Variations Are a Theme: Copy Number Mutations in Sporadic Schizophrenia

30 May 2008. In the latest of a flurry of recent papers examining the role of copy number variations (CNVs) in schizophrenia, researchers report a strong association between de novo CNVs and the sporadic, or nonfamilial, form of the disorder. The paper is part of another flurry as well, being the third major paper this month from the Columbia University groups led by Maria Karayiorgou and Joseph Gogos, close on the heels of their recent DISC1 and 22q11 deletion syndrome mouse model papers (Kvajo et al., 2008; Stark et al., 2008).

Two studies published earlier this year (see SRF related news story) implicated CNVs in schizophrenia, but neither was designed to directly address the relative contribution of inherited versus de novo CNVs to sporadic cases.

One of these studies (Kirov et al., 2008), which reported CNV disruptions of neurexin1 (NRXN1) and amyloid precursor-binding protein A2 (APBA2) in subjects with schizophrenia, compared a sample with equal numbers of sporadic and familial cases to control subjects. In one arm of the second study (Walsh et al., 2008), parents of 83 patients with childhood-onset schizophrenia (COS), an extremely rare and severe form of the disorder (see Q&A with Nitin Gogtay accompanying SRF related news story), were genotyped to identify de novo CNVs "insofar as possible," but the authors conceded that, aside from two mutations that were "presumptively" de novo, 25 other CNVs they reported were "either inherited or of unknown origin."

To focus more closely on the role of de novo CNVs (referred to as "copy number mutations" by the authors) in sporadic cases of schizophrenia, Karayiorgou, Gogos, and first author Bin Xu joined forces with researchers at the University of Pretoria in a study of 1,017 individuals from the genetically homogeneous Afrikaner population of South Africa (Xu et al., 2008). Of these individuals, 200 had been diagnosed with schizophrenia or schizoaffective disorder and 159 were unaffected control subjects. However, both biological parents of all affected and control subjects were also enrolled in the study, allowing the team to confirm that 152 affected individuals were sporadic cases, with no incidence of schizophrenia in first- or second-degree relatives, while 48 were familial cases.

A fine filter
The researchers performed whole-genome scans of the entire sample at a resolution of ~30 kb. They eliminated from consideration any CNVs that had >50 percent overlap with a CNV in any parental chromosome (including across families), thus avoiding the inclusion of common CNVs that might result from high mutation rates in unstable genomic regions and any commonly inherited CNVs that might falsely appear to be de novo without this filtering procedure.

The researchers identified 15 de novo CNVs in the 152 sporadic cases (~10 percent), but only two in the 159 controls (1.3 percent), meaning that de novo mutations were about eight times more common in sporadic cases, a highly significant association (p = 0.00078). Moreover, the group found no de novo CNVs in the 48 familial cases of schizophrenia, indicating that "...the association between de novo [CNVs] seems to be primarily confined to the sporadic cases."

In a comparison of the collective rate of de novo and inherited CNVs in sporadic cases and unaffected controls, the researchers found that sporadic cases were only 1.5 times more likely to harbor inherited CNVs (p = 0.049), and they argue that "only a small portion of [these inherited mutations] should be expected to contribute to the pathogenesis of the sporadic..."
As for the nature of the CNVs, the researchers identified roughly equal numbers of genomic gains and losses in the sporadic cohort, but they cite three microdeletions in the 22q11.2 locus as particularly significant. These mutations, which were found in three different subjects, represent the only recurrent structural variations in the study, and confirm earlier reports by Karayiorgou’s group and others that variations in this locus confer susceptibility to schizophrenia (Karayiorgou et al., 1995; Liu et al., 2002). Of special interest in this regard, a de novo mutation identified at 14q32.13–32.2 includes DICER1, the protein product of which regulates microRNA production. Deletions at 22q11.2 disrupt DCGR8, a key miRNA processing protein, which these researchers recently tied to schizophrenia-relevant deficits in their 22q11DS mouse model (Stark et al., 2008).

The authors also report three de novo CNVs mutating single genes, including a microdeletion affecting RAPGEF6. This gene, a GTP exchange factor that acts with the GTPases Rap1 and Rap2, has been supported as a candidate gene for schizophrenia in both linkage and association studies (Lewis et al., 2003; Chen et al., 2006). Among the other genes affected by the de novo CNVs detected in this study, Xu and colleagues report statistically significant enrichment in neurodevelopmental, small GTPase, and RNA binding/processing pathways among the schizophrenia cases.

"[De novo CNVs] can explain, at least in part, how schizophrenia persists in the population despite the low fecundity of affected individuals. Second, our findings suggest that de novo genetic lesions at many different loci can contribute to schizophrenia, and this heterogeneity may account for the difficulties in finding genetic variants with a major effect on disease risk," the researchers write.—Peter Farley.

Reference:

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Related News: Copy Number Variations in Schizophrenia: Rare But Powerful?
Comment by: Daniel Weinberger, SRF Advisor
Submitted 27 March 2008

The paper by Walsh et al. is an important addition to the expanding literature on copy number variations in the human genome and their potential role in causing neuropsychiatric disorders. It is clear that copy number variations are important aspects of human genetic variation and that deletions and duplications in diverse genes throughout the genome are likely to affect the function of these genes and possibly the development and function of the human brain. So-called private variations, such as those described in this paper, i.e., changes in the genome found in only a single individual, as all of these variations are, are difficult to establish as pathogenic factors, because it is hard to know how much they contribute to the complex problem of human behavioral variation in a single individual. If the change is private, i.e., only in one case and not enriched in cases as a group, as are common genetic polymorphisms such as SNPs, how much they account for case status is very difficult to prove.

An assumption implicit in this paper is that these private variations may be major factors in the case status of the individuals who have them. The data of this paper suggest, however, this is actually not the case, at least for the childhood onset cases. Here’s why: mentioned in the paper is a statement that only two of the CNVs in the childhood cases are de novo, i.e.,
spontaneous and not inherited (and one of these is on the Y chromosome, making its functional implications obscure). If most of the CNVs are inherited, they can't be causing illness per se as major effect players because they are coming from well parents.

Also, if you add up all CNVs in transmitted and non-transmitted chromosomes of the parents, it's something like 31 gene-based CNVs in 154 parents (i.e., 20 percent of the parents have a gene-based deletion or duplication in the very illness-related pathways that are highlighted in the cases), which is at least as high a frequency as in the adult-onset schizophrenia sample in this study...and three times the frequency as found in the adult controls. This is not to say that such variants might not represent susceptibility genetic factors, or show variable penetrance between individuals, like other polymorphisms, and contribute to the complex genetic risk architecture, like other genetic variations that have been more consistently associated with schizophrenia. However, the CNV literature has tended to seek a more major effect connotation to the findings.

View all comments by Daniel Weinberger

Related News: Copy Number Variations in Schizophrenia: Rare But Powerful?

Comment by: William Honer
Submitted 28 March 2008
Posted 28 March 2008

I recommend the Primary Papers

As new technologies are applied to understanding the etiology and pathophysiology of schizophrenia, considering the clinical features of the cases studied and the implications of the findings is of value. The conclusion of the Walsh et al. paper, "these results suggest that schizophrenia can be caused by rare mutations..." is worth considering carefully.

What evidence is needed to link an observation in the laboratory or clinic to cause? Recent recommendations for the content of papers in epidemiology (von Elm et al., 2008) remind us of the suggestions of A.V. Hill (Hill, 1965). To discern the implications of a finding, or association, for causality, Hill suggests assessment of the following:

1. Strength of the association: this is not the observed p-value, but a measure of the magnitude of the association. In the Walsh et al. study, the primary outcome measure, structural variants duplicating or deleting genes was observed in 15 percent of cases, and 5 percent of controls. But what is the association with? The diagnostic entity of schizophrenia, or some risk factor for the illness? Of interest, and noted in the Supporting Online Material, these variants were present in 7/15 (47 percent) of the cases with presumed IQ <80, but only 15/135 (11 percent) of the cases with IQ >80. Are the structural variants more strongly associated with mental retardation (within schizophrenia 47 percent vs. 11 percent) than with diagnosis (11 percent vs. 5 percent of controls, assuming normal IQ)? This is of particular interest in the context of the speculation in the paper concerning the importance of genes putatively involved with brain development in the etiology of schizophrenia.

2. Consistency of results in the literature across studies and research groups: there are now several papers examining copy number variation in schizophrenia, including a report from our group (Wilson et al., 2006). The authors of the present paper state that each variant observed was unique, and so consistency of the specific findings could be argued to be irrelevant, if the model is of novel mutations (more on models below). Undoubtedly, future meta-analyses and accumulating databases help determine if there is anything consistent in the findings, other than a higher frequency of any abnormalities in cases rather than controls.

3. Specificity of the findings to the illness in question: this was not addressed experimentally in the paper. However, the findings of more abnormalities in the putative low IQ cases, and the similarity of the findings to reports in autism and mental retardation, suggest that this criterion for supporting causality is unlikely to be met.
4. Temporality: the abnormalities should precede the illness. If DNA from terminally
differentiated neurons harbors the same variants as DNA from constantly renewed
populations of lymphocytes, then clearly this condition is met. While it seems highly likely
that this is the case, it is worthwhile considering the possibility that DNA structure may vary
between tissue types, or between cell populations. Even within human brain there is some
evidence for chromosomal heterogeneity (Rehen et al., 2005).

5. Biological gradient: presence of a “dose-response” curve strengthens the likelihood of a
causal relationship. This condition is not met within cases: only 1/115 appeared to have more
than one variant. However, in the presumably more severe childhood onset form of
schizophrenia, four individuals carried multiple variants, and the observation of a higher
prevalence of variants overall. Still, the question of what the observations of CNV are
associated with is relevant, since one of the inclusion/exclusion criteria for COS allowed IQ
65-80, and it is uncertain how many of these cases had some degree of intellectual deficit.

6. Plausibility: biological likelihood—quite difficult to satisfy as a criterion, in the context of
the limits of knowledge concerning the mechanisms of illness of schizophrenia.

7. Coherence of the observation with known facts about the illness: the genetic basis of
schizophrenia is quite well studied, and there is no dearth of theories concerning genetic
architecture. However, a coherent model remains lacking. As examples, the suggestion is
made that the observations concerning inherited CNVs in the COS cases are linked with a
severe family history in this type of illness. This appears inconsistent with a high penetrance
model for CNVs as suggested in the opening of the paper (presuming the parents in COS
families are unaffected, as would seem likely). Elsewhere, CNVs are proposed by the authors
to be related to de novo events, and an interaction with an environmental modifier, folate
(and exposure to famine), is posited (McClellan et al., 2006). A model of the effects of CNVs,
which generates falsifiable hypotheses is needed.

8. Experiment: the ability to intervene clinically to modify the effects of CNVs disrupting
genes seems many years away.

9. Analogy: the novelty of the CNV findings is both intriguing, but limiting in understanding
the likelihood of causal relationships.

The intersection of clinical realities and novel laboratory technologies will fuel the need for
better translational research in schizophrenia for many, many more years.

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View all comments by William Honer
The new study by Walsh et al. (2008), as well as recent data from other groups working in schizophrenia, autism, and mental retardation, make a strong case for including copy number variants as an important source of risk for neurodevelopmental phenotypes. These findings raise several intriguing new questions for future research, including: the degree of causality/penetrance that can be attributed to individual CNVs; diagnostic specificity; and recency of their origins. While these questions are difficult to address in the context of private mutations, one potential source of additional information is the examination of common, recurrent CNVs, which have not yet been systematically studied as potential risk factors for schizophrenia.

Still, the association of rare CNVs with schizophrenia provides additional evidence that genetic transmission patterns may be a complex hybrid of common, low-penetrant alleles and rare, highly penetrant variants. In diseases ranging from Parkinson's to colon cancer, the literature demonstrates that rare penetrant loci are frequently embedded within an otherwise complex disease. Perhaps the most well-known example involves mutations in amyloid precursor protein and the presenilins in Alzheimer's disease (AD). Although extremely rare, accounting for <1 percent of all cases of AD, identification of these autosomal dominant subtypes greatly enhanced understanding of pathophysiology. Similarly, a study of consanguineous families in Iran has very recently identified a rare autosomal recessive form of mental retardation (MR) caused by glutamate receptor (GRIK2) mutations, thereby opening new avenues of research (Motazacker et al., 2007). In schizophrenia, we have recently employed a novel, case-control approach to homozygosity mapping (Lencz et al., 2007, Lencz et al., 2007), resulting in several candidate loci that may harbor highly penetrant recessive variants. Taken together, these results suggest that a diversity of methodological approaches will be needed to parse genetic heterogeneity in schizophrenia.

References:


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Related News: Copy Number Variations in Schizophrenia: Rare But Powerful?

Comment by: Ben Pickard
Submitted 31 March 2008

In my mind, the study of CNVs in autism (and likely soon in schizophrenia/bipolar disorder, which are a little behind) is likely to put biological meat on the bones of illness etiology and finally lay to rest the annoyingly persistent taunts that genetics hasn't delivered on its promises for psychiatric illness.

I don't think it's necessary at the moment to wring our hands at any inconsistencies between the Walsh et al. and previous studies of CNV in schizophrenia (e.g., Kirov et al., 2008). There...
are a number of factors which I think are going to influence the frequency, type, and identity of CNVs found in any given study.

1. CNVs are going to be found at the rare/penetrant/familial end of the disease allele spectrum—in direct contrast to the common risk variants which are the targets of recent GWAS studies. In the short term, we are likely to see a large number of different CNVs identified. The nature of this spectrum, however, is that there will be more common pathological CNVs which should be replicated sooner—NRXN1, APBA2 (Kirov et al., 2008), CNTNAP2 (Friedman et al., 2008)—and may be among some of these "low hanging fruit." For the rarer CNVs, proving a pathological role is going to be a real headache. Large studies or meta-analyses are never going to yield significant p-values for rare CNVs which, nevertheless, may be the chief causes of illness for those few individuals who carry them. Showing clear segregation with illness in families is likely to be the only means to judge their role. However, we must not expect a pure cause-and-effect role for all CNVs: even in the Scottish t(1;11) family disrupting the DISC1 gene, there are several instances of healthy carriers.

2. Sample selection is also likely to be critical. In the Kirov paper, samples were chosen to represent sporadic and family history-positive cases equally. In the Walsh paper, samples were taken either from hospital patients (the majority) or a cohort of childhood onset schizophrenia. Detailed evidence for family history on a case-by-case basis was not given but appeared far stronger in the childhood onset cases. CNVs appeared to be more prevalent, and as expected, more familial, in the latter cohort. A greater frequency was also observed in the Kirov study familial subset.

3. Inclusion criteria are likely to be important—particularly in the more sporadic cases without family history. This is because CNVs found in this group may be commoner and less penetrant—they will be more frequent in cases than in controls but not exclusively found in cases. Any strategy, such as that used in the Kirov paper, which discounts a CNV based on its presence—even singly—in the control group is likely to bias against this class.

4. Technical issues. Certainly, the coverage/sensitivity of the method of choice for the "event discovery" stage is going to influence the minimum size of CNV detectable. However, a more detailed coverage often comes with a greater false-positive rate. Technique choice may also have more general issues. In both of the papers, the primary detection method is based on hybridization of case and pooled control genomes prior to detection on a chip. Thus, a more continuously distributed output may result—and the extra round of hybridization might bias against certain sequences. More direct primary approaches such as Illumina arrays or a second-hand analysis of SNP genotyping arrays may provide a more discrete copy number output, but these, too, can suffer from interpretational issues.

The other major implication of these and other CNV studies is the observation that certain genes "ignore" traditional disease boundaries. For example, NRXN1 CNVs have now been identified in autism and schizophrenia, and CNTNAP2 translocations/CNVs have been described in autism, Gilles de la Tourette syndrome, and schizophrenia/epilepsy. This mirrors the observation of common haplotypes altering risk across the schizophrenia-bipolar divide in numerous association studies. It might be the case that these more promiscuous genes are likely to be involved in more fundamental CNS processes or developmental stages—with the precise phenotypic outcome being defined by other variants or environment. The presence of mental retardation comorbid with psychiatric diagnoses in a number of CNV studies suggests that this might be the case. I look forward to the Venn diagrams of the future which show us the shared neuropsychiatric and disease-specific genes/gene alleles. It will also be interesting to see if the large deletions/duplications involving numerous genes give rise to more severe, familial, and diagnostically more defined syndromes or, alternatively, a more diffuse phenotype. Certainly, it has not been easy to dissect out individual gene contributions to phenotype in VCFS or the minimal region in Down syndrome.
We agree with the comments of Weinberger, Lencz and Malhotra, and Pickard, and the question raised by Honer about the extent to which the association may be more to mental retardation than schizophrenia. These new studies of copy number variation represent important advances, but need to be interpreted carefully.

We are now getting two different kinds of data on schizophrenia, which can be seen as two opposite poles. The first is from association studies with common variants, in which large numbers of people are required to see significance, and the strengths of the associations are quite modest. These kinds of vulnerability factors would presumably contribute a very modest increase in risk, and many taken together would cause the disease. By contrast, the “private” mutations, as identified by the Sebat study, could potentially be completely causative, but because they are present in only single individuals or very small numbers of individuals, it is difficult to be certain of causality. Furthermore, since some of them in the early-onset schizophrenia patients were present in unaffected parents, one might have to assume the contribution of a common variant vulnerability (from the other parent) as well.

If a substantial number of the private structural mutations are causal, then one might expect to have seen multiple small Mendelian families segregating a structural variant. The situation would then be reminiscent of the autosomal dominant spinocerebellar ataxis, in which mutations (currently about 30 identified loci) in multiple different genes result in similar clinical syndromes. The existence of many small Mendelian families would be less likely if either 1) structural variants that cause schizophrenia nearly always abolish fertility, or 2) some of the SVs detected by Walsh et al. are risk factors, but are usually not sufficient to cause disease. The latter seems more likely.

We think these two poles highlight the continued importance of segregation studies, as have been used for the DISC1 translocation. In order to validate these very rare “private” copy number variations, we believe that it would be important to look for sequence variations in the same genes in large numbers of schizophrenia and control subjects, and ideally to do so in family studies.

One very exciting result of the new copy number studies is the implication of whole pathways rather than just single genes. This highlights the importance of a better understanding of pathogenesis. The study of candidate pathways should help facilitate better pathogenic understanding, which should result in better biomarkers and potentially improve classification and treatment. In genetic studies, development of pathway analysis will be fruitful. Convergent evidence can come from studies of pathogenesis in cell and animal models, but this will need to be interpreted with caution, as it is possible to make a plausible story for so many different pathways (Ross et al., 2006). The genetic evidence will remain critical.

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The idea that a proportion of schizophrenia is associated with rare chromosomal abnormalities has been around for some time, but it has been difficult to be sure whether such events are pathogenic given that most are rare. Two instances where a pathogenic role seems likely are first, the balanced ch1:11 translocation that breaks DISC1, where pathogenesis seems likely due to co-segregation with disease in a large family, and second, deletion of chromosome 22q11, which is sufficiently common for rates of psychosis to be compared with that in the general population. This association came to light because of the recognizable physical phenotype associated with deletion of 22q11, and the field has been waiting for the availability of genome-wide detection methods that would allow the identification of other sub-microscopic chromosomal abnormalities that might be involved, but whose presence is not predicted by non-psychiatric syndromal features. This technology is now upon us in the form of various microarray-based methods, and we can expect a slew of studies addressing this hypothesis in the coming months.

Structural chromosomal abnormalities can take a variety of forms, in particular, deletions, duplication, inversions, and translocations. Generally speaking, these can disrupt gene function by, in the case of deletions, insertions and unbalanced translocations, altering the copy number of individual genes. These are sometimes called copy number variations (CNVs). Structural chromosomal abnormalities can also disrupt a gene sequence, and such disruptions include premature truncation, internal deletion, gene fusion, or disruption of regulatory or promoter elements.

It is, however, worth pointing out that structural chromosomal variation in the genome is common—it has been estimated that any two individuals on average differ in copy number by a total of around 6 Mb, and that the frequency of individual duplications or deletions can range from common through rare to unique, much in the same way as other DNA variation. Also similar to other DNA variation, many structural variants, indeed almost certainly most, may have no phenotypic effects (and this includes those that span genes), while others may be disastrous for fetal viability. Walsh and colleagues have focused upon rare structural variants, and by rare they mean events that might be specific to single cases or families. For this reason, they specifically targeted CNVs that had not previously been described in the published literature or in the Database of Genomic Variants. The reasonable assumption was made that this would enrich for CNVs that are highly penetrant for the disorder. Indeed, Walsh et al. favor the hypothesis that genetic susceptibility to schizophrenia is conferred not by relatively common disease alleles but by a large number of individually rare alleles of high penetrance, including structural variants. As we have argued elsewhere (Craddock et al., 2007), it seems entirely plausible that schizophrenia reflects a spectrum of alleles of varying effect sizes including common alleles of small effect and rare alleles of larger effect, but data from genetic epidemiology do not support the hypothesis that the majority of the disorder reflects rare alleles of large effect.

Walsh et al. found that individuals with schizophrenia were >threefold more likely than controls to harbor rare CNVs that impacted on genes, but in contrast, found no significant difference in the proportions of cases and controls carrying rare mutations that did not impact upon genes. They also found a similar excess of rare structural variants that deleted or duplicated one or more genes in an independent series of cases and controls, using a cohort with childhood onset schizophrenia (COS).
The results of the Walsh study are important, and clearly suggest a role for structural variation in the etiology of schizophrenia. There are, however, a number of caveats and issues to consider. First, it would be unwise on the basis of that study to speculate on the likely contribution of rare variants to schizophrenia as a whole. It is likely correct that, due to selection pressures, highly penetrant alleles for disorders (like schizophrenia) that impair reproductive fitness are more likely to be of low frequency than they are to be common, but this does not imply that the converse is true. That is, one cannot assume that the penetrance of low frequency alleles is more likely to be high than low. Thus, and as pointed out by Walsh et al., it is not possible to know which or how many of the unique events observed in their study are individually pathogenic. Whether individual loci contribute to pathogenesis (and their penetrances) is, as we have seen, hard to establish. Estimating penetrance by association will require accurate measurement of frequencies in case and control populations, which for rare alleles, will have to be very large. Alternatively, more biased estimates of penetrance can be estimated from the degree of co-segregation with disease in highly multiplex pedigrees, but these are themselves fairly rare in schizophrenia, and pedigrees segregating any given rare CNV obviously even more so.

As Weinberger notes, the case for high penetrance (at the level of being sufficient to cause the disorder) is also undermined by their data from COS, where the majority of variants were inherited from unaffected parents. This accords well with the observation that 22q11DS, whilst conferring a high risk of schizophrenia, is still only associated with psychosis in ~30 percent of cases. It also accords well with the relative rarity of pedigrees segregating schizophrenia in a clearly Mendelian fashion, though the association of CNVs with severe illness of early onset might be expected to reduce the probability of transmission.

Third, there are questions about the generality of the findings. Cases in the case control series were ascertained in a way that enriched for severity and chronicity. Perhaps more importantly, the CNVs were greatly overrepresented in people with low IQ. Thus, one-third of all the potentially pathogenic CNVs in the case control series were seen in the tenth of the sample with IQ less than 80. The association between structural variants and low IQ is well known, as is the association between low IQ and psychotic symptoms, and it seems plausible to assume that forms of schizophrenia accompanied by mental retardation (MR) are likely to be enriched for this type of pathogenesis. The question that arises is whether the CNVs in such cases act simply by influencing IQ, which in turn has a non-specific effect on increasing risk of schizophrenia, or whether there are specific CNVs for MR plus schizophrenia, and some which may indeed increase risk of schizophrenia independent of IQ. In the case of 22q11 deletion, risk of schizophrenia does not seem to be dependent on risk of MR, but more work is needed to establish that this applies more generally.

Another reason to caution about the generality of the effect is that Walsh et al. found that cases with onset of psychotic symptoms at age 18 or younger were particularly enriched for CNVs, being greater than fourfold more likely than controls to harbor such variants. There did remain an excess of CNVs in cases with adult onset, supporting a more general contribution, although it should be noted that even in this group with severe disorder, this excess was not statistically significant (Fisher’s exact test, p = 0.17, 2-tailed, our calculation). The issue of age of onset clearly impacts upon assessing the overall contribution CNVs may make upon psychosis, since onset before 18, while not rare, is also not typical. A particular contribution of CNVs to early onset also appears supported by the second series studied, which had COS. However, this is a particularly unusual form of schizophrenia which is already known to have high rates of chromosomal abnormalities. Future studies of more typical samples will doubtless bear upon these issues.

Even allowing for the fact that many more CNVs may be detected as resolution of the methodology improves, the above considerations suggest it is premature to conclude a substantial proportion of cases of schizophrenia can be attributed to rare, highly penetrant CNVs. Nevertheless, even if it turns out that only a small fraction of the disorder is attributable to CNVs, as seen for other rare contributors to the disorder (e.g., DISC1
translocation), such uncommon events offer enormous opportunities for advancing our knowledge of schizophrenia pathogenesis.

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Related News: Copy Number Variations in Schizophrenia: Rare But Powerful?

Comment by: Ridha Joober, Patricia Boksa
Submitted 2 May 2008
 Posted 4 May 2008

Walsh et al. claim that rare and severe chromosomal structural variants (SVs) (i.e., not described in the literature or in the specialized databases as of November 2007) are highly penetrant events each explaining a few, if not singular, cases of schizophrenia.

However, their definition of rareness is questionable. Indeed, it is unclear why SVs that are rare (<1 percent) but previously described should be omitted from their analysis. In addition, contrary to their own definition of rareness, the authors included in the COS sample several SVs that have been previously mentioned in the literature (e.g., “115 kb deletion on chromosome 2p16.3 disrupting NRXN1”). Furthermore, some of these SVs (entire Y chromosome duplication) are certainly not rare (by the authors’ definition), nor highly penetrant with regard to psychosis (Price et al., 1967). Finally, as their definition of rareness depends on a specific date, the results of this study will change over time.

As to the assessment of severity, it can equally be concluded from table 2 and using their statistical approach that “patients with schizophrenia are significantly more likely to harbor rare structural variants (6/150) that do not disrupt any gene compared to controls (2/268) (p = 0.03)”, thus contradicting their claim. In fact, had they used criteria in the literature (Lee et al., 2007; Brewer et al., 1999) (i.e., deletion SVs are more likely than duplications to be pathogenic) and appropriate statistical contrasts, deletions are significantly (p = 0.02) less frequent in patients (5/23) than in controls (9/13) who have SVs. In addition, the assumption of high penetrance is questionable given the high level (13 percent) of non-transmitted SVs in parents of COS patients. Is the rate of psychosis proportionately high in the parents? From the data presented, we know that only 2/27 SVs in COS patients are de novo and that “some” SVs are transmitted. Adding this undetermined number of transmitted SVs to the reported non-transmitted SVs will lead to an even larger proportion of parents carrying SVs. Disclosing the inheritance status of SVs in COS patients along with information on diagnoses in parents from this “rigorously characterised cohort,” represents a major criterion for assessing the risk associated with these SVs.

Consequently, it appears that the argument of rareness is rather idiosyncratic and contains inconsistencies, and the one of severity is very open to interpretation. Most importantly, it should be emphasized that amalgamated gene effects at the population level do not allow one to conclude that any single SV actually contributes to schizophrenia in an individual. Thus it is unclear how this study of grouped events differs from the thousands of controversial and underpowered association studies of single genes.

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Related News: More Evidence for CNVs in Schizophrenia Etiology—Jury Still Out on Practical Implications

Comment by: Christopher Ross, Russell L. Margolis
Submitted 1 August 2008

The two recent papers in Nature, from the Icelandic group (Stefansson et al., 2008), and the International Schizophrenia Consortium (2008) led by Pamela Sklar, represent a landmark in psychiatric genetics. For the first time two large studies have yielded highly significant consistent results using multiple population samples. Furthermore, they arrived at these results using quite different methods. The Icelandic group used transmission screening and focused on de novo events, using the Illumina platform in both a discovery population and a replication population. By contrast, the ISC study was a large population-based case-control study using the Affymetrix platform, which did not specifically search for de novo events.

Both identified the same two regions on chromosome 1 and chromosome 15, as well as replicating the previously well studied VCFS region on chromosome 22. Thus, we now have three copy number variants which are replicated and consistent across studies. This provides data on rare highly penetrant variants complementary to the family based study of DISC1 (Porteous et al., 2006), in which the chromosomal translocation clearly segregates with disease, but in only one family. In addition, they are in general congruent with three other studies (Walsh et al., 2008; Kirov et al., 2008; Xu et al., 2008) which also demonstrate a role for copy number variation in schizophrenia. These studies together should put to rest many of the arguments about the value of genetics in psychiatry, so that future studies can now begin from a firmer base.

However, these studies also raise at least as many questions as they answer. One is the role of copy number variation in schizophrenia in the general population. The number of cases accounted for by the deletions on chromosome 1 and 15 in the ISC and Icelandic studies is extremely small--on the order of 1% or less. The extent to which copy number variation, including very rare or even private de novo variants, will account for the genetic risk for schizophrenia in the general population is still unknown. The ISC study indicated that there is a higher overall load of copy number variations in schizophrenia, broadly consistent with Walsh et al and Xu et al but backed up by a much larger sample size, allowing the results to achieve high statistical significance. The implications of these findings are still undeveloped.

Another issue is the relationship to the phenotype of schizophrenia in the general population. Many more genotype-phenotype studies will need to be done. It will be important to determine whether there is a higher rate of mental retardation in the schizophrenia in these studies than in other populations.

Another question is the relationship between these copy number variations (and other rare events) and the more common variants accounting for smaller increases in risk, as in the recent O'Donovan et al. (2008) association study in Nature Genetics. It is far too early to know, but there may well be some combination of rare mutations plus risk alleles that account for cases in the general population. This would then be highly reminiscent of
Alzheimer’s disease, Parkinson’s disease, and other diseases which have been studied for a longer period of time.

For instance, in Alzheimer’s disease there are rare mutations in APP and presenilin, as well as copy number variation in APP, with duplications causing the accelerated Alzheimer’s disease seen in Down syndrome. These appear to interact with the risk allele in APOE, and possibly other risk alleles, and are part of a pathogenic pathway (Tanzi and Bertram, 2005). Similarly in Parkinson’s disease, rare mutations in α-synuclein, LRRK2 and other genes can be causative of PD, though notably the G2019S mutation in LRRK2 has incomplete penetrance. In addition, duplications or triplications of α-synuclein can cause familial PD, and altered expression due to promoter variants may contribute to risk. By contrast, deletions in Parkin cause an early onset Parkinsonian syndrome (Hardy et al., 2006). Finally, much of PD may be due to genetic risk factors or environmental causes that have not yet been identified. Further studies will likely lead to the elucidation of pathogenic pathways. These diseases can provide a paradigm for the study of schizophrenia and other psychiatric diseases. One difference is that the copy number variations in the neurodegenerative diseases are often increases in copies (as in APP and α-synuclein), consistent with gain of function mechanisms, while the schizophrenia associations were predominantly with deletions, suggesting loss of function mechanisms. The hope is that as genes are identified, they can be linked together in pathways, leading to understanding of the neurobiology of schizophrenia (Ross et al., 2006).

The key unanswered questions, of course, are what genes or other functional domains are deleted at the chromosome 1, 15, and 22 loci, whether the deletions at these loci are sufficient in themselves to cause schizophrenia, and, if sufficient, the extent to which the deletions are penetrant. Both of the current studies identified deletions large enough to include several genes. The hope is that at least a subset of copy number variations (unlike SNP associations identified in schizophrenia to date) may be causative, making the identification of the relevant genes or other functional domains—at least in principle—more feasible.

Another tantalizing observation is that the copy number variations associated with schizophrenia were defined by flanking repeat regions. This raises the question of the extent to which undetected smaller insertions, deletions or other copy number variations related to other repetitive motifs, such as long tandem repeats, may also be associated with schizophrenia. Identification and testing of these loci may prove a fruitful approach to finding additional genetic risk factors for schizophrenia.

References:


Abstract


Abstract

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Related News: More Evidence for CNVs in Schizophrenia Etiology—Jury Still Out on Practical Implications

Comment by: Daniel Weinberger, SRF Advisor
Submitted 3 August 2008

Several recent reports have suggested that rare CNVs may be highly penetrant genetic factors in the pathogenesis of schizophrenia, perhaps even singular etiologic events in those cases of schizophrenia who have them. This is potentially of enormous importance, as the definitive identification of such a “causative” factor may be a major step in unraveling the biologic mystery of the condition. I would stress several issues that need to be considered in putting these recent findings into a broader perspective.

It is very difficult to attribute illness to a private CNV, i.e., one found only in a single individual. This point has been potently illustrated by a study of clinically discordant MZ twins who share CNVs (Bruder et al., AJHG, 2008). Inherited CNVs, such as those that made up almost all of the CNVs described in the childhood onset cases of the study by Walsh et al. (Science, 2008), are by definition not highly penetrant (since they are inherited from unaffected parents). The finding by Xu et al. (Nat Gen, 2008) that de novo (i.e., non-inherited) CNVs are much more likely to be associated with cases lacking a family history is provocative but difficult to interpret as no data are given about the size of the families having a family history and those not having such a history. Unless these family samples are of comparable size and obtained by a comparable ascertainment strategy, it is hard to know how conclusive the finding is. Indeed, in the study of Walsh et al., rare CNVs were just as likely to be found in patients with a positive family history. Finally, in contrast to private CNVs, recurrent (but still rare) CNVs, such as those identified on 1q and 15q in the studies of the International Schizophrenia Consortium (Nature, 2008) and Stefansson et al. (Nature, 2008), are strongly implicated as being associated with the diagnosis of schizophrenia and therefore likely involved in the causation of the illnesses in the cases having these CNVs. In all, these new CNV regions, combined with the VCFS region on 22q, suggest that approximately five to 10 patients out of 1,000 who carry the diagnosis of schizophrenia may have a well-defined genetic lesion (i.e., a substantial deletion or duplication).

The overarching question now is how relevant these findings are to the other 99 percent of individuals with this diagnosis who do not have these recurrent CNVs. Before we had the capability to perform high-density DNA hybridization and SNP array analyses, chromosomal anomalies associated with the diagnosis of schizophrenia were identified using cytogenetic techniques. Indeed, VCFS, XXX, XXY (Kleinfelter’s syndrome), and XO (Turner syndrome) have been found with similarly increased frequency in cases with this diagnosis in a number of studies. Now that we have greater resolution to identify smaller structural anomalies, the list of congenital syndromes that increase the possibility that people will manifest symptoms that earn them this diagnosis appears to be growing rapidly. Are we finding causes for the
form of schizophrenia that most psychiatrists see in their offices, or are we instead carving out a new set of rare congenital syndromes that share some clinical characteristics, as syphilis was carved out from the diagnosis of schizophrenia at the turn of the twentieth century? Is schizophrenia a primary expression of these anomalies or a secondary manifestation? VCFS is associated with schizophrenia-like phenomena but even more often with mild mental retardation, autism spectrum, and other psychiatric manifestations. The same is true of the aneuploidies that increase the probability of manifesting schizophrenia symptoms. The two new papers in Nature allude to the possibility that epilepsy and intellectual limitations may also be associated with these CNVs. The diagnostic potential of any of these new findings cannot be determined until the full spectrum of their clinical manifestations is clarified.

One of the important insights that might emerge from identification of these new CNV syndromes is the identification of candidate genes that may show association with schizophrenia based on SNPs in these regions. VCFS has been an important source of promising candidate genes with broader clinical relevance (e.g., PRODH, COMT). Stefansson et al. report, however, that none of the 319 SNPs in the CNV regions showed significant association with schizophrenia in quite a large sample of individuals not having deletions in these regions. The Consortium report also presumably has the results of SNP association testing in these regions in their large sample but did not report them. It is very important to explore in greater genetic detail these regions of the genome showing association with the diagnosis of schizophrenia in samples lacking these lesions and to fully characterize the clinical picture of individuals who have them. It is hoped that insights into the pathogenesis of symptoms related to this diagnosis will emerge from these additional studies.

Anyone who has worked in a public state hospital or chronic schizophrenia care facility (where I spent over 20 years) is not surprised to find an occasional patient with a rare congenital or acquired syndrome who expresses symptoms similar to those individuals also diagnosed with schizophrenia who do not have such rare syndromes. Our diagnostic procedures are not precise, and the symptoms that earn someone this diagnosis are not specific. Schizophrenia is not something someone has; it is a diagnosis someone is given. In an earlier comment for SRF on structural variations in the genome related to autism, I suggested that, "From a genetic point of view, autism is a syndrome that can be reached from many directions, along many paths. It is not likely that autism is any more of a discrete disease entity than say, blindness or mental retardation.” These new CNV syndromes manifesting schizophrenia phenomena are probably a reminder that the same is true of what we call schizophrenia.

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