, and 454 sequencing). Owing to the relatively low cost of these methods, at least when compared with Sanger sequencing, an unprecedented amount of genome sequence data has been generated in the past few years, including high-coverage assemblies of individual genomes (84, 149, 185, 188). The 1000 Genomes Project, one of the largest and most comprehensive sequencing efforts currently under way, is tasked with cataloging standing genome-wide genetic variation in a broad range of human populations

(1, 2, 121). These large sequencing projects have sparked parallel efforts to develop effective analytical methods that utilize different aspects of these data for the discovery and characterization of structural genome variants, including not only copy-number changes that are detectable by microarrays but also balanced events such as inversions and translocations that have been invisible to array-based methods. These approaches include de novo sequence assembly, paired-end read mapping, split-read mapping, and read-depth analysis (see **Figure 2**). Not unexpectedly,

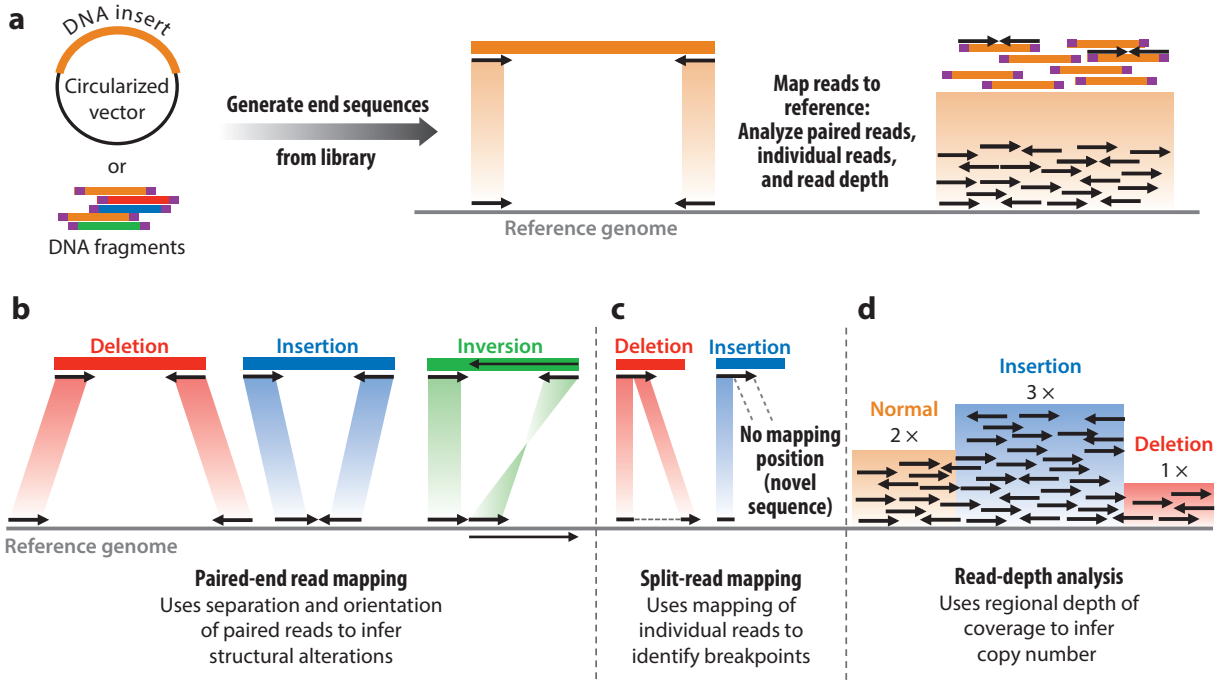


Figure 2

Analysis methods for the identification and characterization of genomic structural variants from massively parallel sequencing data. (*a*) Libraries are constructed from individual DNA samples, and the ends of the cloned DNA inserts/adapter-ligated fragments are sequenced using either traditional Sanger sequencing or next-generation methods. End sequences can then be mapped to the human reference genome, providing mapping data that can be used to infer the position, size, and type of structural variants carried by the individual used to generate the sequencing library with respect to the reference assembly. Several commonly used methods include paired-end read mapping, split-read mapping, and read-depth analysis (panels *b*, *c*, and *d*, respectively). (*b*) In paired-end read mapping, paired reads derived from a single DNA molecule that show relative discordancy in their mapping (in terms of relative separation or orientation of the read pairs) allow the detection of deletions, insertions, and inversions. The maximum size and resolution of the events detected are dictated by the size and distribution of the library that is sequenced. If appropriate mapping parameters are used, paired-end reads located on separate chromosomes can also be used to infer translocation events (not shown). (*c*) Split-read mapping is used to identify instances in which portions of a single read align discontiguously to the reference genome; this approach is useful for directly mapping and characterizing the exact breakpoints of insertion, deletion, and inversion events. The power of this approach is dictated largely by read length. (*d*) Read-depth analysis utilizes the relative sequencing coverage across the genome from a population of individuals to identify regions with significantly altered read depth among individuals, highlighting loci that harbor deletions or duplications. The resolution of this method is strongly influenced by depth of coverage. Importantly, both paired-end and split-read mapping methods often perform poorly where the breakpoints are embedded in segmental duplications owing to the potential for mismapping of reads in paralogous sequences. In such cases, the read-depth approach is often more tractable. Targeted sequencing and assembly in regions harboring novel structural events is another powerful method in structurally complex regions (not shown); de novo assemblies can allow for the delineation of complex event breakpoints as well as complete descriptions at nucleotide resolution of insertion and duplication sequences not represented in the reference genome.

family-based and population-scale screens using these methods have resulted in descriptions of thousands of novel structural variants (1, 2, 67, 79, 121, 172, 193, 194, 196). In addition, the directed application of these approaches has already proven useful in the context of microdeletion/duplication syndromes. For example, recent efforts in the 17q21.31 microdeletion region utilized a suite of methods—including custom microarrays, SNP imputation, large-insert clone mapping/sequencing/assembly, and next-generation sequencing analysis—to construct better haplotype maps of the locus, allowing refined characterization of breakpoints in microdeletion carriers and yielding insights into their underlying mechanisms, the genes impacted, and the local architectures that predispose to the occurrence of these deletions (18, 72, 169).

Methods and analysis pipelines centered on gleaning CNV information from whole-exome sequencing data are also beginning to emerge (56, 91, 98, 134, 147). Exome-based CNV detection methods build on read-depth approaches, such as those mentioned above for whole-genome data. Comparisons made between these methods have revealed some notable discrepancies with respect to both the total number and rate of de novo calls made (56) and in some cases have revealed relatively low concordance rates with high-confidence calls from microarrays, thus indicating that further technological developments are needed in this area (40). Nonetheless, several methods have been shown to have reasonable sensitivity (>75%) when there is sufficient overlap between array probes and exome capture targets (40, 56, 91), and similar results have been observed using whole-genome sequencing calls for comparison (91). Importantly, these exome-based methods are also able to outperform SNP arrays for the detection of smaller CNVs (90, 91). These methods have already been applied to two ASD cohorts (90, 136), demonstrating their power in the research laboratory.

Taken together, the advent and application of exome and whole-genome sequencing highlight the obvious potential benefits to clinical medicine, offering potentially novel approaches complementary to commonly used karyotyping or chromosome array–based methods. The strength of these methods is that they allow parallel assessments of a broad range of variants, from single-nucleotide mutations to large structural variants, including smaller CNVs and translocations that are often missed by standard chromosome arrays. Of course, as with array-based technologies, MPS approaches also come with considerations, and certain methods carry advantages or disadvantages depending on the exact question at hand. For example, exome sequencing provides targeted data for only coding portions of the genome at an increased depth of coverage and at lower cost, enabling researchers to interrogate larger cohorts with increased power. However, the obvious downfall is that, in contrast to whole-genome sequencing, much of the genome is left uninterrogated, and the nature of the data in most cases does not allow for the specific ascertainment of variant characteristics, such as total size or event breakpoint analysis. Thus, future prospects, especially as sequencing costs decrease, may well place more emphasis on whole-genome methods for patient screening.

The utility of whole-genome next-generation sequencing for the detection of lesions underlying genomic disorders is already being demonstrated, including in the introduction of novel noninvasive methods for prenatal screening (74, 85, 132, 164). For example, an approach developed by Srinivasan et al. (164) using cell-free maternal plasma DNA was able to identify a variety of pathogenic lesions (deletions, duplications, and translocations), including a 300-kb microdeletion.

THE EVOLUTIONARY HISTORY OF RECURRENT GENOMIC DISORDERS

Comparative studies of different human populations and closely related primate species have revealed that several loci associated with genomic disorders show evidence of unusual evolutionary pressures. In particular, there are several gene families that are known to have undergone rapid

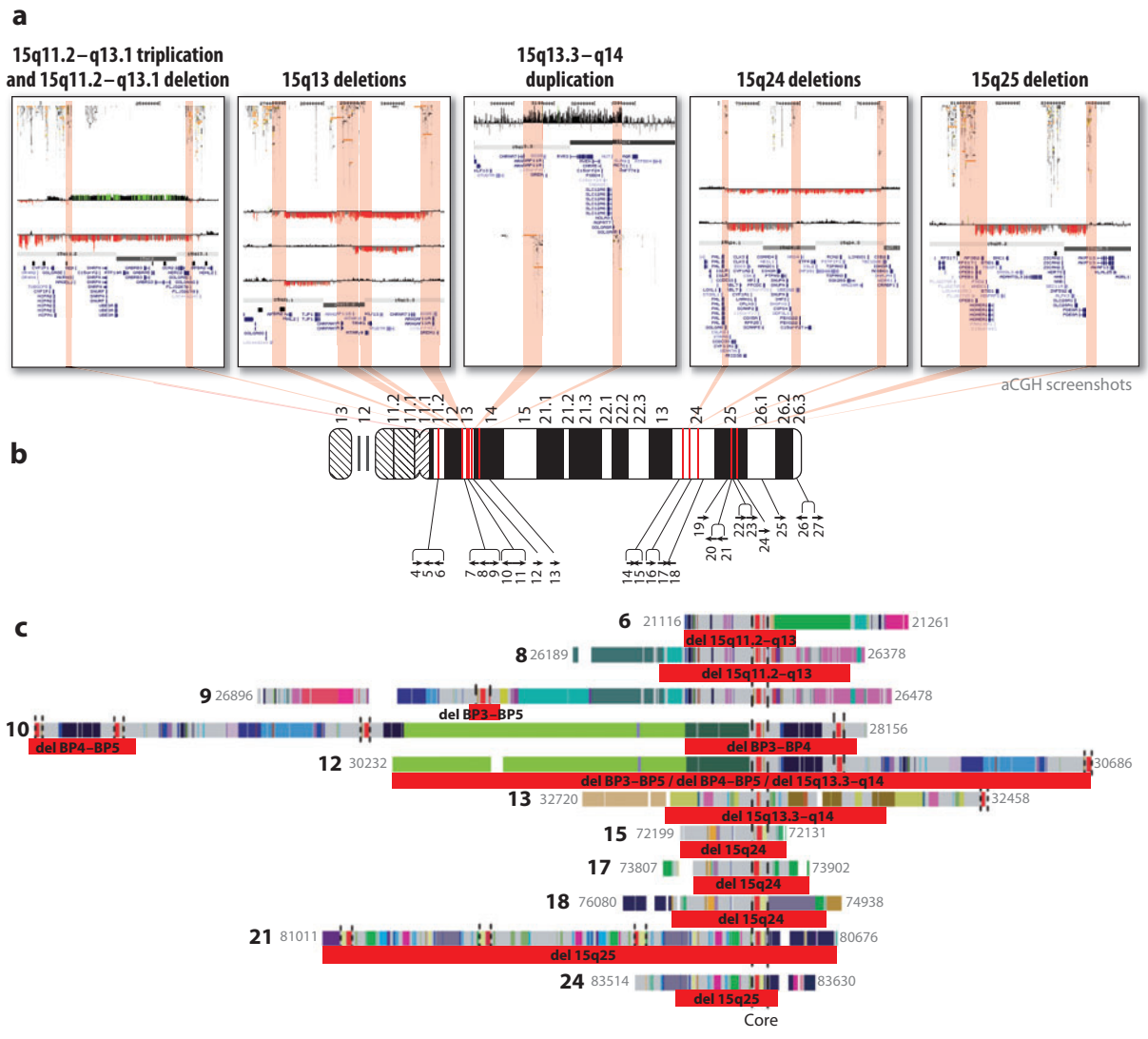
expansion and coding sequence evolution during recent primate evolution and are also associated with recurrent genomic disorders in humans.

Detailed genome-wide analysis of the evolutionary history of SDs shows that many appear to have proliferated in recent human and primate evolution, at the time of the African great ape ancestor, via expansion of key sequences termed core duplicons (75, 107). In many cases, these core duplicons contain transcriptionally active fragments that presumably drive the proliferation of these sequences, and the coding regions of some of these transcripts also show evidence for positive selection driving rapid evolution at the amino acid level (76). Duplication of these cores can often include flanking sequences that hitchhike with them, producing chromosomal regions containing complex mosaics of SD clusters comprising multiple copies of a particular core duplicon (75). This creates a particular pattern in which older duplications are surrounded by younger duplications (107).

Intriguingly, there are several known instances where these core duplicons are located precisely at the breakpoints of recurrent genomic disorders (**Figure 3**). These include gene families such as *NBPF*, which shows the most extreme increase in human-specific copy number of any gene identified to date (135), and *NPIP*, which both has expanded in copy number specifically in the great ape lineage and shows one of the highest levels of amino acid replacement of any human gene (76). Perhaps the most striking example of this phenomenon is provided by the *GOLGA* gene family, which contains several dozen copies scattered over human chromosome 15. Microarray studies of many different chromosome 15 rearrangements, including several recurrent microdeletion/duplication syndromes and more complex rearrangements such as inverted duplications and triplications of chromosome 15, have shown that the breakpoints of all of these appear to coincide precisely with the location of a duplication family containing the *GOLGA* gene (**Figure 3**). The recent proliferation of these highly duplicated gene families therefore seems to have been the key driver in creating local architectures that predispose our genome to recurrent pathogenic rearrangements. Intriguingly, although their evolutionary history strongly suggests that all of these genes have undergone strong positive selection in the primate lineage, and therefore presumably

Figure 3

Gene families that have undergone rapid expansion during recent primate evolution that are associated with several recurrent genomic disorders in humans. (*a*) Studies of multiple chromosome 15 rearrangements by array comparative genomic hybridization (aCGH) show that the breakpoints of these rearrangements coincide with the location of a duplication family containing the *GOLGA* gene. These include both recognized recurrent genomic disorders and rarer rearrangements, including (*left to right*) a triplication of 15q11.2–q13.1 (157), a deletion of 15q11.2–q13.1 associated with Angelman syndrome (157), BP3–BP5 deletions of 15q13, a duplication of 15q13.3–q14 associated with epilepsy, deletions of 15q24 (158), and a deletion of 15q25 associated with congenital diaphragmatic hernia (111). In each image, the locations of duplication blocks containing the *GOLGA* gene (75) are indicated by red shaded regions. Tracks show segmental duplications, cytogenetic bands, assembly gaps, and RefSeq genes. (*b*) This panel shows the locations of 27 assembled *GOLGA*-containing duplication blocks on chromosome 15. Each block is indicated by a black arrow (75), numbered according to its genomic location along the chromosome. Those that coincide with the breakpoints of deletion/duplication events are highlighted (*red bars*). (*c*) *GOLGA* duplication blocks coincide with sites of rearrangement breakpoints on chromosome 15. Red bars below each duplication block indicate the interval in which rearrangement breakpoints occur. Although the presence of structural polymorphisms and cross-hybridization between paralogous sequences makes it difficult to precisely determine the breakpoints by aCGH, in every case data showed that the intervals in which the breakpoints occur overlap a *GOLGA* core. Blocks are numbered, with colored bars denoting the ancestral chromosomal origin of each subelement (75). The core element, which contains the *GOLGA* gene and is shared by all duplication blocks, is highlighted by vertical dashed lines. Note that some blocks contain multiple *GOLGA* sequences. (*d*) At least four different gene families, all of which have undergone rapid amplification of copy number in the past 20–30 million years of primate evolution, are associated with the breakpoints of recurrent genomic disorders in the human genome. This includes *NBPF*, which shows the most extreme increase in human-specific copy number of any gene identified to date (135), and *NPIP*, which both has expanded in copy number specifically in the great ape lineage and shows one of the highest levels of amino acid replacement of any human gene (76). Abbreviation: TAR, thrombocytopenia absent radius. Panels *a–c* have been adapted in part from a figure first published by the Nature Publishing Group (157).



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Gene	Estimated copy number in human genome	Evolutionary characteristics	Chromosomal location	Associated genomic disorders
NBPF	40–60	Rapidly amplified copy number in primates, positively selected at the amino acid level	1q21.1	Deletion/duplication of 1q21.1, TAR syndrome
GOLGA	35	Rapidly amplified copy number in primates	15q	Prader–Willi and Angelman syndromes, deletion/duplication of 15q13, deletion of 15q24, other 15q rearrangements
NPIP	20–30	Rapidly amplified copy number in primates, positively selected at the amino acid level	16p	Deletion/duplication of 16p11.2, deletion/duplication of 16p13.11
TBC1D3	25	Rapidly amplified copy number in primates	17q12	Deletion/duplication of 17q12

have important phenotypic consequences, almost nothing is known about their function. The high frequency of genomic disorders observed in humans can be regarded as a negative side effect resulting from the rapid expansion of these paralogous gene families.

A particularly good example for recurrence of architectures is the 17q21.31 region, which contains an alternative haplotype in humans that includes a large inversion encompassing the recurrent microdeletion region. The inverted H2 haplotype occurs almost exclusively in northern European populations, and population analysis suggests that it has undergone recent positive selection, potentially owing to an association with increased fecundity in carrier individuals (167). Detailed analysis showed that the inverted H2 haplotype represents the ancestral configuration, but, remarkably, this region is highly mutagenic and has undergone multiple inversions at different times during the past ~10 million years, such that it is now polymorphic in both humans and chimpanzees (198).

Despite these rapid changes in the SD architecture of primate genomes, consistent with theoretical predictions, it has recently been demonstrated that genomic disorders are not unique to humans. The advent of whole-genome studies of primates has for the first time identified a chimpanzee with a microdeletion syndrome. Susie, a female chimpanzee from the Biomedical Primate Research Centre, showed abnormal behavioral and phenotype characteristics but had never been formally diagnosed with a specific disease. However, a screen for CNVs in a large panel of 80 great apes (137) found a 1.7-Mb deletion of 17p11.2 (including the *RAI1* gene) present in Susie, corresponding to the region responsible for Smith–Magenis syndrome in humans. This syndrome is characterized by some of the same phenotypes observed in Susie, including aggressiveness, obesity, and renal problems. Surprisingly, the chimpanzee presented a more complex architecture in the region and different deletion breakpoints compared with the human counterpart, suggesting that different duplication blocks that have expanded exclusively in the *Pan* lineage likely catalyze NAHR at this locus (171).

FUTURE PERSPECTIVES

Chromosome microarray testing is a first-line test in the clinic for the diagnosis of patients with ID/DD, ASD, or multiple anomalies, and it has largely replaced traditional karyotype analysis (119). As costs continue to fall and analytical methods evolve, MPS technologies are now poised to replace chromosome microarrays in the near future. Indeed, sequencing-based approaches are already being used in the clinic for noninvasive prenatal diagnosis of fetal aneuploidy (74, 85, 132, 164). Over the past decade, the introduction of first microarrays (40) and then exome sequencing (90, 136) led to marked increases in the proportion of cases of presumptive genetic disease for which an underlying pathogenic mutation can be identified. However, given (*a*) the limited resolution of many array platforms and (*b*) that exome sequencing effectively assays only a small fraction of the genome, we anticipate that the introduction of whole-genome sequencing in the clinic will lead to further improvements in patient diagnosis.

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