Do Faulty Nogo Receptors Allow Axons to Run Amuck in Schizophrenia?

7 January 2009. It was reported several years ago that the gene for Nogo-66 receptor 1 (NGR, or RTN4R), located in the chromosome 22q11 region, may influence genetic predisposition to schizophrenia (Liu et al., 2002). Stephen Strittmatter and colleagues of Yale University in New Haven, Connecticut, find support for this association in a study published in the December 3 issue of the Journal of Neuroscience. NgR1 mediates myelin-associated inhibition of axon growth, and the researchers also report that rare variants of NgR1 found in people with schizophrenia failed to inhibit axonal growth in vitro.

Reduced myelination, oligodendroglial dysfunction, and the reduced expression of several myelination-related genes have been associated with schizophrenia (see SRF related news story and SRF news story). The brains of people with schizophrenia are reported to have abnormal myelination patterns, which may contribute to the disease (for review, see Karoutzou et al., 2008; Segal et al., 2007). The current report lies at the nexus of this line of research and another that seeks to discover why hemizygous deletion of the 22q11 locus confers an increased risk of schizophrenia (Baron, 2001).

Oligodendrocytes in normal brain regulate axonal growth via Nogo protein and its receptor, NgR1. The Nogo receptor is well established to regulate growth cone collapse by binding Nogo associated with myelin, thereby stopping axon growth. In normal development, this process could lead to the establishment of appropriate axonal pathways. In schizophrenia, faulty NgR1 signaling could conceivably cause axonal miswiring.

The Yale researchers, led by first author Stephane Budel, studied several ethnic populations each consisting of people with schizophrenia and an approximately equal number of controls without schizophrenia: 636 Caucasians, 296 African Americans, and 1,122 Chinese. They analyzed seven single nucleotide polymorphisms (SNPs) associated with NGR, and in Caucasians, they were able to identify a group of SNPs, or haplotype, that is significantly associated with schizophrenia. The scientists analyzed whether the results could have been accounted for by genetic variation between the control group and the group with schizophrenia, but no differences between the genetic backgrounds of the two groups were found. In the African American and Chinese groups they found no significant association between changes in the NGR1 locus and schizophrenia.

Strittmatter and his co-workers also examined how changes in NgR1 protein function might contribute to schizophrenia. A detailed examination of DNA from an NIMH collection of samples taken from people with schizophrenia predicted that amino acid substitutions at position R377 in the NgR1 protein were common. The researchers examined the function of two altered forms of NgR1 that had amino acid substitutions at position 377: substituting either glutamine (Q) or tryptophan (W) for arginine (R). They transfected chick retinal neurons, using a herpes virus, causing them to express the altered forms of NgR1. In wild-type chick retinal neurons, growth cone collapse normally occurs in response to Nogo protein, but in the R377Q-NgR1 and R377W-NgR1 cells, Nogo-66 exposure did not collapse growth cone. This effect was also seen
with exposure of the cells to myelin-associated glycoprotein and with myelin. The R377Q-NgR1 cells were less likely to experience growth cone collapse than wild-type cells and R377W-NgR1 cells were the least likely to experience growth cone collapse.

The scientists were also interested in amino acid substitutions R119W and R196H in the NgR1 protein, based on a report from a different research group (Sinbaldi et al., 2004). When these amino acid changes were expressed in NgR1 in chick retinal neurons, neither cells with R119W nor R196H mediated growth cone collapse in response to Nogo-66, myelin associated glycoprotein, or myelin.

Strittmatter and co-workers also tested whether changes in functional NgR1 affect the performance of mice in tests of cognitive function and affect, since both can be altered in people with schizophrenia. They observed impairment of working memory in NGR knockout mice (NGR1-/-), relative to wild-type, using a delayed alternation task. However, they did not see deficits in spatial memory, as NGR knockouts performed similarly to wild-type animals in a water radial arm maze. They also saw no differences in passive-avoidance learning between the two sets of mice by using tests in which mice learned to avoid an electric shock. A light-dark exploration test revealed no differences in anxiety-like behavior between wild-type and NGR knockout mice. Based on these experiments, the cognitive and emotional effects of changing Nogo-66 signaling by eliminating its receptor seem to be restricted to causing problems with working memory.

Based on this report, it seems that in at least some cases of schizophrenia, faulty myelin may be to blame for mixing up neuronal signaling by misdirecting axon growth. The authors conclude that “...one mechanism for increased schizophrenic risk is a failure to restrict anatomical plasticity in the brain.” Myelin-mediated inhibition of axonal sprouting may be a final stage of neuronal development that occurs during adolescence. This fits in with the idea that schizophrenia generally develops around early adulthood. Failure of the NgR1 pathway to inhibit axonal growth in late adolescence could cause abnormal brain connectivity and schizophrenia symptoms. It is interesting that the researchers found these effects in a group of Caucasians specifically. It is conceivable that similar disruptions in axonal remodeling may occur in other ethnic groups based on changes in NgR1 signaling, perhaps via slightly different gene and protein alterations. Stephane Budel told Schizophrenia Research Forum that, “We reached statistical significance only in Caucasians, but cannot rule out that NgR may participate in schizophrenia in other ethnicities.”

Interestingly, a report by Hsu and colleagues in a group led by Joseph Gogos came to quite different conclusions regarding the importance of NgR1 in schizophrenia (Hsu et al., 2007). These researchers found only a weak association between schizophrenia and NgR1 polymorphisms, by using a similar SNP analysis and evaluating samples taken from a family of Afrikaner origin. However, when they examined the behavior of NGR1-/- mice, they failed to see differences in working memory between the Nogo receptor-deficient mice and wild-type mice, as measured using a delayed alternation task. They also failed to see an effect in other schizophrenia-related behavioral tasks, although NGR1-/- mice did appear to have less motor activity than wild-type animals, as measured by an open-field test. The investigators in this study concluded that although NgR1 may not play a major role in conferring schizophrenia susceptibility, it may be one genetic influence that affects risk for schizophrenia in some patients.—Alisa Woods.

Reference:

Comments on News and Primary Papers

Comment by: Takeshi Sakurai, Joseph D. Buxbaum, Patrick R. Hof
Submitted 9 January 2009

Several lines of evidence indicate that oligodendrocytes and myelin are disturbed in schizophrenia (Davis et al., 2003; Segal et al., 2007). However, the relationship of these alterations to the pathogenesis of schizophrenia is still unclear. A recent paper by Budel et al. proposes one possible link between oligodendrocyte and myelin pathology and schizophrenia pathogenesis. The gene for Nogo-66 receptor 1 (RTN4R) is located within the 22q11.2 locus where a hemizygous microdeletion (1.5 Mb) occurs at a frequency of one in 5,000. Twenty to 30 percent of individuals with the deletion develop schizophrenia. Several candidate genes for the schizophrenia phenotype within this locus have been characterized for genetic association, and common variants of the Nogo-66 receptor 1 gene have shown association in one study (Liu et al., 2002), but replication studies have not confirmed the findings using different cohorts (Meng et al., 2007; Hsu et al., 2007). Rare variants for the Nogo-66 receptor 1 gene not found in controls have also been identified in schizophrenia cases (Hsu et al., 2007; Sinibaldi et al., 2004), but their functional or pathological association with schizophrenia has not been demonstrated.

Budel et al. first performed a genetic association study of common variants of Nogo-66 receptor 1 gene and confirmed previous findings in their independent cohort. Because these are intronic variants whose effects on Nogo-66 receptor 1 are not clear, they also searched for rare variants of the gene by direct sequencing of 542 DNA samples from individuals with schizophrenia. They found four previously unreported rare variants that are non-synonymous, giving a rate of non-synonymous rare variants of ~1 percent (eight in 870). These researchers also sequenced 650 control DNA samples and found eight rare non-synonymous variants, which together with previous other studies makes the total number of sequenced control samples 1,250. The overall incidence of coding region variants in controls and schizophrenia does not differ between the two groups. However, a bioinformatic analysis suggested that four out of eight rare variants found in schizophrenia are potentially detrimental to protein function, whereas none of eight rare variants found in controls are, indicating a higher incidence of potentially deleterious variations in the Nogo-66 receptor 1 gene in schizophrenia.

Nogo-66 is a myelin-associated outgrowth inhibitor that is responsible for the inhibition of regeneration of CNS axons. It binds to Nogo-66 receptor 1 which induces repulsive responses from neurons. On this basis, Budel et al. investigated whether these rare variants found in schizophrenia are functional. They found that both the R377Q and R377W variants in the signaling domain could not induce Nogo-66-mediated repulsive response from neurons when they were introduced into neurons that do not express the Nogo receptor. Interestingly, these variants could suppress the repulsive response from neurons that express endogenous Nogo-66 receptor 1, suggesting that they could work as dominant-negative forms. They also showed that Nogo-66 binding domain variants R119W and R196H both showed reduced binding to Nogo-66. Their results demonstrate that some of the rare variants found in schizophrenia (and not in controls) are functional variants, some of which may work as dominant negatives.
They further analyzed Nogo-66 receptor 1 knockout mice for behavioral characteristics relevant to schizophrenia. They found that Nogo-66 receptor 1 knockout mice show impairment in spatial working memory, a promising endophenotype in schizophrenia. This was specific to working memory as no deficits in the radial arm water maze test or passive avoidance test were observed. Nogo-66 receptor 1 knockout mice did not, however, show any changes in PPI, another important phenotype relevant for schizophrenia mouse models.

The authors had shown previously that Nogo-66 receptor-mediated axon growth inhibition is crucial for formation of ocular dominance in the visual cortex in mice, suggesting myelin involvement in brain wiring refinement and restriction of neuronal plasticity (McGee et al., 2005). The prefrontal cortex—believed to be impaired in schizophrenia—completes its myelination during late adolescence and early adulthood, when symptoms of schizophrenia emerge (Benes, 1989). Therefore, their study supports the idea that abnormal myelination may be a risk factor for schizophrenia. Interestingly, another Nogo receptor, pirB, has been identified (Atwal et al., 2008), and PirB is also shown to be involved in ocular dominance formation in the visual cortex (Syken et al., 2006). It would be interesting to look into PirB involvement in myelination and oligodendrocyte differentiation as well as in schizophrenia.

The Budel et al. study leaves a number of issues open. First, the incidence of each rare variant is one in 870 in schizophrenia, and to prove that they are not found in controls would require screening in a much larger number of controls for a reliable genetic study. This is always the problem when we characterize rare variants in association with disorders such as schizophrenia. Second, Hsu et al. also performed a behavioral analysis of their Nogo-66 receptor 1 knockout mice and did not find the working memory deficits that were seen in this study. They both used T maze-based delayed spatial working memory paradigm, but with subtle difference in their protocols. Also, Budel et al. used very strict inclusion/exclusion criteria of animals for the final testing based on animals’ ability during training sessions. Furthermore, as Hsu et al. suggested, there may be differences in mouse genetic background and age, which is not described in this study; Hsu et al. used a C57Blx129 mixed genetic background. Third, as Budel et al. mentioned, a recent study showed that Nogo-66 receptor 1 has synaptic functions, playing a role in glutamate receptor modulation (Lee et al., 2008), and as such it is distinctly possible that the Nogo-66 receptor 1 involvement in pathogenesis of schizophrenia is not related to myelin, but to synaptic deficits. Nevertheless, this study clearly demonstrated that rare variants in Nogo-66 receptor 1 found in individuals with schizophrenia show defects in myelin-mediated neuronal function, which could explain in part the working memory deficits observed in Nogo-66 receptor 1 knockout mice, and may provide a possible causal link between defects in oligodendrocyte function, myelination, and pathogenesis of schizophrenia.

References:


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Comment by: [Hans W. Moises]
Submitted 24 January 2006
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This is another important study supporting the glial growth factors deficiency and synaptic destabilization hypothesis of schizophrenia we proposed in 2002 ([Moises et al., 2002](http://www.schizophreniaforum.org/new/detailprint.asp?id=1479)). The glial synaptic destabilization hypothesis is based on the landmark 1997 paper by [Pfrieger and Barres](http://www.schizophreniaforum.org/new/detailprint.asp?id=1479) and the tripartite synapse model suggested by Philip Haydon and coworkers ([Araque et al., 1999](http://www.schizophreniaforum.org/new/detailprint.asp?id=1479); [Pascual et al., 2005](http://www.schizophreniaforum.org/new/detailprint.asp?id=1479)). In reference to its underlying principle, the glial growth factors deficiency and synaptic destabilization hypothesis might also more conveniently and briefly be designated as the weakened tripartite-synapse hypothesis of schizophrenia.

References:

Moises HW, Gottesman II. Does glial asthenia predispose to schizophrenia? Arch Gen Psychiatry 2004; 61:1170. [Abstract]


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Related News: CNP Findings Strengthen Oligodendrocyte Link to Schizophrenia

Comment by: Daniel Stewart, Kenneth Davis
Submitted 31 January 2006

Peirce's paper is an exciting addition to the white matter hypothesis in schizophrenia. (Note: many of the authors of this paper are colleagues of ours at the Conte Center investigating white matter in schizophrenia at Mount Sinai.) As noted in the news story, findings from a number of different areas are beginning to come together in support of the white matter hypothesis in schizophrenia. Genetic findings in myelin-related genes, as outlined and referenced above, are demonstrating increased susceptibility to schizophrenia. Imaging findings from diffusion tensor studies are demonstrating abnormalities across multiple brain areas (reviewed in Kubicki et al., 2005), with more recent studies showing that specific white matter tracts are not only abnormal in schizophrenia, but are associated with symptomatology and cognitive deficits (Kubicki et al., 2002; Kubicki et al., 2003; Nestor et al., 2004). Postmortem examination is revealing that oligodendrocytes are decreased in number and abnormally spaced in patients with schizophrenia (Hof et al., 2002; Hof et al., 2003). These converging data argue strongly for the involvement of myelin, oligodendrocytes, and white matter in schizophrenia.

We continue to examine various aspects of white matter involvement in schizophrenia with the hope of providing both translational data (i.e., the relationship between symptom severity or independent living and white matter coherence) and further basic science data that may shed some light on upstream events that contribute to myelin and oligodendrocyte deficits. These new data by the Owen and O'Donovan group are a valuable contribution.

References:


Kubicki M, Westin CF, Nestor PG, Wible CG, Frumin M, Maier SE, Kikinis R, Jolesz FA, McCarley RW, Shenton ME. Cingulate fasciculus integrity disruption in schizophrenia: a


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Related News: CNP Findings Strengthen Oligodendrocyte Link to Schizophrenia

Comment by: William Honer
Submitted 4 March 2006
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The Peirce et al. paper represents an important contribution to understanding the possible mechanisms through which genetic risk factors could contribute to the pathophysiology of schizophrenia. Studies of SNPs in candidate genes for schizophrenia are most clearly related to mechanism when the SNP changes amino acid sequence (rarely), or when the SNP changes mRNA expression (commonly postulated, but less often demonstrated). Studies combining SNP and mRNA analyses are challenging, and Peirce et al. provide a novel approach—by measuring the relative amount of mRNA expressed from the variant and the wild-type alleles in brain tissue from heterozygotes. They demonstrated relatively reduced expression from the variant allele. It must be noted however, that these studies were carried out in brain tissue from individuals described as being “free from psychiatric or neurological disorder at time of death” (not schizophrenia samples as suggested by the SRF news story [Editor’s note: since corrected]), and the total expression of CNP mRNA was not determined. While CNP mRNA expression is reported to be lower in schizophrenia, and Peirce et al. demonstrate the variant allele is a risk factor for schizophrenia in studies of genetic association, it remains uncertain to what extent the lower CNP mRNA expression in schizophrenia is related to genetic variation or to other factors. CNP mRNA differences in expression between schizophrenia and control samples appear to be of different magnitude in different brain regions from the same cases (Katsel et al., 2005). This could represent non-genetic effects. However, genetic variation in CNP could also be more or less likely to be expressed in different brain regions. In this regard, the samples used in the Peirce et al. study were mixed, coming from frontal, parietal, or temporal cortex. Studies with larger sample sizes, and of schizophrenia as well as control tissues, will be needed to test these possibilities.

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Related News: OLIG2 Gene Supports Notion of Myelin Abnormalities in Schizophrenia

Comment by: William Honer
Submitted 4 August 2006

This paper demonstrates several important shifts in research strategies for schizophrenia. Many previous studies of candidate genes in the illness have chosen their targets based on concepts of the mechanism of action of antipsychotic drugs, or by virtue of the proximity of a
gene to a genetic linkage site defined with anonymous markers. The choice of candidate gene here is based on a wide range of neurobiological evidence, including studies of gene expression and protein levels. As well, the authors do not limit their study to one gene; instead, they expand their investigation to include plausibly interacting gene targets. Analysis of complex disorders will likely need more than simple models, and the approach here is worth noting.

The gap still remains between the DNA-mRNA approaches and protein analysis. Gene expression is one factor determining mRNA levels. However, especially in human brain tissue samples, many other antemortem and postmortem factors contribute to the measured level of mRNA. The meaning of gene expression measures obtained for oligodendrocyte/myelination-related genes from samples comprising largely gray matter is not entirely certain. The role of oligodendrocytes in gray matter may deserve more attention.

The genetic evidence presented here for an interaction between OLIG2 and ErbB4 is intriguing. A recent paper from Steve Arnold’s group indicated the neuregulin-1–ErbB4 signaling pathway appears to be overactive in schizophrenia, with consequences for NMDA receptor function (Hahn et al., 2006). Although the analysis in their paper focused on neurons, if a similarly overactive pathway was operative in oligodendrocytes, inhibition of myelination might be a predicted outcome (Sussman et al., 2005), with downregulation of a host of oligodendrocyte/myelination-related genes as a consequence. Of further interest, NMDA receptors may have important roles in oligodendrocytes as well as in neurons (Matute, 2006).

References:


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