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SHORT REPORT

Support for involvement of the *AHI1* locus in schizophrenia

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Recently, markers in the Abelson Helper Integration Site 1 (*AHI1*) region were shown to be associated with schizophrenia in a family sample of Israeli-Arabs. Here, we report a study evaluating the relevance of the *AHI1* region to schizophrenia in an Icelandic sample. Seven markers shown to confer risk in the previous report were typed in 608 patients diagnosed with broad schizophrenia and 1504 controls. Odds ratios for the overtransmitted alleles in the Israeli-Arab families ranged from 1.15 to 1.29 in the Icelandic sample. After Bonferroni correction for the seven markers tested, two markers were significantly associated with schizophrenia. Thus, our results are in general agreement with the previous report, with the strongest association signal observed in a region upstream of the *AHI1* gene.
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Introduction

The pathogenesis of schizophrenia involves complex interaction of genetic variants and environmental factors.[1] Several susceptibility loci for schizophrenia have been identified using linkage analysis, and a recent meta-analysis showed greater consistency across studies than previously had been recognized.[2] Linkage to chromosome 6q is seen in several studies[3–5] and although extensive genomic distance is implicated in the different reports, a 6q15-23.2 locus is supported by meta-analysis.[2] A recently published analysis of inbred schizophrenia pedigrees of Israeli-Arab origin lends further support to the linkage on 6q, with a peak marker at 6q23.3.[6,7] Subsequent family-based association study of the same pedigrees, using SNP markers from the 6q23.3 locus, revealed significant association of markers in a linkage disequilibrium (LD) block harbouring the Abelson Helper Integration Site 1 (*AHI1*) gene.[8] The *AHI1* gene is expressed at high levels in both foetal and adult human brain.[9] Mutations in the gene have been shown to cause Joubert Syndrome, an autosomal recessive brain disorder.[10]

In the association analysis by Amann-Zalcenstein *et al*,[8] seven SNP markers were significant at single marker level after Bonferroni correction in the original linkage sample. The same seven markers have now been typed on a large sample of Icelandic schizophrenia patients and controls.

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Subjects and methods

Human samples

Approval for the study was granted by the National Bioethics Committee of Iceland and the Icelandic Data Protection Authority and informed consent was obtained for all participants. For this study, 608 patients with a

broad schizophrenia diagnosis (schizophrenia ($N=574$), schizoaffective disorder ($N=25$), schizotypal features ($N=6$), unspecified functional psychosis ($N=3$)) were identified through referrals to in- and outpatient services. Diagnoses were assigned according to the Research Diagnostic Criteria (RDC)[11] through the use of the lifetime version of the Schizophrenia and Affective Disorders Schedule (SADS-L).[12] Two groups of controls were used: one set ($N=1045$) was chosen randomly from the Icelandic population; a second group ($N=459$) was recruited from longevous Icelanders aged 90 years and older. Demographic and relatedness information for cases and both sets of controls is shown in Supplementary Table 1. Neither control group was screened specifically for the presence of psychiatric illnesses.

Genotyping and statistical analysis

DNA was isolated from whole blood or lymphoblastoid cell lines using an extraction column method (Qiagen Inc., Valencia, CA, USA) and SNP genotyping was carried out using the Centaurus platform (Nanogen Inc., San Diego, CA, USA). Owing to assay failure, markers with $r^2=1$ in the CEU Hapmap sample were chosen as surrogates for two of the original markers. Average marker yield was 95.7% (see Supplementary Tables 2 and 3 for additional information on yield) and no significant deviation from Hardy–Weinberg equilibrium was detected in controls (see Supplementary Table 4 for more information on Hardy–Weinberg equilibrium).

To test the hypothesis that the at-risk alleles identified by Amann-Zalcenstein *et al*[8] were found at elevated frequencies in Icelandic schizophrenics, the signed square-root of a standard likelihood ratio test statistic was used to calculate one-sided *P*-values. Partial information about missing genotypes, available because the seven markers were in LD, was also incorporated into the test statistic using a likelihood approach implemented in the program NEMO.[13] Inclusion of this partial information allowed findings for each marker to be based on the same set of individuals and, therefore, to be more comparable. Results incorporating this partial information were not substantially different from those arrived at using information from only a single marker. Haplotype analyses were carried out using a likelihood procedure in NEMO as described in an earlier publication.[14]

The likelihood ratio test statistic discussed above was computed without taking the relationships both between and within the affected and control groups into account. This was adjusted for by using the Icelandic genealogy to carry out one million simulations of the test statistic under the null hypothesis of no allele frequency difference between cases and controls. The variance of this empirical null distribution was then used in the calculation of *P*-values. See Grant *et al*[15] for further details.

Results

Single marker association results are summarized in Table 1. As there were no significant differences between the two control groups (not shown), results are presented for the combined control group only.

For the overtransmitted alleles in the study of Amann-Zalcenstein *et al*,[8] odds ratios (ORs) ranged from 1.15 to 1.29 in the Icelandic sample. For all seven markers, *P*-values were <0.05, and for two markers, the difference was significant after Bonferroni correction for seven tests.

Five of the seven markers were also used for haplotype association (rs11154801 and rs9647635 were not included as they were nearly equivalent to rs7750586). For 138 haplotypes made up of two to five markers and with frequencies of at least 1%, no association to schizophrenia notably stronger than that found in single-marker analyses was observed (Supplementary Table 5).

The LD structure of the *AHI1* locus in the CEU Hapmap sample is depicted in Figure 1 along with known gene structures and human mRNAs in the region as annotated in the UCSC browser, using Build 36 sequence. The *C6orf217* record has been discontinued since the publication

Table 1 Allelic association to schizophrenia for the seven tested markers in 608 patients and 1504 controls

Marker	*Allele*[a]	*Allelic P-value*	*OR*[b]	*Affected frequency*	*Control frequency*
rs9321501	A	0.017	1.17 (1.01–1.35)	0.553	0.515
rs11154801	C	0.037	1.15 (0.99–1.33)	0.679	0.649
rs7750586	A	0.030	1.15 (0.99–1.34)	0.680	0.648
rs9647635	A	0.038	1.15 (0.99–1.33)	0.678	0.647
rs7739635	C	**0.0032**	1.24 (1.06–1.44)	0.702	0.656
rs9494335, surrogate for A-rs9494332 ($r^2=1.0$[c])	A	0.012	1.20 (1.03–1.40)	0.716	0.678
rs9399158, surrogate for A-rs1475069 ($r^2=1.0$[c])	C	**0.00076**	1.29 (1.10–1.52)	0.743	0.691

P-values are one-sided and corrected for relatedness between subjects but not for number of tests. Owing to assay failure, perfect proxy markers were chosen for two of the original markers, based on Hapmap genotype data for the CEU sample. Allelic *P*-values that remain significant after Bonferroni correction are indicated in bold print.
[a]Overtransmitted in Amann-Zalcenstein *et al*.[8]
[b]Allelic odds ratio assuming a multiplicative model with 95% confidence interval.
[c]In the CEU Hapmap sample.

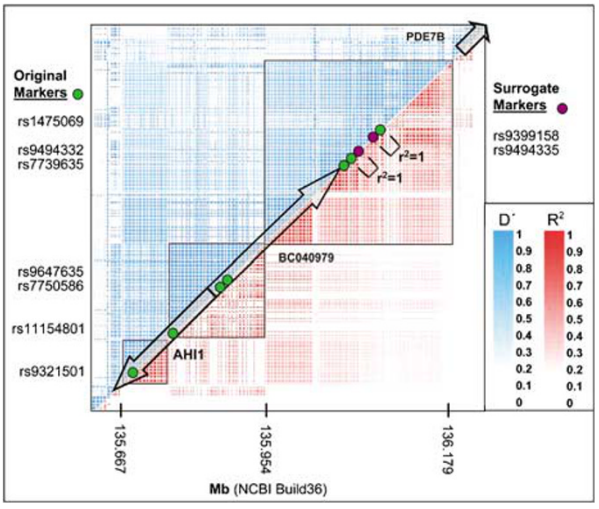

Figure 1 LD around the *AHI1* locus. All seven markers from the Amann-Zalcenstein study[8] are within the same LD block, although substructures can be detected. Two genes, *AHI1* and *BC040979*, are found within the boundaries of the LD block. Furthermore, regulatory elements for the phosphodiesterase 7B (*PDE7B*) gene may be in the LD block. The LD map is derived from Hapmap phase II genotypes (release 19) from the CEU sample.

of Amann-Zalcenstein *et al*,[8] and the hypothalamic mRNA, *BC040979*, now inhabits the region immediately upstream of the *AHI1* gene. Although markers are in LD throughout the locus, three substructures of stronger LD are observed. Pairwise correlation between the markers in the Icelandic data set is summarized in Supplementary Figure 1, and shows a pattern consistent with the CEU Hapmap LD structure. No significant differences in LD were seen between patients and controls (data not shown).

Discussion

Studying an inbred population, Amann-Zalcenstein *et al* were able, by following linkage analysis with family-based association, to identify seven markers strongly associating with schizophrenia.[8] The association was found in an LD block, spanning Mb 135.667–136.179 (NCBI Build 36) on chromosome 6 harbouring the *AHI1* gene. We found all seven significantly overtransmitted alleles from their study in higher frequency in Icelandic schizophrenics than in controls (Table 1). In the Icelandic sample, the ORs for the tested alleles ranged from 1.15 to 1.29, and two of the markers remained significantly associated to schizophrenia after Bonferroni correction for seven tests. The two significant markers, rs7739635 ($P=0.0032$, $RR=1.24$) and rs9399158, a perfect proxy for rs1475069, ($P=0.00076$, $RR=1.29$) are located in a substructure of the large LD block containing the *AHI1* gene (Figure 1). This substructure, spanning Mb 135.954–136.179, is upstream of the *AHI1* gene and contains one uncharacterized mRNA isolated from hypothalamus, and may contain regulatory elements for both the *AHI1* and phosphodiesterase 7B (*PDE7B*) genes. Thus, although our findings are in line with the previous report, the strongest association signal is observed in a substructure of the large LD block initially implicated in the disease.

The *AHI1* gene has already been pointed out as a plausible schizophrenia susceptibility gene at this locus. The *BC040979* transcript is a candidate as well; however, it needs further characterization. Finally, the *PDE7B* gene encodes a cAMP-specific phosphodiesterase, thought to be involved in neuronal cAMP regulation.[16,17] Although not in LD with the significant markers, *PDE7B* should not be ruled out as a candidate, as the region showing significant association is upstream of *PDE7B* and therefore may contain regulatory elements for the gene.

In summary, we have presented results from a case–control association analysis that are in line with data presented by Amann-Zalcenstein *et al*,[8] showing that the *AHI1* locus contributes to schizophrenia. Still, three neighbouring genes, *AHI1*, *BC040979* and *PDE7B* can be affected by variants in this region and none of these genes can be excluded.

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Electronic-Database Information

We obtained SNP genotypes for 30 Utah trios of Northern-European origin from the HapMap project database (http://www.hapmap.org/), and information on chromosomal coordinates and human gene annotation from the UCSC Genome Browser (http://genome.ucsc.edu/).

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