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Association of Neuregulin-1 gene polymorphisms with neuro-cognitive features of schizophrenia patients from South India: A pilot study

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ABSTRACT

Numerous genes have been associated with schizophrenia. In particular, Neuregulin-1 (*NRG1*) has been widely implicated. Specifically, a 7-marker haplotype called HAP_{ICE} in *NRG1* is correlated with schizophrenia in many ethnic groups. However, the association of these markers with endophenotypes has shown many contrasting results. India, which harbours one sixth of the global population, is poorly represented in these studies. The present pilot study is aimed to identify association of *NRG1* genetic variants with schizophrenia and neuro-cognitive features of the disorder. For this, 148 patients and 156 controls were enrolled and four *NRG1* SNPs (rs7812451, rs35753505, rs62510682 and rs6994992) were genotyped. Significant associations of SNPs rs7812451 (Allele-A; OR:2.7, $P < 0.0001$) and rs35753505 (Allele-C; OR:1.7, $P = 0.0015$) with schizophrenia was observed. The CC genotype of rs35753505 was associated with poor response to visual and working memory tests while GG genotype of rs62510682 with good response to verbal fluency, working memory and many executive-function tests.

1. Introduction

Schizophrenia is a debilitating, complex psychiatric disorder that affects approximately 1% of the global population (Ayuso-Mateos, 2010). It is recognized that the disorder has a 60–80% genetic etiology (Sullivan, 2005; Edward and Alastair, 2014). Schizophrenia has heterogeneous heritable clinical features called endophenotypes, such as neuro-cognitive functions, electrophysiological measures, neuro-developmental markers, etc. (Almasy and Blangero, 2001).

The symptoms of schizophrenia are broadly classified into three domains: positive symptoms, negative symptoms and cognitive deficits. Positive symptoms (also called productive symptoms) include hallucinations, delusions, distorted perceptions of reality, etc., while the negative symptoms (deficit features) include asociality, alogia, anhedonia, blunted affect, etc. (Kay et al., 1987). Cognitive impairment is observed in > 98% of affected individuals (Sabb et al., 2008). It is shown that IQ, working memory, attention, executive functions, social cognition, verbal learning and memory are the commonly impaired cognitive functions in schizophrenia. Such impairment is shown to be upto 55% heritable (Sabb et al., 2008). Numerous polymorphisms in genes involved in dopamine signalling, serotonin signalling, ion channels, neurodevelopment, glutamate system and energy metabolism have been associated in cognitive impairment among both schizophrenia patients and healthy adults (Zai et al., 2017). The wide range of cognitive functions and the variety of genes, molecules and pathways involved in their dysfunction is a major challenge in endophenotype classification. Hence, the National Institutes of Health (NIH) has started a Research Domain Criteria (RDoC) project that aims to develop a framework for accurate construct of human behaviour and functioning in health and disease states (RDoC, 2017).

Genetic linkage and genome-wide association studies have shown several chromosomal loci to be associated with the disorder (Levinson, 2003). Specifically, the 8p22–p21, which houses the *NRG1* gene, is significantly associated with the disorder (Stefansson et al., 2002). *NRG1* codes for Neuregulin protein, which mediates cell-cell signalling and plays a critical role in the development of multiple organ systems and neural plasticity (Shamir et al., 2012).

A 7-marker haplotype (5 SNP + 2 microsatellite) in the *NRG1* gene

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Table 1
Frequency of alleles of the 4 NRG-SNPs among 148 cases and 156 controls and their associated Odds Ratio their association from PGC2 database.

NRG-SNP	Allele	Case (%)	Control (%)	OR (95% CI); P value	OR, P value in PGC database
rs7812451 (G/A)	A	33.8	16	2.7 (1.8–3.9); < 0.0001	N.A
	G	66.2	84		
rs35753505(T/C)	C	41.9	29.5	1.7 (1.2–2.4); 0.0015	1; P = 0.68
	T	58.1	70.5		
rs62510682 (G/T)	G	50	48.1	1.1 (0.8–1.5); 0.6354	0.97; P = 0.024
	T	50	51.9		
rs6994992 (C/T)	T	45.9	39.1	1.3 (1–1.8); 0.0882	1; P = 0.61
	C	54.1	60.9		

Note: The minor alleles are underlined.

has been correlated with Schizophrenia in Iceland populations; hence named HAP_{ICE} (Stefansson et al., 2002). Numerous studies have correlated this haplotype (or markers within this haplotype) with schizophrenia in different populations, predominantly in European ethnic groups (Stefansson et al., 2003; Bakker et al., 2004; Corvin et al., 2004). Genetic variants in *NRG1* has been associated with impaired verbal learning and processing speed, abstracting and spatial processing (Greenwood et al., 2011; Voineskos et al., 2013). Many studies have shown associations of the HAP_{ICE} SNPs rs62510682, rs35753505 and rs6994992 with schizophrenia (Kukshal et al., 2013; Mostaid et al., 2017). rs3575505 has shown association with semantic verbal fluency in Schizophrenia and language, visuospatial functions and attention/speed in bipolar disorder (Kircher et al., 2009; Rolstad et al., 2015). But studies on association of these SNPs with cognitive features in schizophrenia patients from South India, specifically the state of Tamil Nadu are not available.

India is characterised by enormous genetic diversity. In particular, South India harbours various populations that are genetically distinct from each other (Arunkumar et al., 2012). Given the high burden of schizophrenia, it is imperative to understand the genetic predisposition to the disorder in Indian populations. Looking at this direction, the present study is aimed at deciphering the association of *NRG1* SNPs with neuro-cognitive features of schizophrenia in Tamil Nadu, a southern state of India.

2. Materials and methods

2.1. Sampling

148 schizophrenia patients and 156 controls attending the psychiatric clinic of Government Rajaji Hospital, Madurai, India, were enrolled for the study. Patients satisfying the ICD-10 criteria for schizophrenia, first onset and treatment-naive, and belonging to the age group 18–45 years were enrolled for the study. Healthy individuals with no clinical symptoms (age, gender and demography matched) were treated as controls. Patients with any systemic diseases, mental retardation, congenital diseases, and those who were unwilling to participate in the study were excluded.

4 ml venous blood was collected from every volunteer and a detailed questionnaire was administered to each after obtaining a signed informed consent. The study methodology was approved by the Human Ethical Committee of Madurai Medical College, Madurai.

2.2. Neuro-cognitive evaluation

Neuro-cognitive functions were evaluated in all the patients using a series of cognitive tests described in Supplementary Table 1.

2.3. Genetic analysis

DNA was extracted from each sample using standard salting out method. The study was initially planned to identify 3 SNPs from the HAP_{ICE} haplotype, as studies describing their allele frequency in South India (Tamil Nadu) were sparse. The SNPs rs62510682 and rs6994992 were genotyped using PCR-RFLP method described in Supplementary text-1. The HAP_{ICE} SNP rs35753505 was genotyped by direct sequencing of the region using the primers TGCATTAGAACTAGAACTTGCGTG and TCATAAATGTCATCTGTGGTTGTCT in a subset of 20 samples. Sequencing of this region resulted in the identification of another SNP rs7812451 which was also taken up for analysis. Further genotyping of these 2 SNPs were performed using custom-made Taqman assays.

2.4. Statistical analysis

The distribution of alleles was expressed as frequencies. Odds ratio, Fisher's exact tests and ANOVA were performed using R statistical package v.3.1 (R). Correction for multiple testing was made in ANOVA for cognitive test results using post-hoc Bonferroni test.

3. Results & discussion

A total of 148 schizophrenia cases and 156 controls were recruited for this pilot study. Four *NRG1* SNPs namely rs7812451(G > A), rs35753505(T > C), rs62510682(G > T) and rs6994992(C > T) were genotyped in these samples. Three of the 4 SNPs (rs35753505, rs62510682 and rs6994992) of the present study are part of the HAP_{ICE} haplotype (Stefansson et al., 2002). Sequencing of region around rs35753505 resulted in the identification of rs7812451 in the samples. The mean age group of cases and controls were not significantly different from each other (31.6 ± 7.7 and 31.1 ± 8.6 respectively).

3.1. Distribution of SNPs in the study subjects

Three out of the 4 SNPs studied were in Hardy-Weinberg equilibrium, while the SNP rs62510682 had a significantly higher frequency of heterozygotes. The homozygous genotypes GG and TT of rs7812451 and rs35753505 respectively were significantly higher in controls (Supplementary Table 2). On the other hand the frequency of the homozygous minor allele genotype rs6994992(TT) and rs35753505(CC) were significantly higher in the cases. Allelic distribution showed a significant association of SNPs rs7812451 (OR:2.7, 95% CI: 1.8–3.9, P < 0.0001) and rs35753505 (OR:1.7, 95% CI: 1.2–2.4, P = 0.0015) with schizophrenia (Table 1). Thus, of the 3 HAP_{ICE} SNPs, only rs35753505 (C allele) showed significant association with the disorder in the present study, which is in line with a recently published large scale meta-analysis (Mostaid et al., 2017). The PGC2 database did not present the rs7812451 marker and the other 3 SNPs were not associated in their GWAS (Table 1) (Psychiatric Genomics Consortium, 2014). None of these SNPs were also present in ExAC database. Thus, to our knowledge association of the SNP rs7812451 with Schizophrenia has not been reported earlier. The prevalence of the minor allele 'A' was almost twice that of the controls. The occurrence of this allele in Indian populations of 1000 genome project (BEB, GIH, ITU, PJL and STU) was similar to that of the controls. Thus, the