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Neuronal cell adhesion genes

Key players in risk for schizophrenia, bipolar disorder and other neurodevelopmental brain disorders?

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The major mental disorders, schizophrenia and bipolar disorder, are substantially heritable. Recent genomic studies have identified a small number of common and rare risk genes contributing to both disorders and support epidemiological evidence that genetic susceptibility overlaps between them. Prompted by the question of whether risk genes cluster in specific molecular pathways or implicate discrete mechanisms, we and others have developed hypothesis-free methods of investigating genome-wide association datasets at a pathway-level. The application of our method to the 212 experimentally-derived pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database identified significant association between the cell adhesion molecule (CAM) pathway and both schizophrenia and bipolar disorder susceptibility across three GWAS datasets. Interestingly, a similar approach applied to an autistic spectrum disorders (ASDs) sample identified a similar pathway and involved many of the same genes. Disruption of a number of these genes (including NRXN1, CNTNAP2 and CASK) are known to cause diverse neurodevelopmental brain disorder phenotypes including schizophenia, autism, learning disability and specific language disorder. Taken together these studies bring the CAM pathway sharply into focus for more comprehensive DNA sequencing to identify the critical genes, and investigate their relationships and interaction with environmental risk factors in the expression of many seemingly different neurodevelopmental disorders.

Key words: schizophrenia, bipolar disorder, genome-wide association, molecular pathway analysis, cell adhesion molecules, neurexin-1

Abbreviations: GWAS, genome-wide association studies; SRT, SNP-ratio test; KEGG, Kyoto Encyclopedia of Genes and Genomes; CAMs, cell adhesion molecules; ASDs, autistic spectrum disorders

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Taken together the major mental disorders, schizophrenia and bipolar disorder, affect almost 2% of the adult population and are a major cause of global disability. In the absence of a clear understanding of the pathophysiological processes involved, diagnosis is based on a clinical assessment of symptoms (e.g., delusions, hallucinations, depression and mania) and course of illness. Modern treatments, which emerged in the mid-twentieth century, although efficacious for many, were discovered serendipitously and are far from perfect as both disorders are still associated with substantial morbidity and premature mortality. The statistics are sobering: people with severe mental illness die on average 25 years earlier than those in the general population. Intensive investigation of known mood stabilizers (e.g., lithium) and antipsychotic medications (e.g., haloperidol and clozapine) has yet to translate into better understanding of aetiology or a breakthrough in development of new therapies leading to improved outcome.

What unites these disorders is that they are substantially heritable.[1] The emergence of molecular genetics offered the exciting possibility of identifying the causative genes as a key to understanding the biology involved. As has been the case with most complex genetic disorders, progress has been slow but has gathered pace as our ability to investigate the genome has improved and collaborative efforts have generated large sample sizes. Within five years we have advanced from looking through a narrow aperture at hundreds of polymorphic markers, in a

selected region or widely scattered across the chromosomes in studies involving hundreds of individuals, to being able to examine most common genomic variation (>85%), captured by a million genetic markers in large-scale studies of thousands of patients. A number of common risk variants, albeit of small effect, have emerged from genome-wide association studies (GWAS) in schizophrenia and bipolar disorder, respectively.[2-6] Some genes (e.g., ZNF804A,[2] CACNA1C[6]) have been implicated in both disorders and there may be many more. Indeed, the genetic architecture of these disorders may include a substantial polygenic component involving thousands of common alleles of very small effect, many of which increase susceptibility to both schizophrenia and bipolar disorder.[3] The bad news is that confirming such small effects may not be feasible even with very large sample sizes (n>100,000 patients). Having only a partial picture of the genes involved is likely to be a substantial barrier to translational research.

What is more biologically informative than linking a gene or chromosomal region to a disorder is knowing if these small effects are concentrated in genes relating to a specific mechanism or implicating specific molecular pathways. Many groups have been developing pathway-based approaches to analyzing genome-wide association datasets and our SNP-ratio test (SRT) is just one such approach.[7-13] The SRT can be applied flexibly to different pathway resources as a hypothesis-free test, which simply identifies whether there are more nominally significant SNPs in genes mapping to that pathway in cases in a GWAS dataset than to genes mapping to all other indicated pathways.[7] Applying this approach to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database of 212 experimentally-derived pathways we captured information on 4,760 genes.[14] We performed a discovery analysis in the International Schizophrenia Consortium dataset, with nominally significant pathways being replicated in an independent, large schizophrenia GWAS dataset.[4,5] Pathways with confirmed association were then tested in a bipolar disorder GWAS dataset to investigate overlap between these conditions.[15] The first step analysis identified 47 nominally significant pathways of which five were associated in the independent schizophrenia dataset. Of these five, only the cell adhesion molecule (CAM) pathway (hsa04514) ($p = 0.001$) exceeded a conservative testing for multiple testing. Of note, one of the other five identified pathways, the 'Tight Junction' pathway (hsa045130) overlaps with the CAM pathway at a molecular level and is involved in many of the same functions. In all, 28 CAM genes of 110 contributing to the analysis had significantly associated SNPs in both schizophrenia datasets and 14 SNPs across 6 CAM genes (NRXN1, CDH4, GLG1, HLA-DQA2, CNTN1 and PDCD1LG2) shared the same risk alleles (see **Fig. 1**). Association at a pathway-level was confirmed with bipolar disorder although only approximately half of the genes implicated in the discovery analysis showed evidence of association in the bipolar disorder dataset. Further work is required in additional samples to investigate whether this difference represents a lower total burden of contributory risk variants in bipolar disorder (a less severe phenotype) or whether different genes or mechanisms within the pathway may explain phenotypic differences between the disorders.

That cell adhesion molecules (CAMs) would be implicated in the aetiology of schizophrenia and bipolar disorder is hardly surprising. There is strong evidence, particularly for schizophrenia, to support a neurodevelopmental contribution to aetiology and known (albeit weak) environmental risk factors include maternal starvation, infection and birth trauma (reviewed in ref. 16). There is growing support, including from neuropathological studies, to suggest that psychosis may be a disorder that involves aberrant brain wiring or dysconnectivity (reviewed in ref. 17). Cell adhesion molecules have an important role in brain development including in axonal/dendrite growth, synapse formation and plasticity. Neuronal CAMs are also known to have an important role in neurotransmission, including at glutamatergic and GABAergic synapses, which have been implicated in schizophrenia.[18]

So what can we learn from the 28 CAM genes contributing to the pathway-level association in our analysis? The individual effect sizes at each gene involved are small. Taking stock of evidence from other common disorders (e.g., type 2 diabetes) these early findings are unlikely to be helpful for risk prediction or diagnosis.[19] Their principle utility may be in understanding the aetiology of schizophrenia, bipolar disorder and possibly other neurodevelopmental brain disorders. So what do we know about these genes? Many of the genes involved in this pathway have been implicated in neuropsychiatric disorders or other brain phenotypes.[20] For example, this is the case for two of the five genes where SNPs directly replicate across GWAS samples. The sixth gene, HLA-DQA2 maps to the major histocompatibility complex (MHC) region on chromosome 6p, which has been implicated in neuronal plasticity, but presents challenges for genetic mapping.[21] Disruption of the NRXN1 gene has been reported in both schizophrenia and autism cases or families (see below). Axonal neurexins form transmembrane complexes with neuroligins on dendrites and are required for the formation of synaptic contacts and for efficient neurotransmission-including maintaining postsynaptic NMDA receptor function. The gene is a direct interactor of the cytoskeleton membrane scaffolding protein gene CASK (OMIM:300171), which is associated in both schizophrenia samples in our study, but is assigned to the "Tight Junction" pathway in KEGG. CASK may have a role in synaptic plasticity by coupling synaptic vesicle exocytosis to neuronal cell adhesion.[22] CDH4 is a classical cadherin thought to be involved in brain segmentation and neuronal outgrowth. This locus has not received particular support from prior linkage or association studies, but curiously association between a SNP at this gene and total cerebral brain volume was the only genome-wide significant finding to emerge from a GWAS study of brain aging using MRI and cognitive assessment of 705 healthy participants from the Framingham study.[23] Reduced brain volumes are a recognized feature of schizophrenia and this may point to a role in maintenance rather than formation of neuronal connections.

Several other recent genomic studies have provided additional support for

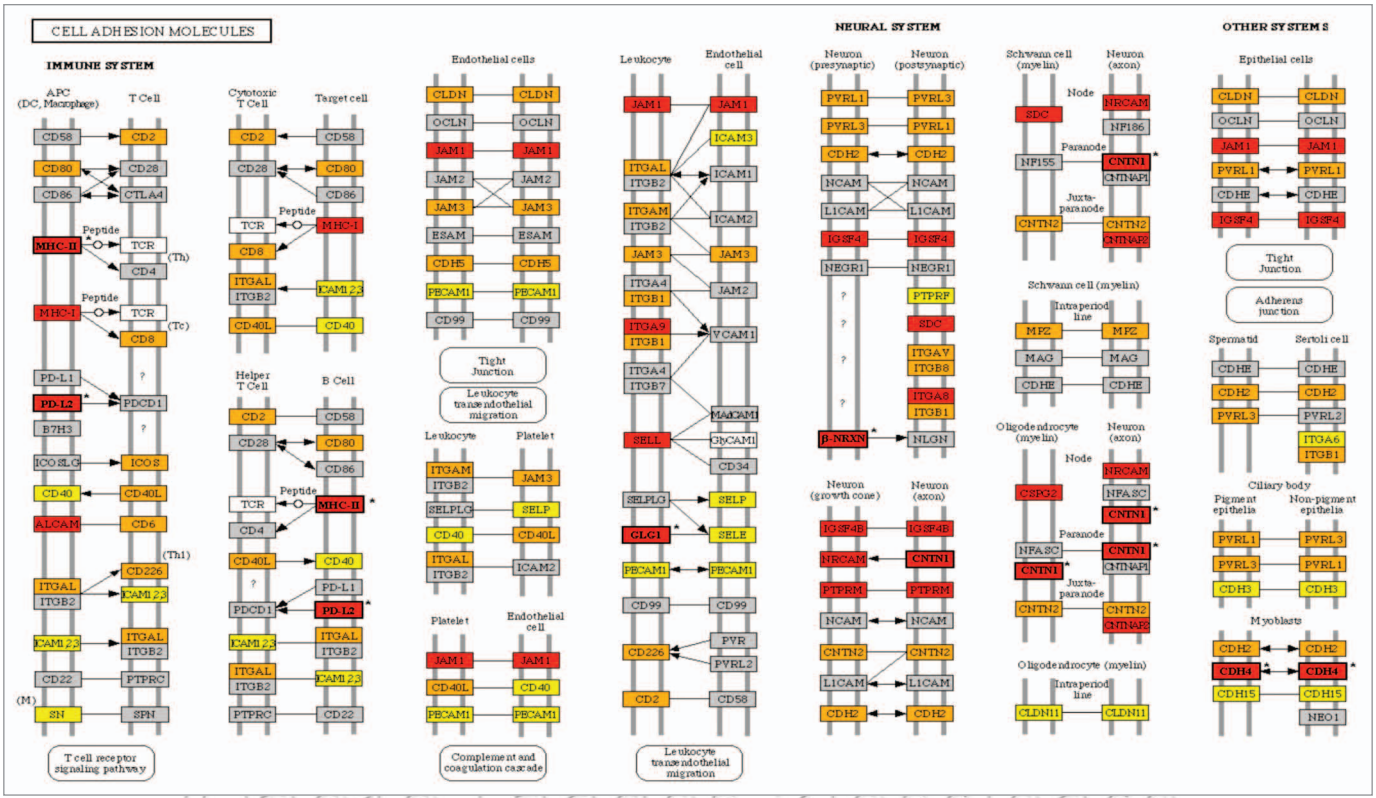

Figure 1. Genes involved in KEGG Cell Adhesion Molecules pathway (www.genome.jp/dbget-bin/www_bget?pathway+hsa04514) Color scheme: red, significant SNPs in gene in both ISC and GAIN datasets; red with emboldened border and gene name, replication of significant SNPs* in gene in ISC and GAIN datasets; orange, significant SNPs in gene in ISC dataset only; yellow, significant SNPs in gene in GAIN dataset only; grey, no significantly associated SNPs in gene for either dataset/No. SNPs in gene tested/Gene not included in SRT *Replication = same risk allele associated with SZ in the two datasets.

involvement of CAMs in both bipolar disorder and autistic spectrum disorders (ASDs). A recent GWAS study in bipolar disorder and subsequent replication efforts have provided some support for association with CDH7.[24] A GWAS study in ASDs identified association with the chromosome 5p14.1 region containing other members of the cadherin superfamily, CDH 9 and CDH 10.[25] Wang and colleagues went further and in a strategy not dissimilar to our approach applied two pathway-based association analyses to their genotype data. In the first they identified that a group of 25 cadherin genes showed more significant association with ASDs than all other genes ($p = 0.02$), a signal that was enriched by including eight neurexin family genes ($p = 0.004$). The result was confirmed using a second formal pathway-association method.[25] Although the methodologies differed, the genes NRXN1, CNTNAP2 and CDH4 were common to the risk pathways identified across the ASDs by Wang and colleagues in their study and in our investigation of schizophrenia and bipolar disorder.

These findings add to growing molecular evidence for overlap between childhood-onset neurodevelopmental disorders (e.g., ASDs) and disorders of typical onset in adolescence or early adulthood like schizophrenia and bipolar disorder (reviewed in ref. 26). The studies performed by our group and Wang and colleagues only investigated common SNP variation and there is increasing realization of the potential importance of rare, highly penetrant structural variation in the aetiology of neurodevelopmental brain disorders. A number of microdeletion/microduplication syndromes have been identified that are associated with ASDs, schizophrenia, intellectual disability, specific language delay and other neurodevelopmental phenotypes (reviewed in ref. 27). Many of these disrupt genes involved in CAM pathways. For instance, disruption of NRXN1 has been reported in cases of both autism and schizophrenia; CASK deletions are reported in individuals with learning disability and brain malformation phenotypes; and disruption of CNTNAP2 has been reported in autism, language disorder and schizophrenia. Taking NRXN1 specifically, many of its post-synaptic binding partners (the neuroligins) and their postsynaptic density interactors (e.g., SHANK3) have been identified as rare causes of ASDs (reviewed in ref. 28).

Together these studies raise the possibility that susceptibility to ASDs and psychotic disorders may involve overlapping molecular aetiology where an accumulation of small effects from many common genetic risk variants or more highly penetrant mutations induce neuronal dysconnectivity by disrupting CAM function. This raises exciting new avenues for research into the mechanisms and

processes involved. The next key step may be large-scale DNA sequencing of these genes and their interactors to identify whether smaller structural or point mutations, undetectable in the current studies, also contribute to risk. By neccessity these studies will require detailed phenotypic information and encompass different phenotypes to explore how genetic risk is expressed as seemingly different neurodevelopmental endophenotypes. This can inform functional studies and identification of potential therapeutic targets. Finally, although the common risk genes identified by our analysis are of limited clinical utility, more highly penetrant mutations although individually rare may be diagnostically significant across these neurodevelopmental disorders as is already being realised in ASDs.[29]

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