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Brief report

Lack of association of NALCN genetic variants with schizophrenia

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ABSTRACT

NALCN (sodium leak channel, non-selective) is located on chromosome 13q (suggested linkage region for schizophrenia). We analyzed 21 polymorphisms in 464 schizophrenia subjects, 220 controls subjects and 119 small nuclear families. We observed nominal association with rs9518320 and rs9518331, suggesting that *NALCN* is not related to schizophrenia risk.

1. Introduction

Schizophrenia affects about 1.0% of the population worldwide. Evidence increasingly suggests that schizophrenia is a disorder of brain development and plasticity (Eichhammer et al., 2004). Genetic studies have recently begun to identify strong candidate risk genes for schizophrenia, and neurobiological studies of the normal and variant forms of these genes are advancing (Chumakov et al., 2002). Linkage and association studies have implicated several loci in the genome that likely harbor genes conferring risk for schizophrenia. A meta-analysis suggested the existence of susceptibility genes on chromosomes 8p, 13q and 22q (Badner and Gershon, 2002). Among other genes, the 13q region contains *G72* (or *DAOA* at 13q33.2). Several individual replication studies and a meta-analysis have supported the association of *G72* with schizophrenia, although the associated alleles and haplotypes are not identical across studies and some polymorphic variants are located outside of the gene.

In this region, 13q33.1, 4.1Mb upstream of *G72* is located in the *NALCN* (also known as *VGCNL1*). NALCN is a highly conserved protein in mammals (99% identity between human and rat). Close homologues are also found in invertebrates (Humphrey et al., 2007; Yeh et al., 2008). *NALCN* mRNA expression has been shown in the cerebral cortex and hippocampus in all neurons and layers (Lee et al., 1999; Lu et al., 2007). *NALCN* mutant mouse neonates do not display gross abnormalities in embryonic development, righting responses, spontaneous limb movement, and toe/tail pinch responses, but do not survive beyond 24 h after birth (Lu et al., 2007). *NALCN* encodes a voltage-independent, non-selective, non-inactivating cation channel permeable to sodium, potassium and calcium when exogenously expressed in HEK293 cells (Lu et al., 2007). *In vivo*, the NALCN channel appears to be the main source of the background sodium leak in the hippocampal neurons at rest and is important for neuronal excitability (Lu et al., 2007). Both hippocampal activity and neuronal excitability are processes strongly altered in schizophrenia (Eichhammer et al., 2004; Oxley et al., 2004). Because the function of *NALCN* is consistent with that manifested in some schizophrenia symptoms, and its location is within a suggestive chromosomal linkage region for schizophrenia, we hypothesized that *NALCN* may show a genetic association with schizophrenia. To test this hypothesis, we performed an association study using case–control and family-based approaches.

2. Methods

All recruitment and clinical assessments were conducted with written informed consent in accordance with the Declaration of Helsinki and approval of the institutional ethics review board. We recruited 464 subjects (male/female ratio = 2; age 36 ± 8, 82% Caucasians) with a DSM-III-R or DSM-IV diagnosis of schizophrenia at the Centre for Addiction and Mental Health ($n = 321$), Case Western Reserve University ($n = 94$) and

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Table 1
Genetic association of *NALCN* variants with schizophrenia risk. A1/2 = allele 1/2. HWE = Hardy–Weinberg equilibrium. MAF = minor allele (A2) frequency. LD = linkage disequilibrium block. T = transmitted. NT = non-transmitted. %T = percentage of transmissions.

Variant	A1	A2	HWE	MAF	Case–control sample							Family-based sample							
					LD	Case		Control		χ^2	*P*	LD	Allele 1			Allele 2			$\chi2$	*P*
						A1	A2	A1	A2				T	NT	%T	T	NT	%T		
rs9554752	C	T	0.05	0.36	–	600	328	278	162	0.28	0.60	–	81	80	50.3	45	46	49.5	0.02	0.90
rs17677552	G	A	0.04	0.32	1	630	298	297	141	0.00	0.98	1	90	88	50.6	42	44	48.8	0.07	0.79
rs686141	G	A	0.99	0.26	1	696	232	319	121	0.97	0.32	1	102	108	48.6	28	22	56.0	0.89	0.35
rs12867417	G	A	0.45	0.20	1	745	183	349	91	0.17	0.68	1	117	114	50.7	22	25	46.8	0.23	0.63
rs658213	T	C	0.62	0.24	1	712	212	319	119	2.88	0.09	1	111	113	49.6	24	22	52.2	0.11	0.75
rs614728	C	T	0.87	0.30	1	655	273	297	141	1.08	0.30	1	108	106	50.5	27	29	48.2	0.09	0.76
rs9513851	G	A	0.57	0.06	1	871	57	413	25	0.10	0.75	–	142	146	49.3	9	5	64.3	1.21	0.27
rs9518307	T	C	0.95	0.06	–	870	58	411	29	0.06	0.81	–	142	147	49.1	9	4	69.2	2.06	0.15
rs12584031	T	A	0.99	0.18	–	765	163	360	80	0.08	0.78	–	130	127	50.6	15	18	45.5	0.31	0.58
rs1452112	G	T	0.13	0.25	2	683	245	338	102	1.63	0.20	–	104	102	50.5	30	32	48.4	0.08	0.77
rs7317836	G	T	0.51	0.30	2	653	275	309	131	0.00	0.96	–	102	107	48.8	38	33	53.5	0.47	0.49
rs9518320	A	G	0.21	0.30	–	667	261	290	150	5.06	0.03	2	95	97	49.5	38	36	51.4	0.08	0.78
rs9518331	G	T	0.19	0.31	–	660	266	290	150	4.05	0.04	2	94	96	49.5	40	38	51.3	0.07	0.79
rs2584531	T	C	0.01	0.37	–	587	341	269	171	0.57	0.45	2	90	91	49.7	47	46	50.5	0.02	0.90
rs3916906	C	T	0.04	0.33	3	637	291	282	158	2.80	0.09	2	96	96	50.0	42	42	50.0	0.00	0.99
rs9518349	G	T	0.14	0.41	3	547	381	262	178	0.05	0.83	2	70	77	47.6	63	56	52.9	0.75	0.39
rs10508059	C	T	0.37	0.18	–	765	163	357	83	0.34	0.56	–	128	121	51.4	15	22	40.5	1.53	0.22
rs7328287	C	T	0.64	0.41	4	550	376	259	181	0.04	0.85	3	72	76	48.7	52	48	52.0	0.27	0.61
rs496238	C	T	0.52	0.35	4	609	319	286	154	0.05	0.82	3	84	85	49.7	43	42	50.6	0.02	0.89
rs9554772	G	A	0.84	0.43	–	528	396	250	190	0.01	0.91	–	72	78	48.0	60	54	52.6	0.56	0.46
rs1748680	C	T	0.42	0.22	–	726	202	346	94	0.03	0.87	–	118	122	49.2	25	21	54.4	0.42	0.52

Hillside Hospital ($n=49$). Another 119 subjects with schizophrenia diagnosis were recruited together with at least one parent (65 dyads and 54 complete trios). The Structured Clinical Interview for DSM (SCID) Axis I Disorders was administered to all subjects and diagnosis was supplemented by a review of medical records. The diagnosis was established via consensus incorporating two physicians. Further, we collected 220 controls (146 male and 73 females, mean age 36 ± 8, 85% Caucasians). The controls were screened for current or past history of major psychiatric disorders or substance misuse, and excluded if either was detected.

We analyzed 21 tag SNPs in *NALCN* selected using tagging algorithm implemented on Haploview 4.1. Genotyping was performed at The Centre for Applied Genomics (Toronto, ON, Canada) using Illumina platform. We did not find any Mendelian errors in the family-based sample. Genetic and clinical data were matched using automated database. Single marker association in case and control data and Hardy–Weinberg equilibrium (HWE) assessment were performed using χ^2 tests. Linkage disequilibrium, haplotype analysis and 1000 permutations were performed using Haploview 4.1. Family-based associations were performed using transmission disequilibrium test (TDT) implemented on Unphased 2.0. We meta-analyzed results from case–control and family-based samples as described elsewhere (Souza et al., 2010).

3. Results

We had genotype call higher than 99.5% for all variants in this study. All polymorphisms presented minor allele frequency higher than 0.05 (Table 1). Two variants (rs17677552 and rs2584531) presented HWE $P<0.05$ in the case–control sample. No variants deviated from HWE across the probands of the family-based sample (data not shown). Single marker analysis showed two variants nominally associated with schizophrenia in the case–control sample (rs9518320 permuted $P=0.152$ and rs9518331 permuted $P=0.211$) (Table 1). However, we did not observe significant transmission disequilibrium in the family-based sample for any of the 21 *NALCN* variants. Moreover, we found no significant results after meta-analyzing the results from both case–control and family-based samples (data not shown).

We observed four linkage disequilibrium blocks in the case–control sample and three linkage disequilibrium blocks in the family-based sample (Table 1). Due to the size of this gene and the low linkage disequilibrium observed across the evaluated variants, we only performed haplotype analysis using variants in the same linkage disequilibrium block. Case and controls had no significant difference between haplotype frequencies. Likewise, we found no haplotypes with transmission disequilibrium in our family-based sample (data not shown).

4. Discussion

This exploratory study examined the association of 21 SNPs in *NALCN* with schizophrenia. We have found two nominally significant single marker associations (rs9518320 and rs9518331) in the case–control sample that do not remain significant after permutations. Corrections for multiple testing have been a controversial issue and considering the exploratory nature of this study, without pre specified hypotheses for most of our SNPs, we assume no clear structure in the multiple tests. Therefore, our statistically significant results should properly be regarded as "exploratory", with confirmatory studies needed (Bender and Lange, 1999). Bonferroni correction for multiple testing on individual variant associations renders all the associations non-significant (Bonferroni corrected $P<0.005$). Another limitation of this study is the sample size that may lead to type II error. Genome-wide association studies have not implicated *NALCN* polymorphisms (or *G72*) in the risk to schizophrenia (Duan et al., 2010). Although *NALCN* plays a clear role in the synaptic activity (Jospin et al., 2007; Yeh et al., 2008) and changes during this process have been associated with schizophrenia, our results suggest that the *NALCN* variants may not be involved with the manifestation of schizophrenia but further work is required to confirm this hypothesis.

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