From Fractured Genomes to Broken Minds

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In recent years, we have dramatically changed our view of human genome, from a collection of DNA base pairs that was largely quite stable to one whose very structure can change. We've learned that higher-order structural features, such as specific configurations of repeated base pair sequences, can predispose for DNA rearrangements.[1]

One of the most intriguing types of DNA rearrangements is copy-number variants (CNVs), deletions or duplications of parts of the genome. While CNVs range in size from a few hundred base pairs to several mega-bases affecting the copy number of one to dozens of juxtaposed genes, they are not identifiable by conventional light microscopy. It was not until a few years ago that improved technology enabled us to perform high-resolution genome-wide surveys of CNVs in individual genomes. These surveys revealed a large amount of copy number variation (at least 12,000 CNVs overlapping more than 1,000 genes), most of which represent benign polymorphic changes. CNVs are classified as rare (occurring at a frequency of <1 percent in the population) or common; collectively they cover at least 12-13 percent of the genome in the general population.

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This unanticipated degree of genomic instability and abundance of structural variation has led to the proposition that, in addition to their known role in a number of uncommon syndromes (such as Angelman syndrome, which is due to a microdeletion at chromosome 15q11), CNVs might also contribute to more common diseases. Many recent studies have established an important role for rare CNVs, both inherited and *de novo* (arising as new mutations in the parental germline), in the origin of neurodevelopmental disorders such as autism spectrum disorders, intellectual dysfunction, epilepsy, and attention deficit disorder, as well as psychiatric disorders, most notably schizophrenia.[2] Two lines of evidence support the idea that CNVs contribute to the risk of these disorders: first, the DNA of people with these disorders show more instances of these rare deletions and duplications relative to controls; second, they also show a higher prevalence of *de novo* CNVs relative to controls.

The vast majority of disease-causing CNVs are very rare, since they are weeded out by natural selection. But, when present, pathogenic CNVs confer significant increases in disease risk, ranging from double to 60-fold. We know that collections and combinations of rare CNVs increase disease risk, but identifying individual high-risk areas has been, with very few exceptions, challenging. Disease-associated CNVs are usually so rare that we need large numbers of patient samples to identify one at a convincing level of statistical evidence.

CNVs are not the only type of rare disease variants but were the first variants to be found to be associated to psychiatric illness because they were large, easily detectable, and able to be assessed by a variety of current technologies. Over the past few years, after many unfruitful decades, genetics research has made significant strides in identifying many additional genetic risk factors for psychiatric and neurodevelopmental disorders, in part due to new-generation exome and genome sequence technologies (Exomes are the set of protein-coding sequences of a genome; about 1 percent of the genome.). These risk factors include rare, inherited or *de novo*, smaller-scale "mistakes" in DNA sequence, such as single nucleotide loss-of-

function variants (nonsense, splice site, and frameshift mutations predicted to abolish protein function), which also have a strong effect on disease risk, as well as more common but weaker inherited single nucleotide polymorphisms.

In the case of schizophrenia, for example, a recent collaborative study including most of the data generated in all genetic studies of schizophrenia to date found definitive evidence for eight such CNV risk loci, which collectively, are carried by a small fraction (1-2 percent) of people with schizophrenia.[3] This group of CNVs is the tip of the iceberg: Hundreds of other pathogenic CNVs have evaded detection so far either due to lack of statistical power or due to limitations in our current techniques.

The major mechanism by which CNVs change traits is by altering gene dosage, that is by changing the number of copies of a gene or genes whose function depends on its expression level. In a deletion CNV, for example, the single remaining functional copy of the affected gene(s) cannot provide sufficient gene product to preserve the standard trait. However, even CNVs that confer large increases in disease risk do not emerge in isolation. Secondary modifiers in the genetic background of each person are necessary to determine the trajectory (health or disease) and symptom severity of a disease-predisposing CNV. Such modifiers could be genetic events (i.e. another CNV, disruptive single nucleotide variants in a gene or genes in the same or a related signaling pathway, contributions of common variants), an environmental influence, or a random event. Secondary modifiers may in principle aggravate the effect of a primary mutation, but they may also suppress it. This pattern of extreme genetic heterogeneity as well as incomplete penetrance (the chance that a CNV carrier will develop the disorder, which is typically less than 50 percent) is not specific to CNVs but also occurs with other rare mutations. This variable expression reflects the complex genetic architecture of mental disorders and the need for convergence of multiple "hits" in each person for them to progress to full blown disease.

Although individual CNVs are responsible for only a very small percentage of cases of the broader disease, they are collectively very common and their relatively high propensity to cause disease makes them valuable tools in creating reasonable and reliable disease models. Such models faithfully recapitulate specific aspects of the disease either in other organisms (such as mice) or human neuronal cell lines (derived using induced pluripotent stem cell techniques).

By using disease models of specific CNVs together with new technologies for analysis in genetics and neuroscience, we can more efficiently identify deviant neural circuit patterns and mechanisms that could be generalized over a variety of genetic causes.[4] We have started translating our current knowledge of rare CNVs into functional studies that help us understand both the genetics and the neurobiology of schizophrenia in more depth, facilitating development of innovative diagnostic tools and new therapies. Below I discuss four areas of research which I believe hold great promise along these lines.

Biological Pathways and Neuronal Activity Patterns

In aggregate, disease-causing CNVs affect a variety of genes in hundreds of distinct areas that contribute to the genetic risk of schizophrenia and autism. This is likely due to the complexity of the neural substrates affected in these disorders, which offers a large mutational target comprising many genes. One important question is whether this diverse genetic risk converges on a smaller number of biological pathways or neural circuits to generate a common pattern of clinical dysfunction or symptoms.

Indeed, despite the genetic diversity there is substantial bioinformatic evidence that genes disrupted by CNVs predisposing to schizophrenia and other neurodevelopmental disorders functionally converge on a highly interconnected network of synaptic proteins, including synaptic cell adhesion molecules, scaffolding proteins as well as glutamatergic ionotropic receptors, and associated protein complexes. This level of description, though, fails to capture how such changes manifest themselves within brain circuits and how they relate to specific symptoms that differentiate, for example, schizophrenia from other neuropsychiatric and neurodevelopmental syndromes.

Our laboratory has proposed that the differentiating level will be that of neural circuits, specifically how the dynamics of intricately connected neuronal populations are affected by CNVs and other pathogenic mutations.[6] We have also described alterations in the long and short-range connectivity, stability, and plastic properties of neuronal circuits emerging as a result of 22q11.2 deletions, a prominent schizophrenia-predisposing CNV and the strongest known genetic risk factor for schizophrenia.[5] These

alterations may lead to unreliable internally-driven or stimulus-driven neuronal activation patterns that may trigger the constellation of schizophrenia symptoms, such as perceptual distortions, loose associations, and cognitive deficits. In that respect, models of CNVs provide a tractable way to investigate how lower-level deviations in genetic, molecular, cellular, and synaptic mechanisms converge to alter the stability of local neuronal assemblies and influence their activity and connectivity patterns. We expect that these and other such studies will offer valuable mechanistic insights and provide novel drug targets, which may be tailored to individual or functionally related groups of CNVs, a novel paradigm in psychiatry.

Genetic Interactions

Work from our group and others on mouse models that carry the equivalent of human CNVs suggests that diseases are due to contributions of more than one neighboring gene within the CNV. Moreover, genetic interactions that amplify or mask the effect of individual genes have been clearly described among neighboring genes within large pathogenic CNVs. Such findings of additive or synergistic interaction of multiple genes within the region suggest that the genetic contribution of CNVs may also involve a degree of "micro-complexity" that could be instructive for our ongoing efforts to decipher the pattern and understand the consequences of the more extensive genetic "macro-complexity" inherent in the genetic architecture of mental disorders. In addition to studies in mouse models, recent human genetic studies have also confirmed that "micro-complexity" is a general principle underlying the disease risk associated with large CNVs.[7]

Thus, multiple, neighboring, modest-effect genes are needed to confer the typically very large increase in schizophrenia risk in the case of large CNVs. Even in this case however, analysis of individual genes from the region could be helpful, to dissect the mechanisms and provide anchors for translational studies. The ultimate test for the effectiveness of potential therapeutic interventions, though, will be in the context of the entire disease-associated CNV.

Overlaps Between Disorders

Multiple CNVs, which affect many different genes, contribute to the genetic risk of autism or schizophrenia. There also is a substantial overlap in copy number risk areas between the two disorders. In some instances, CNVs do not have high diagnostic specificity for psychiatric or neurodevelopmental disorders: A specific CNV might boost risk for many different diseases. In other instances, while CNV loci may overlap between two disorders, the actual variants associated with each of them, such as deletions versus duplications of the same region, may vary.

Distinct types of disorders have been reported in comparisons of people with rare deletions versus duplications of the same genomic regions. For example, a prominent CNV implicated in schizophrenia is a deletion on chromosome 22q11.2—while a reciprocal duplication predisposes to autism. Also, recurrent deletions and duplications of a genomic region of chromosome 16p11.2 have been linked to autism and schizophrenia, respectively.

The discovery of this overlap has raised several fascinating questions, concerning not only the phenotypic boundaries between major neurodevelopmental and psychiatric disorders as they are currently classified but also the mechanistic factors involved in determining who will develop what disorder. Detailed understanding of these questions remains elusive and it will likely require research at the level of the dynamics of affected neural circuits.

Our group recently provided some initial insights using a bioinformatics approach focusing on growth of dendrites and dendritic spines.[8] Most excitatory glutamatergic synapses in the human brain are formed on dendritic spines, and structural aberrations of the spines have been implicated in several psychiatric disorders. We investigated the likely impact on the growth of dendrites or dendritic spines by a gene disrupted by a CNV based on the corresponding dosage change (deletion or duplication).

Our analyses in schizophrenia and autism based on already known mutant phenotypes of CNV-associated genes revealed that CNVs associated with autism should primarily lead to an increase in spine or dendritic growth, while most schizophrenia-associated CNVs should lead to a decrease in growth of dendrites or spines. This finding is consistent with a spine density increase in autism and decrease in schizophrenia as observed in postmortem brain and animal model analyses. The correlation is not perfect and changes in spine and dendritic growth are not the only factors contributing to these diseases, but this analysis suggest that reciprocal CNVs associated with different psychiatric and neurodevelopmental disorders may lead, at least on average, to opposite functional consequences.

Offering Protection?

Finally, identifying mutations or variants that confer protection against disease via loss-of-function effects is another area of research that holds great promise for devising therapies to restore or prevent some or all of disease symptoms by inhibiting specific molecular processes. A well-established example from cardiology is provided by studies of the PCSK9 locus, where therapeutic agents designed to inhibit PCSK9 were developed in response to the detected protective effects of PCSK9 loss of function variants on LDL-C levels and risk for coronary artery disease.

Recent genome-wide scans in people with and without signs of schizophrenia have provided intriguing evidence for multiple "protective" CNVs, (i.e. those that enriched in controls rather than in patients) including duplications of the 22q11.2 genomic interval. It is probable that some of the undiscovered rare alleles affecting risk for schizophrenia confer protection rather than risk. Identifying them unequivocally will be tough due to the scarcity of such alleles in the human genome; it will require exceedingly large cohorts of patients and healthy controls and possibly insights from animal model approaches. Despite these difficulties, finding protective CNVs could prove an attractive roadmap for therapeutic targets.

The discovery of CNVs and their role in the development of mental illness signaled the beginning of the end of a protracted period of famine in our understanding of the proximal causes of mental illness. In addition to illuminating the path from fractured genomes to broken minds and opening the door to novel therapeutics, the discovery of CNVs has clear potential to influence more directly everyday clinical practice. Testing for CNVs is now affordable and is being used in clinical genetics and neurodevelopmental disorders clinics. Given the rapid pace of discovery in psychiatric genetics, it is likely that testing will also soon be expanded to psychiatric care in the coming years.

References and Further Reading

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