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Research Article

Neureglin 3 gene intronic polymorphisms rs10883866, rs6584400, rs1937970 and rs677221 in patients with Schizophrenia

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Abstract
Background: Schizophrenia is a progressive disease presenting with insufficiencies in thought processes, perceptions, and emotional responsiveness with early onset. There are no laboratory tests or specific biomarkers established so far to diagnose the disease, so clinical and psychiatric analysis are used worldwide. Abnormal neurodevelopment, genetic predisposition and environmental factors are the main factors associated with this disease. The Neuregulin 3 *(NRG3)* gene encodes the Neuregulin 3 protein which binds to the extracellular domain of ERBB4 receptor tyrosine kinase and controls the proliferation, differentiation, and migration of neural progenitor cells.
Methods: Purpose of this study was to find the *NRG3* gene polymorphisms i.e., rs10883866, rs6584400, rs1937970 and rs677221 in Schizophrenic patients (n=114) and then compared with healthy controls (n=114). For this purpose, blood samples were taken after the informed consent of patients and the controls followed by DNA extraction and genotyping through PCR-RFLP and PCR-ARMS.
Results: The minor allele frequencies of the SNPs rs10883866, rs6584400, rs1937970 and rs677221 in schizophrenic patients were 21.1%, 28.1%, 68.1% and 62.2% respectively. The SNPs rs10883866 and rs6584400 in protective effect toward the Schizophrenia whereas, the other two SNPs showed any statistically significant difference in the allelic distribution between cases and controls. Haplotype block analysis revealed that C-G haplotype in block 1, A-G in block 2 had frequencies of 62.4% and 60.7% in cases. The haplotype G-A in block 1 showed the protective effect towards the disease Schizophrenia with the *p-value* 0.011.
Conclusion: *NRG3* has association with the disease schizophrenia. More studies should be conducted including different phenotypic traits of schizophrenia and meticulous clinical evaluation of the patients with larger sample size is suggested to find out the association of NRG3 gene with the schizophrenia.
Key Words: Schizophrenia, Neuregulin 3 gene, Polymorphism

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INTRODUCTION

Schizophrenia disease is presented with insufficiencies in thought processes, perceptions, and emotional responsiveness. The disease has an onset usually in late adolescence or early adulthood [1]. Schizophrenia is diagnosed clinically by psychiatric analysis and by observing patient's abnormal social behavior. There are no laboratory tests or specific biomarkers that can be used to make a diagnosis of this disease [2]. Abnormal neurodevelopment, genetic predisposition and environmental factors are considered to be the main factors associated with this disease [3]. The developmental process suggests that environmental insults, early during the development of fetus, such as viral or bacterial infections, nutritional deficiencies, exposure to neurotoxins and obstetrics complications may result in the person developing neuropsychiatric disorders including schizophrenia later in life [4].
The neuro-developmental theory of schizophrenia suggests that the basic pathology of schizophrenia occurs during the development of brain and that these developmental brain abnormalities do not become evident until a person reaches adolescence [5]. This is supported by the detection of neuroanatomical and cyto-architectural abnormalities in the brains of patients. Problems of neuronal migration are seen in the brains of these patients as is evident by out-of-place and grouped neurons that are particularly seen in the entorhinal cortex, suggesting an early developmental abnormality. Smaller cell bodies and fewer dendritic spines are seen in pyramidal neurons of the hippocampus and neocortex of schizophrenia patients [6, 7].

Heritability of schizophrenia is strongly exhibited by family, twin and adoption studies. Inheritance mode is complex and does not follow the Mendelian law. Family studies demonstrated a higher risk of developing schizophrenia in the individuals who have first or second degree relative affected with this disease as compared to general population [8]. It has been reported that the risk of developing schizophrenia in second degree relatives is about 2–4 %, which increases in first degree relatives to 10–15 % and up to 45% in monozygotic twin [9]. Several diseases are associated with DNA variations have been identified by international consortia working on genetics of this disease. These variations are spread all over the human genome and are present in genes that regulate specific function. Although each individual variant itself has a little effect, it has been predicted that common variations when taken as a whole account for about half of the genetic variance in schizophrenia.

The neuregulin proteins are expressed in neurons and perform a wide variety of functions in the developing nervous system. Neuregulin 3 (*NRG3*) belongs to this family and is present on the long arm of chromosome 10 (10q23.1) [10, 11]. *NRG3* gene has 12 exons: first five of these exons encode the extracellular epidermal growth factor domain of the protein, whereas exons 8 to 12 encode the cytoplasmic portion of the protein. In both fetal and adult human brain, thirteen different *NRG3* splice variants have been identified that classify this protein into 4 different classes. Differential regulation of *NRG3* isoforms is considered to affect neurodevelopmental pathways and play a role in the development of schizophrenia [12]. It has been found that expression of Class 1 isoforms of *NRG3* is increased to 40% in the dorsolateral prefrontal cortex of schizophrenia patients as compared to controls. Similarly, the expression of Class IV *NRG3* isoforms is also increased by 50% in individuals with schizophrenia as compared to normal controls, while Class II and III transcripts did not show differences in expression. Neuregulin are reported to be important in regulating the growth and differentiation of glial, epithelial, and muscle cells [13, 14].

NRG3 is a growth factor that binds to the extracellular domain of ERBB4 receptor tyrosine kinase. This binding activates tyrosine phosphorylation of ERBB4 receptor and modulates the neuronal migration and differentiation [15]. *NRG3* plays pleiotropic roles in the development, plasticity of the brain and is essential for the embryonic cerebral cortex development. It does so by controlling the proliferation, differentiation, and migration of neural progenitor cells [16]. The four SNPs analyzed in the present study were rs10883866, rs6584400, rs1937970 and rs677221. The two SNPs rs10883866 and rs6584400 are located in intron 1 while rs1937970 and rs677221 lie in intron 2.

METHODS

Clinical Sampling

A total of 114 diagnosed schizophrenic patients fulfilling the criteria of DSM IV and of both genders were recruited from Department of Psychiatry in the tertiary care hospital of Lahore, Pakistan. For comparison purposes, 114 healthy subjects with no personal and family history of schizophrenia or any other psychiatric disorder were also included in the study as healthy control subjects. After an informed written consent, blood samples were collected from the schizophrenic patients and normal subjects in EDTA containing vials.

Genotyping of Samples

The DNA was isolated by salting out procedure and estimation of its quality and quantity was done using Nanodrop. Genotyping for SNPs rs10883866 and rs658400 was done by PCR-RFLP using the TspR1 and Hinf1 restriction enzyme method as mentioned in supplementary Table 2. The Components and concentrations of ARMS-PCR reaction are given in supplementary Table 3. The SNPs rs1937970 and rs677221 were genotyped by the ARMS-PCR. The primer sequences are given in supplementary tables 4-5. The components and concentrations of PCR and RFLP reaction are given in supplementary Table 2 and 1. PCR-RFLP and ARMS-PCR products were resolved at 2% while ARMS-PCR products were resolved on 3% TAE agarose gel along with 50bp DNA size marker based on molecular weight.

Data Analysis

Hardy–Weinberg equilibrium was tested to check the deviation of genotype count using a chi-square goodness-of-fit test using OEGE-Online Encyclopedia for Genetic Epidemiology Study. Chi-square test evaluated statistical differences in genotypic and allelic distribution between schizophrenia patients and control subjects at significance level of 0.05. The effect of different alleles was evaluated by calculating odds ratio (OR) and their 95% confidence intervals (95% CI) using IBM SPSS software (version 20.0, SPSS Inc). p-value < 0.05 was considered statistically significant. Genetic modeling was done using SNPStats software and Haplotype analysis was done using SHEsis online software.

RESULTS

Demographics of Cohort

There were 57.7% male patients and 42.3% were females. The age of the patients ranged from 18 years to 80 years with a mean age of 35 ±11.6 years. Most patients (62.0%) were in the age group of 25-40 years and in this age group, percentage of female patients (70.0%) was greater than males. Majority of patients (70.8%) were product of consanguineous marriages. According to their age groups, they were further stratified into three groups, below 25 years, 25-40 years, and above 40 years. The mean age of the study population was 35±11.6 years. Age distribution showed that 62.0% of patient's ages were 25-40 years. However, when male to female ratio was compared to each age group, it was found that around 70.0% females (n= 35) were affected during the age group 25-40 years.

Genotyping for *NRG3* SNPs by PCR-RFLP

Supplementary figures 1-4 show gel images of the genotyping results for SNPS. The allelic and genotypic frequencies of the four SNPs are given in the Table 6 and 7. The heterozygous CG genotype of rs10883866, rare homozygous AA genotype of rs6584400 and rare CC homozygous genotype of rs677221 showed the protective influence of genotype over schizophrenia with ODDs of, because its frequency in control group was significantly more than patient population with ODDs ratios and CI as (0.32 [0.20-0.52] *p=0.042,* (0.390 [0.201-0.756] *p=0.014 and* 0.258 [.105-.633] *p-value 0.0013* respectively.

Haplotype Analysis and Pairwise Linkage Disequilibrium:

Linkage analysis was done for all the possible pairwise combinations of 4 SNPs with minor allele frequency ≥ 1%. Figure 1 exhibits the visual depiction of the degree of LD which was determined by the standard disequilibrium coefficient D' and r^2 values. Two LD blocks were defined using these D' and r^2 values. Each block contained two SNPs i.e. rs10883866 and rs6584400 were included in block 1 whereas rs1937970 and rs677221 were present in block 2. The LD plot showed that the SNPs within each block were moderately linked as shown in Figure 1(A) and (B).

Haplotype block analysis revealed eight major haplotypes, four for the two SNPs of each block as shown in the supplementary Table 8. In block 1, C-G haplotype was the most occurring with the frequency of 62.4% in cases and 46.8% in controls whereas, in block 2 A-G was the most frequent haplotype of all with the frequency of 60.7% in cases and 48.52% in controls. The haplotype G-A in block 1 showed the protective effect towards the disease schizophrenia with the *p-value* 0.011. In block 2, no single haplotype showed any effect toward the disease's phenotype. The major haplotypes of each block, their corresponding frequencies and the odds ratios are described in supplementary Table 8.

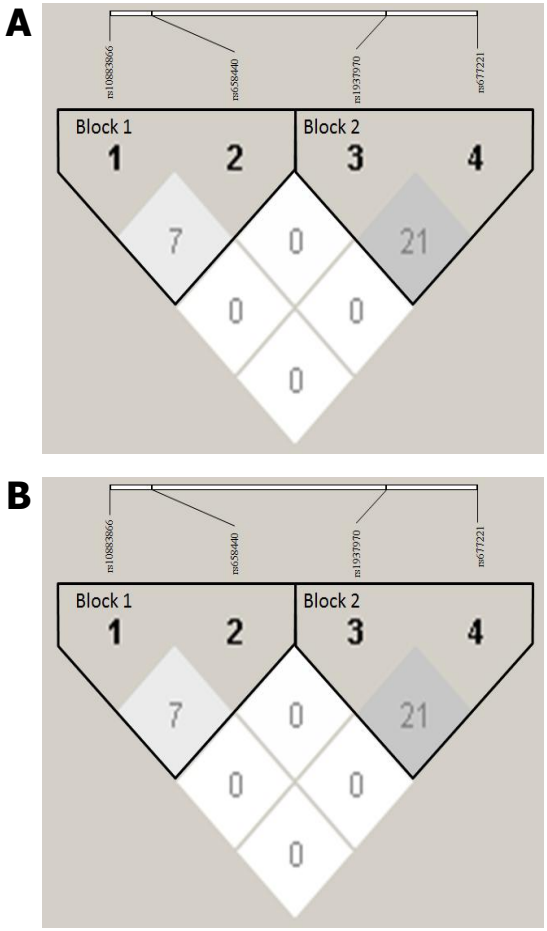

Figure 1: Linkage disequilibrium analysis for NRG3variants rs10883866 (1), rs6584400 (2), rs1937970 (3) and rs677221 (4). White bar indicates the span of NRG3 gene area having these four SNPs. The two pentagons represent the two blocks and the boldfaced numbers in blocks represent the htSNPs. Figure 3.7 (A) presents the LD plot constructed based on D' values and the level of LD is indicated with the color scheme given at its bottom right. Figure 3.7 (B) shows the LD plot based on r^2 values.

DISCUSSION

Linkage studies in different populations have identified the 10q22-q23 chromosome region as potentially harboring risk genes for schizophrenia [17]. This region includes members of the neuregulin family, which comprises four epidermal growth factor-like molecules: *NRG1, NRG2, NRG3, and NRG4* [18].These molecules interact with the ERbB receptor tyrosine kinase family, and among them, *NRG1* and *NRG3* have been most strongly implicated in schizophrenia pathogenesis [19]. *NRG3*, a key member of the neuregulin family, binds specifically to the ErbB4 receptor and activate through ligand-stimulated phosphorylation [20]. Given with its predominant expression in brain regions such as the hippocampus, amygdala, and thalamus, *NRG3* plays a crucial role in neuronal development, particularly in cortical cell migration and patterning during embryonic brain formation [21].

This variant has been the focus of increasing attention in the field of schizophrenia research, as it has been consistently associated with the disorder across multiple populations and ethnic groups [22]. The rs10883866 variant is thought to play a role in the regulation of gene expression, potentially impacting the function of genes involved in neurodevelopment, neurotransmission, and other cellular processes relevant to the pathophysiology of schizophrenia [23, 24]. Beyond genetic contributions, emerging evidence suggests that epigenetic mechanisms, such as DNA methylation, may also contribute to the association between rs10883866 and schizophrenia [24].

The non-association of rs10883866 with schizophrenia in the present study aligns with findings from the Han Chinese and Ashkenazi Jewish populations. However, both studies demonstrated that when analyzing "delusion" as a quantitative trait of schizophrenia, rs10883866 showed significant association with this clinical feature. The present study focused on schizophrenia as a discrete phenotype and did not investigate specific clinical traits, which could explain the lack of association. In contrast, research on White Americans of European ancestry revealed an association between rs10883866 and both schizophrenia risk and the severity of delusions, along with increased expression of *NRG3* isoforms in the brains of affected patients [25]. Similarly, Australian studies found significant associations between rs10883866 and schizophrenia subtypes characterized by delusions and relatively intact cognition [26].

Regarding rs6584400, the current study found no association with schizophrenia, corroborating findings in Korean population [27]. However, studies in Han Chinese, Ashkenazi Jewish, and European populations reported associations between rs6584400 and delusional traits in schizophrenia [10, 14]. Notably, in German patients, rs6584400 was significantly associated with psychotic symptoms and cognitive performance [28]. These findings suggest that while rs6584400 may not contribute directly to schizophrenia, it could influence specific cognitive or behavioral traits. In the case of rs677221 and rs1937970, the present study found no significant association with schizophrenia, consistent with previous research on White American populations [25]. However, studies in Han Chinese populations reported that rs677221 remained associated with schizophrenia after multiple testing corrections [14], highlighting the variability in genetic associations across populations.

Overall, the current study adds to the evidence suggesting that *NRG3* SNPs may not be directly associated with schizophrenia in populations. Genetic heterogeneity, population-specific factors, and methodological differences likely contribute to the discrepancies observed between studies. Additionally, it appears that *NRG3* SNPs may be more strongly linked to specific schizophrenia traits, such as delusions, rather than to the disorder itself. Future research with larger sample sizes and comprehensive clinical assessments of schizophrenia traits is needed to clarify the role of *NRG3* in schizophrenia and identify other potential genetic contributors to this multifactorial disorder. Provided with further evidence, genetic testing covering NRG genes may provide an instrumental way forward in diagnosis and pathogenesis assessment of schizophrenia.

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