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Research article

A *cis*-eQTL in *AHI1* confers risk to schizophrenia in European populations

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HIGHLIGHTS

- *Rs11154801* was significantly associated with *AHI1* expression in healthy individuals.
- *Rs11154801* showed marginal association with schizophrenia.
- The sample size in the current study was large.

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ABSTRACT

Schizophrenia is a devastating mental disorder, with heritability as high as 80%. Although genome-wide association studies have identified multiple promising risk variants of schizophrenia, they could only explain a small portion of the disease heritability, and other variants with low to moderate effect remain to be identified. Abelson helper integration site 1 (*AHI1*) is highly expressed in mammals throughout the developing brain, with lower expression continuing into adulthood. Besides, previous evidence suggested that AHI1 expression was changed in schizophrenia patients. Furthermore, association signal between *AHI1* variants and schizophrenia has been reported in several European samples. In the present study, we first analyzed two expression quantitative trait loci (eQTL) datasets in healthy individuals and investigated the associations of eQTL of AHI1 with schizophrenia in independent European samples. We observed that a *cis*-eQTL of *AHI1*, *rs11154801*, showed significant association with AHI1 expression in both datasets ($P < 5E\text{-}05$). Genetic evidence exhibited that *rs11154801* was significantly associated with schizophrenia risk in both the discovery sample (9394 cases and 12462 controls, $P = 0.046$, OR = 0.958, 95% CI = 0.918-0.999) and the replication sample (3240 cases and 14786 controls, $P = 0.024$, OR = 0.949, 95% CI = 0.870-0.990). When the discovery and replication samples were pooled together, this association was further strengthened ($P = 0.004$, OR = 0.949, 95% CI = 0.916-0.983). These results suggested that AHI1 is likely a risk gene for schizophrenia, at least in European populations.

1. Introduction

Schizophrenia is a devastating mental disorder characterized by psychotic features (delusions and hallucinations), dysfunction in normal affective responses and impaired cognitive functions, the prevalence of which is approximately 1% among world populations [1,2].

Family, twin, and adoption studies suggest that both genetic and environmental factors contributed to the etiology of schizophrenia, with high heritability up to 80% [3]. Through candidate gene association studies and genome-wide association studies (GWAS), multiple promising risk loci have been identified for schizophre-

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nia [4,5]. Next-generation sequencing, including exome sequencing and whole-genome sequencing, also detected a lot of schizophrenia susceptibility genes, especially rare variants [6]. However, all the identified risk variants could only explain a small portion of the disease heritability. Moreover, the underlying mechanisms by which genetic risk variants contribute to this disorder remain largely unknown.

Accumulating evidence strongly suggests that inherited variation that impacts on gene expression rather than protein structures/functions play an important part in susceptibility to schizophrenia [7], and schizophrenia susceptibility alleles are enriched among those loci affecting gene expression, which are also called expression quantitative trait loci (eQTL), as well [8]. Based on this observation, Luo et al. recently integrated genome-wide association studies (GWAS) and human brain eQTL data, and successfully identified two SNPs, rs2859365 (P = 1.40E-07) and rs1150711 (P = 6.85E-10) in *ZNF323* to be significantly associated with schizophrenia [9]. Nevertheless, other common risk variants with potentially small effect size, reflected as less significant association result, and/or with infrequent risk alleles, remain to be detected.

Abelson Helper Integration Site 1 (*AHI1*) is highly expressed in the cerebellum and cerebral cortex of adult human brain [10]. Nonsense and missense mutations in human *AHI1* were reported to cause Joubert Syndrome, an autosomal recessive brain disorder, the characteristics of which included mental retardation, ataxia and oculomotor apraxia [10]. Amann-Zalcenstein et al. found that the strongest linkage signal of schizophrenia in an Arab-Israeli sample harbors *AHI1* [11]. Similarly, Ingason et al. investigated seven loci in a cohort of Icelandic samples and detected significant association between AHI1 variants and schizophrenia [12], which was replicated in a larger European sample [13]. Analysis of two cohorts of schizophrenia samples from Spain and Germany reveled that *rs7750586* and *rs911507* were significantly associated with schizophrenia as well [14]. Moreover, associations between common variants of *AHI1* and affective disorders and autism have been also reported, while mental disorders share genetic components [15,16]. All these lines of evidence showed that *AHI1* is potentially a susceptibility gene for schizophrenia, which was further supported by functional findings. *Ahi1* knock-out mice displayed an anxiolytic-like phenotype, which might be due to reduced connectivity of the amygdala with other brain regions involved in processing of anxiogenic stimuli and inhibitory avoidance learning as revealed by fMRI [17,18]. Moreover, in *Ahi1* knock-out mice, neurotransmitters including serotonin and dopamine were significantly decreased in multiple brain regions (cortex, hipocampus, hypothalamus, brainstem, and amygdale) after postnatal day 10 [19].

In the present study, we focused on eQTL SNPs and performed genetic analyses of *AHI1* gene with schizophrenia. In detail, we first investigated eQTL SNPs that are located in or nearby the *AHI1* gene locus in two expression quantitative trait loci (eQTL) datasets in healthy individuals; and then explored whether these eQTL were associated with schizophrenia risk in independent European samples.

2. Methods and materials

2.1. Healthy subjects for eQTL analysis

In order to identify potential *cis*-eQTL SNPs for *AHI1*, we screened two publicly available databases. For both databases, lymphoblastoid cell lines (LCLs) were derived from peripheral blood lymphocytes on each participant. Cells were harvested at log phase and total RNA was extracted for genome-wide eQTL analysis.

The first database included 270 individuals with genotype data from the International HapMap Project, including 30 Caucasian trios of northern and western European origin (CEU), 30 Yoruba trios from Ibadan, Nigeria (YRI), and 45 unrelated Chinese individuals from Beijing University (CHB) as well as 45 unrelated Japanese individuals from Tokyo (JPT) [20]. Expression of genes was quantified with Illumina's human whole-genome expression (WG-6 version 1) arrays. Linear regression model was applied to analyze those potentially *cis*-acting SNPs. To determine the significance of the regression P values, 10,000 permutations were used for correction of multiple comparisons. We searched potential *cis*-eQTL within 100 kb upstream and downstream from *AHI1* gene locus on an online eQTL Browser (http://www.ncbi.nlm.nih.gov/projects/gap/eqtl/index.cgi) which presented SNPs exhibiting associations with gene expression (P-value < 0.05).

These *cis*-eQTL across *AHI1* identified in the first database were screened in another database for verification. The second database was comprised of 400 children from families recruited through a proband with asthma (http://www.sph.umich.edu/csg/liang/asthma/) [21]. All the subjects were of British decent. The Affymetrix HG-U133 Plus 2.0 chip was applied to quantify global gene expression. The association between SNPs and local gene expression was determined using the FASTASSOC component of MERLIN, and sex was also included in this model. The same P-value threshold (P < 0.05) was applied.

2.2. Case-control samples

Recently, the Psychiatric Genomics Consortium (PGC) schizophrenia working group performed a large-scale GWAS on schizophrenia in populations of European ancestry [22]. The discovery cohort included 21856 individuals, 9394 cases and 12462 controls; the replication cohort consisted of 8442 cases and 21,397 controls from 14 European countries. Detailed information, including sample source, diagnostic criteria, genotyping method and statistical analysis were seen in the original manuscript [22]. We extracted the results of all available common SNPs (minor allele frequency (MAF) > 0.05) within 100 kb upstream and downstream from *AHI1* gene locus from the discovery cohort (9394 cases and 12462 controls) as our screening data.

For replication analysis, we enrolled nine independent case-control samples, which genotyped common variants of AHI1 in 3240 cases and 14786 healthy individuals from two studies [13,23]. All the subjects were of European ancestry except one Korean sample from Porcelli et al. sutdy [23]. Briefly, the nine independent samples were: 1) the German sample (495 cases and 1272 controls); 2) the Icelandic sample (589 cases and 11491 controls); 3) the UK sample (93 cases and 88 controls); 4) the Finnish sample-general (59 cases and 147 controls); 5) the Finnish sample-isolate (123cases and controls); 6) Italian sample (84 cases and 89 controls); 7) the Dutch sample (713 cases and 643 controls); 8) the Scottish sample (658 cases and 661 controls) [13] and 9) the Korean sample (426 cases and 345 controls) [23]. All the samples had no overlap with each other. All replication samples showed no overlap with our discovery samples. Totally, 12634 patients of schizophrenia and 20429 healthy controls were included in this study. Notably, a total of 12 cohorts of European samples were recruited in Ingason et al. (2010) study [13]; however, 4 of them overlapped with those used in the GWAS, they were Danish samples (456 cases and 995 controls), Norwegian samples (264 cases and 181 controls), German (Bonn, 483 cases and 367 controls) and British samples (479 cases and 2936 controls), which were thus excluded in the replication analysis in the current study. All studies received ethical approvals from their institutions, and subjects in each study provided written informed consent for participation.