

## On the spot: very local chromosomal rearrangements

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### Abstract

Over the last decade, the detection of chromosomal abnormalities has shifted from conventional karyotyping under a light microscope to molecular detection using microarrays. The latter technology identified copy number variation as a major source of variation in the human genome; moreover, copy number variants were found responsible for 10-20% of cases of intellectual disability. Recent technological advances in microarray technology have also enabled the detection of very small local chromosomal rearrangements, sometimes affecting the function of only a single gene. Here, we illustrate how high resolution microarray analysis has led to increased insights into the contribution of specific genes in disease.

### Introduction: the detection of chromosomal abnormalities

Karyotyping, a technique to visualize banded chromosomes under a light microscope, allows for the genome-wide detection of chromosomal rearrangements with a resolution up to 5 Mb, roughly corresponding to the size of a single chromosome band. This technique led to the discovery of aneuploidies, such as Down's syndrome, caused by an additional chromosome 21, as well as large structural variants, including interchromosomal translocations. The introduction of targeted methods, such as fluorescence in situ hybridization (FISH) and multiplex ligation-dependent probe amplification (MLPA), enabled the detection of smaller rearrangements with a resolution up to 150 kb -100 bp in selected regions of the genome. More recently, the development of microarrays has enabled the detection of small aberrations on a genome-wide scale [1-3]. The use of genome-wide arrays in populations of healthy controls led to the discovery of copy number variation. Copy number variants are defined as deletions or duplications not detectable by conventional karyotyping, with a size of at least 1,000 bp [4]. Currently, this technique detects very local, small chromosomal rearrangements of only a few kb in addition to large copy number variants in a routine basis. Such small aberrations may affect only a single gene and, thus, create

unique opportunities to study gene function. This report highlights some recent breakthroughs in genomic research made possible by the detection of such small abnormalities and looks ahead to how the increased resolution of arrays can further contribute to gene discovery.

### Copy number variants in the healthy population

Somewhat surprisingly, the percentage of our genome that can harbour copy number variants is still the subject of debate. The first large-scale microarray study utilising a 26 K tiling path bacterial artificial chromosome (BAC) array platform estimated that 12% of the genome is subject to copy number variants [5]. Because of the probe size of at least 100 kb, these platforms typically overestimate individual copy number variant size and, consequently, also the total copy number variant content in our genome. However, after studying more than 2,000 individuals on higher resolution arrays containing over 550,000 probes, Shaikh *et al.* [6] and Itsara *et al.* [7] concluded that 16-19.4% of the genome is subject to copy number variants. This higher proportion is mainly a consequence of the increase in sample size compared to the previous study, as the total copy number variant content was composed of many small and rare copy number variants. Consequently, it would be reasonable to expect that the estimation of 3.7% copy number variable

content in the genome, which was obtained by analysing just 41 HapMap individuals on a NimbleGen array set containing ~42 million oligonucleotide probes – the microarray platform with the highest possible resolution today, would increase to a similar high percentage if the study population was sufficiently large to catalogue rare variants [8].

Copy number variants identified by major studies on healthy populations, such as the HapMap project, which is aimed at cataloguing normal genetic variation in man, are accessible through the Database of Genomic Variants (DGV). This database currently holds 37 microarray studies, and is, thus, a rich catalogue of human variation in healthy individuals. According to the DGV statistics consulted in June 2012, about 50% of the human reference genome may be copy number variable in healthy individuals [4]. At the moment, it is uncertain whether the cumulative variation present in the database correctly represents the copy number variant content in our genome or whether this is an overestimation. For instance, some samples, most notably the HapMap samples, have been analysed multiple times, sometimes with strikingly different outcomes in copy number variant content between different studies.

Whatever the exact percentage is, it has been demonstrated that copy number variants are a primary source of human genetic variation [9]. For a comparison, the 1,000 Genomes Project identified about 36.8 million single nucleotide polymorphisms (SNPs), covering only 1.3% of the genome [10]. Moreover, per generation locus-specific *de novo* mutation rates are 100 to 1,000 times higher for copy number variants than for SNPs, which further emphasizes the role of copy number variants in the evolution and variation of the human genome [10-12].

### **Copy number variants in disease**

In our hands, specific causative copy number variants were found in about 10-20% of patients with intellectual disability in routine microarray screening, which is in line with published data [13]. Because of this high diagnostic yield, microarray analysis is gradually replacing karyotyping as the routine diagnostic test for genetic abnormalities in many clinical laboratories.

### **Very local chromosomal rearrangements**

When specific copy number variants cause consistent clinical manifestations across multiple patients, a microdeletion or microduplication syndrome can be defined. These syndromes can be divided into two groups: recurrent and non-recurrent microdeletion or microduplication syndromes, which differ in the clustering of the copy

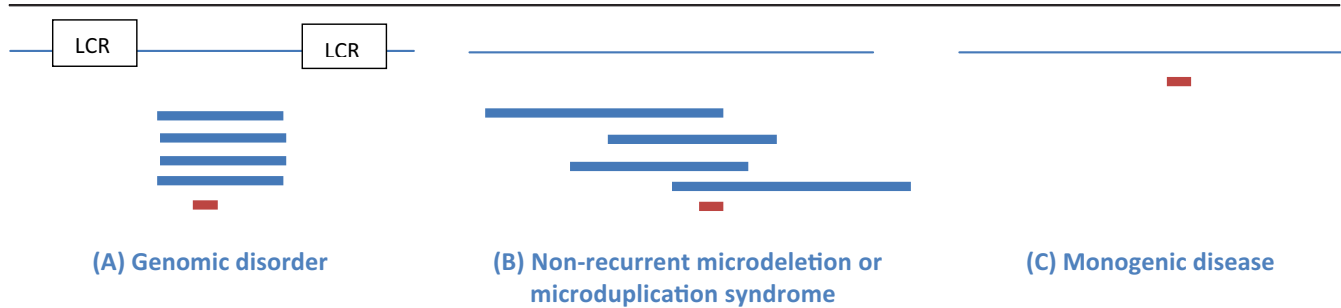
number variant breakpoints. So far, over 50 clinically recognizable microdeletion and microduplication syndromes associated with intellectual disability have been reported [13].

Genomic disorders [14-16] are recurrent microdeletion and microduplication syndromes, characterized by copy number variants with an identical size and clustered breakpoints, explained by the presence of low copy repeats flanking the copy number variants. The local chromosomal architecture makes these regions prone to non-allelic homologous recombination (NAHR) [17], resulting in the deletion or duplication of the sequences in between the low copy repeats. The 17q21.31 microdeletion syndrome was one of the first defined genomic disorders identified through reverse phenotypics. It is characterized by mild to severe intellectual disability, hypotonia, friendly behaviour and specific facial dysmorphisms, as well as epilepsy, cardiac defects and urologic anomalies [18-20]. The deletion that causes this phenotype spans 900 kb and contains six genes.

Non-recurrent microdeletion and microduplication syndromes are characterized by copy number variants in the same chromosomal region with non-identical breakpoints. The associated copy number variants arise by means of different recombination-repair mechanisms, such as non homologous end joining (NHEJ), fork stalling and template switching (FoSTeS) and break induced replication (BIR), all based on the occurrence of microhomology at the breakpoints [17,21]. Non-recurrent disorders are frequently located in chromosomal regions that contain repetitive sequences. Examples include the Miller-Dieker syndrome, characterized by type I lissencephaly, severe psychomotor delay, hypotonia, epilepsy, microcephaly, feeding problems and typical facial features. The causal deletions vary in size up to 2.5 Mb, but all share a 400 kb region containing nine genes at 17p13.3 [22,23].

### **Very local chromosomal rearrangements lead to the genetic dissection of microdeletion and microduplication syndromes and gene identification**

The specific phenotypes associated with microdeletion or microduplication syndromes are commonly attributed to an alteration in expression of dosage-sensitive genes in the copy number variant region. However, for many microdeletion and microduplication syndromes, there is much debate on the exact contribution of specific genes to the clinical manifestation of the patient. The increased resolution of current array platforms has enabled the identification of atypical copy number variants affecting only part of the genomic region associated with the phenotype. By careful study of the clinical manifestations of patients

**Figure 1. The added value of a very small copy number variation in genetic discovery**

Schematic representation of the added value of a very small copy number variant (red) in the genetic dissection of recurrent and non-recurrent microdeletion and microduplication syndromes (A and B) in relation to previously reported copy number variants (blue), and for causative gene identification in monogenic diseases (C).

with small copy number variants, the role of individual genes in the disease has been elucidated in some cases (illustrated in figure 1 A-C).

For instance, two patients presenting with the typical symptoms of the 17q21.31 microdeletion syndrome were identified with a much smaller deletion comprising only two out of the six genes typically deleted [24]. Mutation analysis of these two candidate genes, *MAPT* and *KANSL1*, was performed in patients with the clinical characteristics of this disorder but without the typical deletion. Point mutations in the chromatin modifier gene *KANSL1* were found in these patients, and it was concluded that the clinical characteristics of this genomic disorder can be caused by defects in this single gene [24,25]. Similarly, a long search to determine which of the genes deleted in the Prader-Willi syndrome are key contributors to the phenotype is nearly finished following the identification of a patient with a small copy number variant inside the Prader-Willi syndrome canonical region [26]. The child manifested most of the major symptoms of Prader-Willi syndrome and harboured an interstitial paternal deletion encompassing only the SNORD116 C/D box small nucleolar RNA (snoRNA) cluster.

In other cases, specific small copy number variants in the critical genomic region of microdeletion or microduplication syndromes can rule out the involvement of suspected candidate genes. We recently identified a pure hemizygous deletion of the *CLIP2* gene, located within the Williams-Beuren syndrome canonical region [27]. *CLIP2*, along with *GTF2I* and *GTF2IRD1*, were known as the key candidate genes for the characteristic cognitive and facial abnormalities associated with the Williams-Beuren syndrome. We could not demonstrate any abnormalities in two healthy siblings carrying the *CLIP2* deletion and, thus, concluded

that haploinsufficiency of this gene alone is not sufficient to cause any of the cognitive or facial features of this syndrome.

In many non-recurrent microdeletion and microduplication syndromes, careful correlation of local chromosomal rearrangements to the clinical characteristics of the carriers has led to the identification of the disease gene within the candidate region. Talkowski *et al.* [28] recently pinpointed the *MBD5* gene as the single causal locus for the intellectual disability, epilepsy and autism spectrum disorder (ASD) associated with the 2q23.1 microdeletion syndrome. This correlation was deduced by comparing the molecular and phenotypic data of 65 patients for which *MBD5* was the only affected gene in common.

Causative genes for several other genomic disorders have also been identified using the same approach, including *MEF2C* in the 5q14.3 microdeletion syndrome [29], *SATB2* in the 2q33.1 microdeletion syndrome [30], *HDAC4* in the brachydactyly mental retardation syndrome [31], *SHANK3* in the Phelan-McDermid syndrome/22q13 deletion syndrome [32], and *CREBBP* in the Rubinstein-Taybi syndrome [33]. Although these examples illustrate the importance of very local rearrangements in the study of genomic disorders, it should be noted that not each contiguous gene syndrome is due to deficits in only a single gene.

Very small local rearrangements can also pinpoint a disease gene outside regions of microdeletion or microduplication syndromes. *MYCN* was identified as causative for the Feingold syndrome following the identification of copy number variants encompassing this gene in patients with the clinical characteristics of this syndrome [34]. In a similar way, the *FOXF1* gene was identified as causative for alveolar capillary dysplasia [35]. Other examples include

two studies flagging *NRXN3* and *CMIP* as putative autism genes following the identification of rare microdeletions, including these genes in autistic patients [36,37].

### More causative gene identifications lie ahead of us

With the introduction of higher resolution arrays in routine diagnostics, we can expect an increasing number of very local chromosomal rearrangements to be identified. These small copy number variants detected in patients are submitted to databases, such as DECIPHER, ECARUCA and ISCA, along with phenotypic information [38-44]. A closer look at the data submitted, for instance to DECIPHER, suggests that many more causative gene identifications may lie ahead of us. In this database, currently containing publicly available data from 6,000 patients, about 80 *de novo* single gene aberrations are present, consisting of 55 deletions and 25 duplications. Using the DAVID annotation tools [45], we found that 55% of these genes are expressed in the brain and 15% are involved in the functioning of the glutamate or N-methyl-D-aspartate (NMDA)-receptors (unpublished observations). Furthermore, genes involved in neurogenesis, differentiation and coding for developmental proteins are enriched in this set. These data clearly suggest a role for at least some of these small copy number variants in neurological disease.

As some microdeletion or microduplication syndromes cannot be attributed to a single gene defect, careful study of the clinical presentation in relation to the affected gene(s) is necessary to decide whether the observed abnormality is causative for (part of) the phenotype of the patient [46]. Genotypic and phenotypic information stored in copy number variant databases are critical for the accurate interpretation of these results. However, the information in these databases should be used with caution as misannotated entries appear to be present. For example, two individuals included in the DGV carry a deletion of the *ELN* gene, causing supravalvar aortic stenosis. In addition, single gene deletions of the *KANSL1* gene, causal for the 17q21.31 microdeletion syndrome, are present in this database. There are two possible explanations for this observation. First, these regions might be false positive results, as each microarray technology inherently yields a low amount of false positive results. Second, the variants may be *bona fide* but are not associated with the expected clinical phenotype in these individuals, either due to genetic background or to incomplete penetrance. In either case, the user should be aware of the associated phenotypes before filtering copy number variants against these databases.

### Future directions

As the resolution of the arrays in routine diagnostics continues to increase, it can be expected that more very local rearrangements will be found. The interpretation of

the clinical consequences of such copy number variants will remain challenging. It is, therefore, important to integrate clinical information, functional annotation of affected genes, and published cases in interpretational workflows. This report illustrates how small copy number variants that affect a single gene only led to the identification of disease genes inside or outside regions of known microdeletion or microduplication syndromes. Thanks to the introduction of high-resolution microarray analysis in routine diagnostics in many laboratories, many more small copy number variants will be detected, and we expect that many more exciting discoveries lie ahead of us.

### Abbreviations

ASD, autism spectrum disorder; BIR, break induced replication; DGV, Database of Genomic Variants; FISH, fluorescence in situ hybridization; FoStEs, fork stalling and template switching; MLPA, multiplex ligation-dependent probe amplification; NAHR, non-allelic homologous recombination; NHEJ, non homologous end joining; NMDA, N-Methyl-D-aspartate; snoRNA, small nucleolar RNA; SNP, single nucleotide polymorphism.

### Competing interests

The authors declare that they have no competing interests.

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