G72 Protein Shows Up in Mitochondria

24 August 2007. Nearly 5 years ago, the first report appeared linking the G72/G30 locus on chromosome 13q to schizophrenia (Chumakov et al., 2002). That first paper also reported that a protein encoded by the G72 gene bound to and activated the enzyme D-amino acid oxidase (DAO) in vitro, earning the protein the name “D-amino acid oxidase activator” (DAOA) in some quarters.

A new study from Joseph Gogos and Maria Karayiorgou at Columbia University, New York, outlines a different scenario of how the G72 gene could contribute to schizophrenia. The results, published earlier this month in the online edition of Molecular Psychiatry, suggest that LG72, the protein encoded by the longest splice variant derived from G72, is found only in mitochondria, and not in peroxisomes where it might interact with DAO.

The researchers found that overexpression of LG72 causes mitochondrial fragmentation in several cell lines. In immature primary neurons, enforced expression of G72 caused increased dendritic arborization. The investigators did not replicate the previously reported association of LG72 with DAO, leading them to suggest that the protein may instead play a previously unrecognized role in neurons to modulate mitochondrial function.

A case of right suspect, wrong weapon?

DAO is one of a number of enzymes that oxidizes serine, an agonist of NMDA-type glutamate receptors. The findings of Chumakov and colleagues raised the possibility that changes in G72 expression could affect serine levels to lower NMDA receptor activity, an idea that fit nicely with the hypothesis that schizophrenia is a disease of glutamate hypofunction. In further support of the G72-serine connection, the original paper showed a genetic interaction between the G72 locus and DAO, the gene for DAO, on chromosome 12q24.

Since then, follow-up studies (see SRF related news story, as well as the meta analysis of Detera-Wadleigh and McMahon, 2006) have generally upheld the association between the G72/G30 locus and both schizophrenia and bipolar disorder; the results with DAO have been less consistent (for example, see Corvin et al., 2007 and Shinkai et al., 2007). In addition, the exact gene and variant responsible for the effect, as well as its protein product and function, have remained obscure (for review, see Abou Jamra et al., 2006).

To establish the subcellular localization of the G72 protein, first author Mirna Kvajo overexpressed G72 in COS7 cells and in rat hippocampal neuron cells. The LG72 protein has no homology to known proteins, but has high α helix content, suggesting it is membrane-bound. Both immunofluorescent staining of intact cells and Western blotting of cell fractions revealed LG72 in the mitochondrial compartment. Deletion of the first 25 N-terminal amino acids abolished mitochondrial localization of the protein. Endogenous LG72 was not detected by immunostaining in any of seven cell lines tested, with the exception of rare staining in HeLa cells, where mitochondrial localization was confirmed. The researchers also note that a protein band consistent with the presence of endogenous LG72 was detected by Western blotting of
membrane-enriched fractions of human amygdala lysates.

The presence of overexpressed LG72 affected mitochondrial morphology, the investigators found. The protein was distributed in mitochondrial vesicles, and the mitochondria themselves appeared fragmented. The changes were not associated with induction of apoptosis, or loss of mitochondrial membrane potential in cell lines, suggesting that G72 might regulate normal mitochondrial morphology, involving the fission and fusion of organelles. In primary rat hippocampal neurons, expression of LG72 resulted in mitochondrial fragmentation and an increase in dendritic branching. The dendrites contained more mitochondria after LG72 expression, which the authors speculate may have enabled the higher degree of branching.

To look for association of LG72 and DAO in intact cells, the investigators overexpressed both proteins in COS cells. As reported, DAO localized to peroxisomes. There was no indication of colocalization with LG72, which was found only in mitochondria, nor did the two proteins appear physically associated by coimmunoprecipitation experiments. In addition, they found no increase in endogenous DAO activity in another cell line after LG72 overexpression. “Taken together with the lack of detectable subcellular colocalization and physical interaction, this result makes it unlikely that G72 can directly and robustly regulate DAO activity in the tested mammalian cell lines,” they conclude. The authors point out that the original study identified the G72/DAO protein-level interaction using a yeast two-hybrid screen and in vitro enzyme assays, and did not confirm the results in cells. Thus, it is possible that the interaction does not occur under physiological conditions.

“If our negative findings are confirmed in further studies, the 'NMDA-hypofunction' rationale behind the hypothesis that G72 is a susceptibility gene for schizophrenia would be weakened,” the authors write. “Nevertheless, the issue of whether or not the existing association studies support a role of variants in the vicinity of the G72 gene in schizophrenia and bipolar disorder susceptibility should be judged independently of the underlying functional rationale, especially because this gene was identified initially via a nonhypothesis-driven positional cloning approach.”

In other words, the genetic evidence itself warrants an intensive study of G72 function. If that function turns out to reside in mitochondria, it would fit a different but equally interesting theory of neurological disease. That is, of course, the hypothesis of mitochondrial dysfunction, which has been invoked in neurodegenerative diseases, and more recently, in schizophrenia and bipolar disorder (Ben-Shachar, 2002; Stork and Renshaw, 2005; Konradi et al., 2004)—Pat McCaffrey.

Reference:

Comments on Related News

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Comment by: Patricia Estani
Submitted 23 April 2006
☑ I recommend the Primary Papers

http://www.schizophreniaforum.org/new/detailprint.asp?id=1387
I would caution that G72 has not been shown to be an actual gene, and in the four years since Chumakov and colleagues' report, the biochemistry has not been reproduced.

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Reply to comment by Dr Scolnick

We agree that caution is required regarding the assumption that the genetic association at this locus is causally related to the DAOA "gene," and this is the reason that in the paper we have referred to the "DAOA/ G30 locus." Establishing robust genetic association in a restricted region of the genome is clearly the first step on a path to characterizing the biological and phenotypic relationships associated with the variation. It is entirely possible that pathologically relevant variation occurs at the DAOA/G30 locus that does not involve a protein product of the DAOA DNA sequence.

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The DAOA/G30 locus is a paradigm of association in psychiatric genetics, where positive reports are followed by both confirmation of association and failures to associate, with the observers of the glass being half-full commenting that it is unlikely that replication would occur spuriously multiple times, and those seeing the glass as half-empty (or three-quarters empty) emphasizing allelic inconsistencies, lack of identified causative SNPs, and in the case of DAOA/G30, lack of conclusive evidence of a gene expressed in brain. Clearly, we are just scratching the surface of understanding the reasons for any association signal in this region of the genome. It is important to remember that the DAOA/G30 locus was cloned from a region that has shown linkage in a number of studies, giving prior probability to association analyses, and that association has been reported in samples from a number of corners of the world. Expression may be restricted to discrete times in development and may not be present in abundance in middle-aged brains. It is also possible, as noted by Mike Owen, that the association signal reflects variation that impacts on a gene or genetic network not yet fully characterized.

This study by Craddock and colleagues makes the case for variation in the gene being related to nondiagnostic aspects of psychopathology, consistent with the reasonable expectation that genes for mental illness will not respect DSM-IV boundaries. Nevertheless, the confirmation of a role for this locus in the pathophysiology of psychiatric disorders will not be based on...
statistics but on evidence that genetic variation impacts on the biology of brain functions related to the psychopathology in question. We recently reported evidence that the SNP in the 2002 report by Chumakov et al., 2002 that showed association in both of their clinical samples—M10—is associated with cognitive function in a large family sample and with physiologic activation of the medial temporal lobe measured with fMRI even in normal subjects (Goldberg et al., 2006). These associations were not found for SNPs that were negative in the Chumakov et al. study, and the pattern of association with risk alleles was in the direction of abnormalities associated with schizophrenia and with pharmacological NMDA antagonism. In our view, there is dense smoke in the DAOA locus, though fire has yet to be conclusively observed.

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