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Combined analysis of exon splicing and genome wide polymorphism data predict schizophrenia risk loci

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ABSTRACT

Schizophrenia has a strong genetic basis, and genome-wide association studies (GWAS) have shown that effect sizes for individual genetic variants which increase disease risk are small, making detection and validation of true disease-associated risk variants extremely challenging. Specifically, we first identify genes with exons showing differential expression between cases and controls, indicating a splicing mechanism that may contribute to variation in disease risk and focus on those showing consistent differential expression between blood and brain tissue. We then perform a genome-wide screen for SNPs associated with both normalised exon intensity of these genes (so called splicing QTLs) as well as association with schizophrenia. We identified a number of significantly associated loci with a biologically plausible role in schizophrenia, including MCPH1, DLG3, ZC3H13, and BICD2, and additional loci that influence splicing of these genes, including YWHAH. Our approach of integrating genome-wide exon intensity with genome-wide polymorphism data has identified a number of plausible SZ associated loci.
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1. Objectives

Schizophrenia (SZ) is a complex mental disorder of brain functioning with patients presenting a wide spectrum of phenomena including delusions, hallucinations, disorganised speech and behaviour, negative symptoms and cognitive impairment. Heritability estimates on the order of 0.8 indicate a major genetic component (see Cardno and Gottesman, 2000; Sullivan et al., 2003 for an overview), but the genetic effects are likely to be heterogeneous between subgroups of affected individuals and to be of small effect individually (Need et al., 2009). Recent genome wide association studies (GWAS) have identified SZ risk loci on several chromosomes (Kirov et al., 2009; Athanasiu et al., 2010; Ripke et al., 2011) supporting the notion that the combination of numerous variants, each of small effect size, contribute to disease etiology (Manolio et al., 2008).

As with most other complex diseases, genetic variants showing robust and validated association with SZ currently explain only a small fraction (<2%) of the heritability (Visscher et al., 2011). However, it is has been estimated that 23% of the variation in liability to SZ may be captured by common SNPs (Lee et al., 2012), suggesting that more powerful or alternative methods of analysis may be required to identify additional variants contributing to the risk of SZ.

It is well known that SNPs can alter expression levels of genes (expression quantitative trait loci or eQTLs (Emilsson et al., 2008)), and a recent study showed that SNPs with evidence of association with SZ are more likely to be eQTLs, suggesting that SZ risk is mediated in part by these genetic variants (Richards et al., 2012). At the sub-transcript level there is a novel and under-reported class of variant known as splicing (s)QTLs (Kwan et al., 2008): variants associated with altered expression level of exons within a gene (i.e., differential splicing), resulting in heritable isoforms. Alternative splicing of messenger RNA is predicted to affect greater than 80% of all human multi- exon genes (Pan et al., 2008; Wang et al., 2008) and provides a mechanism by which a relatively small number of genes can produce a diverse range of proteins. A recent study

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showed that there is a substantial amount of allelic heterogeneity for regulatory effects on a single gene and these effects are independent for exons within the same gene (Lappalainen et al., 2013), and that transcriptional activity and transcript usage is often controlled by different regulatory elements of the genome. Alternatively spliced genes are important entities in pathways relevant to schizophrenia, including the dopaminergic signal transduction pathway (Dal Toso et al., 1989; Giros et al., 1989; Monsma et al., 1989; Talkowski et al., 2010) and the Neuregulin/ERBB signalling pathway (Steinthorsdottir et al., 2004; Law et al., 2007; Sundvall et al., 2008). Alternatively spliced genes such as Neuregulin (*NRG1*; >17 transcripts from one gene) have been studied extensively in the context of SZ; however there has been no previous assessment of genome wide alternative mRNA splicing events with regard to SZ.

Here we describe a novel approach to identifying genetic loci associated with SZ, utilising genome-wide exon expression array intensity data and SNP genotype data. The proposed method comprises three stages (see Fig. 1):

- In stage one, we measured exon intensity data for all known genes using data collected from 122 blood samples (79 cases, 43 controls) and a separate cohort of 74 brain tissue samples (37 cases and 37 controls). Exons with significantly different normalised exon intensities between SZ cases and controls were identified, with the requirement that differences should be consistent in both blood and brain tissue samples.
- In stage two we hypothesised that observed exon intensity differences may reflect the effects of genetic variants in trans-acting genes that influence the splicing machinery. We therefore performed a genome-wide association analysis (GWAS) for common SNPs tagging such regulatory variants and hence associated with exon intensity measured in stage 1.
- In stage three, SNPs identified in stage two were tested for association with SZ in a larger independent dataset. We proposed that SNPs showing significant association with SZ in the final stage would represent genetic effects on exon-splicing and thus exon intensity (sQTLs).

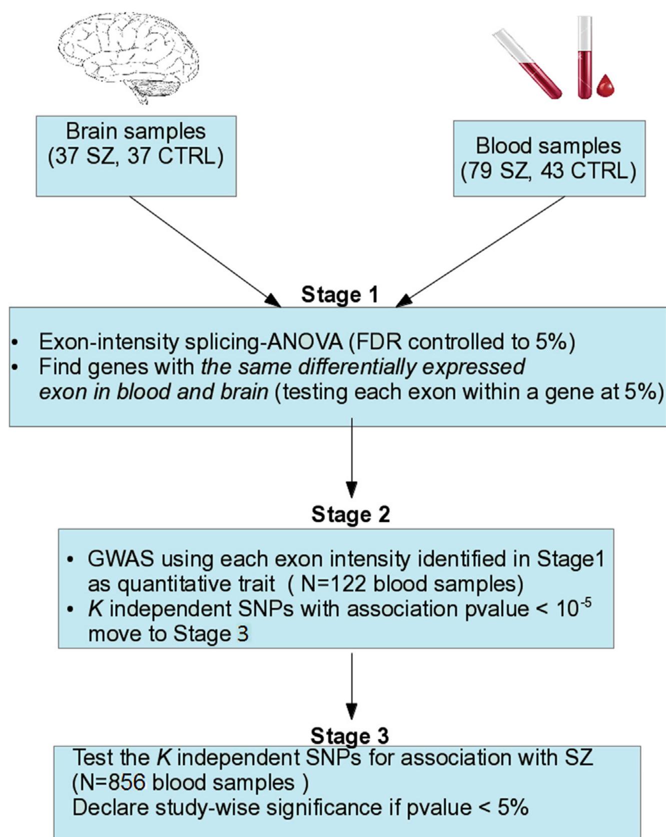

Fig. 1. Summary of the three stage method of identifying splicing QTLs associated with SZ.

This method expands on our previous approach of looking for “consistency” across multiple analyses (Oldmeadow et al., 2011) which has shown promise as an alternative to larger and larger sample sizes. In our current study we have a valuable but limited collection of expression data from post-mortem brain tissue of SZ cases and controls. It is unlikely that we would be able to get such collections numbering in the tens of thousands or more, as in current GWAS meta-analyses; how then to best make use of this data? We proposed this approach of consistency as a way of using small collections and “triangulating” between different samples and different methods to identify valid signals.

2. Materials and methods

2.1. Exon-expression profiling

Peripheral blood mononuclear cells (PBMCs) were collected by the Australian Schizophrenia Research Bank (ASRB) ($n = 86$ schizophrenia/schizoaffective disorder cases and $n = 51$ healthy controls) from which RNA/DNA was extracted. We restricted analyses to the 122 samples with genotype data available (described below). All ASRB participants were assessed as previously described (Loughland et al., 2010) using the Diagnostic Interview for Psychosis, a structured clinical interview yielding diagnoses according to DSM-IV criteria (Castle et al., 2006). Non-psychiatric controls were excluded if there was a personal or family history of either psychosis or bipolar disorder. Post-mortem dorsolateral prefrontal cortex (Broadmann Area 46; BA46) tissue samples were collected by the NSW Tissue Resource Centre (University of Sydney) ($n = 37$ matched schizophrenia/schizoaffective disorder cases and controls) and has been previously described (Weickert et al., 2010). All post-mortem cases had diagnoses of schizophrenia or schizoaffective disorder according to DSM-IV criteria. Subjects with a significant history of drug or alcohol abuse or neurological disorder were excluded, as were control subjects if there was a history of alcoholism or suicide.

Two hundred and fifty nanograms of RNA was obtained from the ASRB for the PBMC samples. Two hundred and fifty nanograms of RNA was also extracted from the grey matter of the BA46 tissue and processed using the Applause WT-Amp Plus ST assay (NuGEN Technologies, San Carlos, CA), according to the manufacturer’s instructions. Five micrograms of amplified cDNA was fragmented, labelled and hybridised to Affymetrix Human Exon 1.0 arrays. Arrays were scanned using Affymetrix GeneChip Scanner 3000. CEL files were generated and exported to Affymetrix Expression Console software for normalisation. Normalised files were imported to GeneSpring GX11 (Silicon Genetics, Redwood City, CA) for analysis.

2.1.1. Genome-wide genotyping

DNA extracted from the ASRB’s PBMCs samples was also obtained for this study; there were 934 individuals with a blood sample and verified diagnoses of schizophrenia or schizo-affective disorder. Genotyping was performed using the Illumina 610-Quad Bead-Chip array, with genotype quality control (QC) performed using PLINK (Purcell et al., 2007). A total of 598,821 SNPs were genotyped, of which 13,423 were excluded due to call rate <0.95,

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