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Copy Number Variation and Schizophrenia

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Over the last 12 months, a series of major articles have reported associations with schizophrenia of copy number variants at 1q21, 15q11.2, 15q13.3, 16p11.2, 22q12, and *Neurexin 1* loci. These are rare high-penetrant mutations that increase risk not only of schizophrenia but also of a range of other psychiatric disorders including autism and mental retardation. In some cases, the same phenotype can occur irrespective of whether the copy number variant causes a deletion or duplication. Some of these mutations occur at very high rates in human populations, but because of reduced fecundity associated with major psychiatric disorders the overall frequency in the population remains low. These new findings raise fundamental clinical and scientific questions concerning classification of major neuropsychiatric disorders, modes of inheritance, diagnostics, and genetic counseling. Although the loci identified so far account for only a small proportion of cases, many more are likely to be discovered over the next few years. A major focus of research will be to identify the key, the genetic and environmental determinants of schizophrenia risk in carriers of these copy number variants, and to discover whether their rates of mutation are unstable or fixed.

Key words: schizophrenia/genome/CNV

It is over 40 years since traditional cytogenetics using light microscopy first showed that variations in chromosome copy number and other structural changes could cause disease in humans. The earliest psychiatric disorder implicated was Down syndrome caused by the presence of an additional copy of chromosome 21. However, many other visible deletions and duplications were later identified associating with disease, as well as good number of apparently benign variations present in normal individuals. The classic examples in schizophrenia are velo-cardio-facial syndrome (VCFS) due to a deletion at 22q11 associated with psychosis in 30% of cases and disruption of the *DISC1* gene in a balanced 1q43:11q14 translocation associated with schizophrenia and other forms of major mental illness in a large Scottish pedigree.[1–3]

Recently new whole-genome scanning methods have made it possible to interrogate the genome at a resolution intermediate between that of cytogenetic analysis (greater than 2–5 million base pairs) and DNA sequencing (1–700 base pairs). It is these intermediate sized deletions and duplications that we refer to in this article by the term copy number variants (CNVs). Estimates vary, but upward of 25% of the human genome are reported to harbor CNVs. The majority are rare and found in only a very small number of individuals. However, a substantial fraction of the others, which are over 50 kilobases in size are recurrent. They have a very high mutation rate, are often to be found in rapidly evolving gene-rich unstable areas of the genome, and are flanked by long stretches of low-copy DNA repeats (LCRs), also called segmental duplications. The high mutation rate is due to a process called nonallelic homologous recombination (NAHR) between neighboring LCRs. The current total of 17 000 different CNVs at over 5000 sites, mostly detected with scanning technologies with resolution limits bigger than 30 kilobases, is almost certainly an underestimate (Database of Human Variants. projects.tcag.ca/variation). CNVs are of course at least 10 times less common than the insertion/deletion structural variants of less than 1000 bases that are also scattered throughout the genome; these are second only in frequency to single nucleotide polymorphisms (SNPs). Genome wide technologies have not yet been developed to analyse insertion/deletion variants, and so their overall role in health and disease remains unclear.[4]

The last 2 years has witnessed an explosion of interest in human CNVs. This has been in large part driven by advances in genome scanning technology. The discovery of CNV association with neuropsychiatric disorders, especially autism and schizophrenia, has in turn raised a number of fascinating new clinical and scientific questions, especially concerning the phenotypic boundaries between major neuropsychiatric disorders as they are currently classified, their modes of inheritance, the implications of this new information for diagnostics and genetic counseling, the genetic and environmental factors that determine the penetrance and expressivity of CNV-associated psychiatric phenotypes, and finally the relationship between human behavior itself and human genomic evolution.

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A detailed description of the different technologies and platforms that have been developed to interrogate the genome for CNVs can be found in a recent revue.[5] Array comparative genomic hybridization (CGH) and commercial SNP genotyping platforms from Affymetrix and Illumina are the most extensively used technologies.

Several genome wide searches for CNVs in schizophrenia have now been reported. Kirov et al[6] selected 45 male and 48 female unrelated proband/ parents schizophrenia trios from Bulgaria. Using array CGH technology and screening out known CNV variants, they identified 13 novel CNVs. The most interesting was a deletion on chromosome 2p16.3, which spans the promoter and first exon of the *Neurexin1* gene. The mutation was also present in the mother and another affected sibling. Partially overlapping deletions disrupting the same gene had previously been identified in association with mental retardation and autism.[7–12] Multiple deletions disrupting *Neurexin1* gene associating with schizophrenia have now been confirmed in a large case-control series from the SGENE consortium (manuscript submitted). Disruption of *Neurexin1* gene now looks like a definite high-impact genetic risk factor for schizophrenia as well as for other psychiatric phenotypes.

Walsh et al[13] reported an excess of CNVs in patients with schizophrenia. In a first test group of 150 individuals with schizophrenia and 268 controls, they found variants that deleted or duplicated genes in 15% of cases and 5% of controls. In a replication cohort of childhood-onset schizophrenia, they found that 28% had rare CNVs. The most interesting genes disrupted were *ERBB4* gene, which codes for a receptor for *Neuregulin1*, a gene already implicated in schizophrenia, and *Neurexin1* gene disrupted in identical twins concordant for schizophrenia. Two childhood-onset cases also harbored recurrent microduplications of a 500-kb region on chromosome16 p11.2 flanked by LCRs. This duplication had already been associated with autism and also found in 2 individuals with bipolar disorder.[14,15]

Xu et al[16] analyzed 359 schizophrenia subjects and both of their biological patients recruited from an Afrikaaner population in South Africa. In sporadic cases, they detected 19 CNVs of which 17 were de novo. Three of these were de novo chromosome 22q11.2 microdeletions. The frequency of de novo CNVs in sporadic schizophrenia cases was 10% vs 1.3% in controls. By contrast, they found none in 48 familial cases with a first- or second-degree relative with schizophrenia.

Stefansson et al[17] hypothesized that CNVs conferring risk of disorder such as schizophrenia may be under negative selection pressure because of the reduced fecundity of affected individuals. They identified 66 de novo CNVs by examination of 9878 sets of transmissions from unaffected parents to normal offspring. These, they suggested, would be among the commonest de novo CNVs in normal Caucasian populations. They then tested these 66 CNVs for disease association in a sample of 1433 patients with schizophrenia and 33 250 controls. They found 3 CNV deletions that were nominally associated with schizophrenia. These they proceeded to examine in a follow-up sample of 3285 cases and 7951 controls. All 3 deletions were significantly associated with schizophrenia. The deletions sizes were 1.38 Mb at 1q21, 450 kb on 15q112, and 1.57 Mb on 15q13.3. Although not part of the set of 66 de novo CNVs in the discovery cohort, the chromosome 22q11 deletion also significantly associated with schizophrenia. Interesting candidate genes were alpha7 nicotinic receptor gene (*CHRAN7*) at 15q13.3 that is targeted to axons by *Neuregulin1* and was already implicated in schizophrenia and also mental retardation and the *CYFIP1* gene at the 15q11.2 deletion interval. It interacts with fragile X mental retardation protein, and in fragile X syndrome the fragile X mutation results in a reduction in expression levels of the *CYFIP1* gene.[18] The CNVs significantly associating with schizophrenia on chromsomes1q21, 15q11.2, and 15q13.3 are all flanked by large LCR sequences. All showed less clustering in the normal Icelandic population than would be expected if they were selectively neutral. Because of this negative selection, due to reduced fecundity, these CNVs are maintained at low frequency in the normal population in spite of a very high mutation rate of perhaps 1 in every 10 000 meioses.

In a related article, the International Schizophrenia Consortium performed a genome-wide survey of rare CNVs in 3391 patients with schizophrenia and 3181 ancestrally matched controls.[19] They also identified the previously unknown associations with large deletions in 1q21.1 and 15q13.3 loci as well as the known VCFS deletion at the 22q11.2 locus. They found that the overall burden of rare CNVs greater than 100 kb in length was significantly increased in schizophrenia. When CNVs were then classified by number of genes they contained, the gene count was highly enriched in schizophrenia cases vs controls.

De novo and inherited 1q21 deletions and duplications were also recently reported[20] as enriched in a large series (5218) of cases with various forms of mental retardation including autistic spectrum. An earlier study of a 1000 patients with mental retardation, a recurrent deletion CNV at the 15q13.3 locus was associated with mental retardation and seizures syndrome in 9 individuals including one with autism.[21]

What are the implications of these new findings? All the studies have strengths and weaknesses, but when combined, in contrast to the often contradictory findings in schizophrenia DNA linkage and SNP allelic association studies, they are internally consistent and consistent with each other.

First of all, they show beyond doubt that carriers of some de novo and inherited CNVs, especially when they can be shown to disrupt gene function, are at high risk of developing schizophrenia. In the great

majority of cases, the schizophrenia is phenotypically indistinguishable form schizophrenia as it presents to psychiatric clinics the world over. In a small proportion, there may be associated features such as learning difficulties or VCFS, and in these cases the schizophrenia can be considered syndromic.

Second, deletions and/or duplications of CNVs at 1q21, 15q11.2, 15q13.3, 16p11.2, and *Neurexin 1* loci substantially increase the risk of a broad range of major psychiatric disorders in addition to schizophrenia. These include mental retardation, autism, bipolar disorder, and attention deficit/hyperactivity disorder. Bleuler in his famous 1911 book suggested that autistic withdrawal should be considered part of the core phenotype of schizophrenia.[22] Gradually starting with Kanner[23] in 1943, autism and other mental disorders have come to be looked upon as more or less distinct with separate clinical presentation and associated environmental and genetic risk. These new findings do not suggest that current clinical symptom-based classification systems are invalid; they do however suggest that in certain instances a number of clinically distinct psychiatric disorders may share a common etiology.

Third, the findings help to explain why some cases of schizophrenia appear familial and other cases appear sporadic. Sporadic cases are much more likely to be caused by de novo events. The high rate of de novo events probably also explains the well-known observation of higher risk of schizophrenia in the children than in the siblings of schizophrenia probands.

Fourth, these new discoveries have important implications for genetic counseling. Risk of schizophrenia in siblings of individuals with schizophrenia is far less if the proband carries a de novo CNV mutation. Much work will need to be done to catalogue the full-range psychiatric phenotypes associated with both de novo and inherited CNVs at these new loci and at the many more new loci likely to be reported in the next few years. A major difficulty will be determining the size of the risk at the individual loci and deciding which lesions are truly causative. Easiest to study will be the relatively common recurrent CNVs at loci flanked by LCRs where the mutations are probably similar in size. The challenge becomes more formidable at loci not flanked by LCRs such as *Neurexin I* where sizes and sites of disruption of the gene vary. The genes themselves may be complex. *Neurexin 1* gene may have up to 2000 alternative transcripts.[24] With rarer CNVs, it may be very difficult to determine which are truly causative. Interestingly, many CNVs disrupt several genes, only one of which may predispose to schizophrenia. Others may however impact on the physical health of individuals with schizophrenia and could in part explain the poorer health outcomes associated with schizophrenia.

Fifth, what proportion of the overall genetic burden of schizophrenia can be accounted for by CNVs? To date, only CNVs larger than 50–100 kb have been assayed, and at least half the loci, as indicated earlier, cannot be interrogated by the platforms used in the studies performed to date.[25] So far, they account for perhaps 2%–4% of schizophrenia. As many more novel loci are identified by newer high-resolution platforms, they may account for 10–20%. These optimistic assumptions concerning further new CNV detection are in direct contrast to allelic association studies in schizophrenia, where increasing marker density unfortunately seems unlikely to identify associations not already identified in schizophrenia by current genotyping platforms.

Sixth, determining the precise nature of the CNVs and what the genetic/epigenetic and environmental factors are that influence their penetrance and expressivity and cause them to increase risk for a broad range of neurodevelopmental disorders will be an exciting challenge for neuropsychiatric research in the coming years.

Finally, why are CNVs in humans especially associated with neuropsychiatric and behavioral phenotypes? Part of the explanation is probably that so many genes are involved in brain development and function, disruption of any one of which could cause schizophrenia. However, this is not the whole story. Genes involved in brain development and synaptic plasticity are definitely overrepresented at sites of CNVs.[26] The ideas are still controversial, but genome instability in humans is tolerated at sites rich in genes involved in brain development and function. This instability is recent in origin and corresponds to the period of rapid brain evolution in hominids. In fact, this rapid evolution may not have occurred without genome instability. There is a gradient with CNVs occurring more often in humans and chimpanzees than in old world monkeys through to rodents, chickens, and Drosophila. Varki et al[27] have proposed that genomic instability in these neural gene rich regions is better tolerated in humans owing to the buffering caused by our increasing independence from instinctive behavior. In other words, transmission of skills by learning and culture has allowed human behavior to decouple from strict genetic control, and this in turn has allowed a relaxation of selection on and so loss of specificity of function of genes involved in human neural systems.[27] Are CNV mutation rates themselves unstable? This is a key question with implications for schizophrenia and autism. It is possible that environmental stressors such as famine and/or severe psychogenic stress may increase CNV mutation rates either globally or at targeted genomic regions,[28] and this may account for the increased rates of adult schizophrenia observed in people conceived to parents exposed to these extreme circumstances.[29,30]

All these exciting new discoveries have raised more questions than they have answered. Many more are likely to be made over the next few years. This will certainly not be the last review to be published on the subject of copy number variation and schizophrenia.

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