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Genetic Association Between NRXN3 Variants (rs12879016 & rs2270964) and Risk of Schizophrenia (SCZ)

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ABSTRACT

There hasn't been much study on the link between schizophrenia (SCZ) and neurexin 3 (NRXN3) genetic variations. The first association study of the NRXN3 rs12879016 G>C,T and rs2270964 C>A,T single-nucleotide polymorphisms (SNP)in a Saudi population is shown here.

We used real-time PCR to assess the genotypes for the polymorphisms in one hundred seventeen patients with SCZ and Seventy-eight healthy controls. We used endonuclease digestion of amplified genomic DNA to establish the genotypes for the polymorphisms in 117 patients with DSM-IV and PANSS (Positive and Negative Syndrome Scale) evaluations of schizophrenia and 78 healthy controls.

We aim to find out if there is a relation between NRXN3 variations (rs12879016 and rs2270964) and susceptibility of developing SCZ in a Saudi population.

We hypothesis that SCZ can be related to patients with NRXN3 variations (rs12879016 / rs2270964) among Saudi population.

According to our result both rs12879016 and rs2270964 polymorphisms were shown to be unrelated to the risk of schizophrenia.

Keywords: NEUREXIN3, SCHIZOPHRENIA (SCZ), rs12879016, rs2270964, VARIANTS.

INTRODUCTION

Schizophrenia is a dangerous mental disorder that influences a person's thinks, feelings, and attitudes (i.e., hallucinations and delusion). SCZ patients appear detached from reality, which distresses them as well as their family and friends. SCZ patients are characterized by recurrent or persistent episodes of psychosis Hallucinations (usually hearing voices), delusions, paranoia, and disorganized thinking are common symptoms. Apathy, social detachment, and diminished emotional expression are other symptoms.(Owen, Sawa and Mortensen, 2016a)

There is no single etiology of SCZ, according to researches. SCZ is thought to be caused by a combination of genes and a variety of environmental circumstances. The onset and progression of SCZ may be influenced by psychosocial variables. Heavy cannabis use is linked to a higher chance of developing the disease.(Khan, Martin-Montañez and Muly, 2013)

In the case of SCZ, symptoms not treated can be disabling and persistent. In general, SCZ is usually diagnosed at the end of the years of teens to the first years of the thirties. It is earlier in males than females. (McGrath *et al.*, 2008)

However, delivering effective treatment, which is available nowadays at the right time and in a suitable continuous way, can control the disease. Medication (antipsychotic medications),

psychoeducation, family interventions, cognitive-behavioral therapy, and psychosocial rehabilitation(e.g., life skills training) are all treatment choices for patients with SCZ.(Khan, Martin-Montañez and Muly, 2013)

SCZ is prevalent with 0.33% to 0.75% worldwide (Saha *et al.*, 2005; Moreno-Küstner, Martín and Pastor, 2018).

According to the Saudi Arabian Ministry of Health, SCZ or schizotypal and delusional disorders cause 22.4 % of mental health (157,801 patient inpatients & outpatients) mental and behavioral abnormalities (Ministry of Health (2019) Health statistical year book. Riyadh: Ministry of Health., 2019). Latino Americans are more than three times as likely as Euro-Americans to be diagnosed with SCZ. At the same time, African Americans continued to be the group most likely to be diagnosed with SCZ, four times more likely than European Americans. (Minsky *et al.*, 2003; Schwartz and Blankenship, 2014)

SCZ is one of the top fifteen diseases that can cause disability worldwide (Vos *et al.*, 2017). Many people become less functioning in society because of the disorder, and some become homeless.

Supporting evidence has connected schizophrenia's high heritability to a combination of common genes with small effects and a few rare alleles with significant effects.(Owen, Williams and O'Donovan, 2009). In recent years, genome-wide association studies have discovered thirteen genetic SNPs that contribute to Schizophrenia(Purcell *et al.*, 2009; Ripke *et al.*, 2011a; Sklar *et al.*, 2011; Steinberg *et al.*, 2011) and have exceeded a genome-wide significance threshold $P < 5 \times 10^{-8}$.(Pe'er *et al.*, 2008)

Ten genes have been confirmed by the results of these studies (i.e., CSF2RA, HIST1H2BJ, NOTCH4, NRGN, SHOX, SMARCA2, TCF4, ZNF804A, PRSS16, and PGBD1)(O'Donovan *et al.*, 2008; Koga *et al.*, 2009; Purcell *et al.*, 2009; Riley *et al.*, 2009; Del Re *et al.*, 2014). However, they impose only minor increases in risk and account for just a small amount of heredity.(Manolio *et al.*, 2009; Del Re *et al.*, 2014)

Genes that code for dopaminergic system proteins have been studied extensively in investigations of SCZ susceptibility. Evidence suggests that central dopamine pathways have a role in the pathogenesis of the condition (Cordeiro, Da Silva and Vallada, 2012), like medicines that lower dopamine levels improve psychotic symptoms while treatments that raise dopamine levels worsen them. (Money *et al.*, 2010)

NRXN3 consists of twenty-four exons and very large introns. (OMIM Entry - * 600567 - NEUREXIN III; NRXN3). This gene is found in chromosome 14 (*14q24.3-q31.1) and* has the most expression of NRXN3 in the brain. NRXN3 codes for a protein that functions as a receptor and a cell adhesion molecule in the neurological system.(Hishimoto *et al.*, 2007)

Many behavioral traits, including alcoholism and autism spectrum disorders, have been linked to genetic variation in this gene.(Fagerberg *et al.*, 2014)

According to this study, we will try to find if there is a relation between NRXN3 genetic variations

(rs12879016 G>C,T and rs2270964 C>A,T) and susceptibility risk of SCZ among the Saudi Arabian population.

SCHIZOPHRENIA

SCZ is a functional psychotic condition marked by delusional beliefs, hallucinations, and disruptions in cognition, perception, and behavior.

SCZ is a serious psychiatric condition with far-reaching consequences for both individuals and society. Roughly 20% have chronic symptoms and disability.(Barbato, 1998) Unemployment is at an all-time high of 80–90%(Marwaha and Johnson, 2004; Kooyman *et al.*, 2007), and life expectancy has been cut by 10–20 years. One of the most difficulties facing modern medicine is figuring out the etiology and pathogenesis of SCZ, as well as discovering novel, more effective, and acceptable therapies.

However, there has been great advancement in the application of genetics, epidemiology, and neuroscience to SCZ during the last decade.

HISTORY OF SCZ

Swiss psychiatrist Eugen Bleuler initially defined it in 1908 to characterize the functional separation of personality, thinking, memory, and perception. During a presentation at a psychiatric conference in Berlin on 24 April 1908, he coined the word, which he later published. (Watt, 1987; Fusar-Poli and Politi, 2008)

Bleuler went on to write a book about his new disease idea in 1911, which was finally translated into English in 1950. (Adityanjee *et al.*, 1999)

Some claim that sickness has always existed and was just 'discovered' in the early twentieth century. The credibility of this assertion hinges on the ability to diagnose previous occurrences of craziness as 'schizophrenia' retrospectively. Others believe that the term "schizophrenia" refers to a culturally determined clustering of mental symptoms.(Berrios, Luque and Villagrán, 2003) What is certain is that at the start of the twentieth century, the traditional idea of insanity had splintered into 'diseases' (psychoses) (Berrios, 1987), including paranoia, dementia praecox, manic-depressive insanity, and epilepsy.(Berrios and Hauser, 1988)

Manic-depressive insanity was renamed bipolar disorder, while dementia praecox was renamed SCZ, paranoia was called delusional disease, and manic-depressive insanity was renamed manic-depressive insanity (epilepsy was transferred from psychiatry to neurology).

CLINICAL MANIFESTATION OF SCZ

Positive symptoms (delusions and hallucinations; so-called psychotic symptoms in which there is a loss of contact with reality), negative symptoms (impaired motivation, reduction in spontaneous speech, and social withdrawal), and cognitive impairment characterize SCZ. (As a group, patients with SCZ perform more poorly than controls over a wide range of cognitive functions though there is much individual variability). (Joyce and Roiser, 2007) Positive symptoms usually relapse and fade away, while some individuals have long-term psychotic symptoms. Negative and cognitive symptoms are often chronic and linked to long-term social dysfunction. The first episode of psychosis usually occurs in late adolescence or early adulthood, although it is commonly preceded by a prodromal phase or "at-risk mental state" (Lieberman *et al.*, 2001; Addington and Heinssen, 2012) and in some cases, premorbid cognitive and/or social functioning abnormalities date back many years. (Lewandowski, Cohen and Öngur, 2011)

In some cases, however, commencement occurs suddenly in previously healthy people.

TABLE1: SCZ SYMPTOMS:

Positive symptoms	
Lack of insight	Failure to recognize that symptoms aren't real or disease-related.
Delusions	• Patients believe they are being persecuted in some way. Are they a storyline danger, or are they essential to the plot? • Patients with passivity believe that their opinions or activities are unimportant. being ruled by someone or something outside of yourself • Others - illusions can arise from any topic, for example,

	spectacular, sexual, or religious for example
Hallucinations	Perception in the absence of a stimulus Touch, smell, taste, or visual hallucinations are all possibilities. Auditory hallucinations are the most common sort of hallucination.
Thought disorder	A lack of capacity to communicate in a rational and consistent manner. "Knight move" - ideas go in one way, then abruptly turn around at right angles, like a chess knight, leaving no logical chain of thought behind**.**
Negative symptoms	
Avolition-apathy	Anhedonia, asociality, and motivation
Diminished expressiveness	Nonverbal and verbal communication

EPIDEMIOLOGY OF SCZ

According to world Health organization (WHO) SCZ affects around 0.3–0.7% of the population at some time in their lives (van Os and Kapur, 2009) or 21 million people globally as of 2011 (About one of every 285).(*Schizophrenia*, no date). SCZ appears to have occurred with considerable consistency across time throughout the last half-century.(Häfner and An Der Heiden, 1997)

While it is stated that SCZ affects people at equal rates world(Jablensky *et al.*, 1992), the frequency and incidence of the disease vary significantly between countries(Kirkbride *et al.*, 2006) as well as at the local and neighborhood level.(Kirkbride *et al.*, 2007)

Males are diagnosed with SCZ 1.4 times more frequently than females, and symptoms typically present earlier in men.(Picchioni and Murray, 2007) Males' peak onset ages are 20–28 years, while females' peak onset ages are 26–32 years. (Castle *et al.*, 1991) The disease may begin in childhood before the age of thirteen. (American Psychiatric Association, 2013; Da Fonseca and Fourneret, 2018)] Late-onset can occur between the ages of 40 and 60, and very late-onset can occur after 60. (Murante and Cohen, 2017)

The average age of first hospital admission for SCZ treatment is between 25 and 35. According to studies, people with lower incomes are diagnosed with their condition later after the development of symptoms than people with higher incomes.

The average age of first hospital admission for SCZ treatment is between 25 and 35. According to studies, people with lower incomes are diagnosed with their condition later after the development of symptoms than people with higher incomes. As a result, those from lower socioeconomic strata who suffer as a result are more likely to be untreated for their sickness.(Owen, Sawa and Mortensen, 2016b)(Häfner and An Der Heiden, 1997)

Women are thought to present with SCZ 4–10 years later than their male counterparts. (Häfner *et al.*, 1991) Females' onset ages are trimodal, peaking at 22.4, 36.6, and 61.5 years old.

In contrast, men have a bimodal age of onset with peaks at 21.4 and 39.2 years old, according to wide criteria for diagnosing SCZ. (Castle, Sham and Murray, 1998)

This new post-menopausal peak in late-onset SCZ in women questions the disease's etiology and sparks a debate regarding SCZ "subtypes," with men and women being prone to various types. This is confirmed by the fact that the disease manifests differently in men and women. (Kulkarni *et al.*, 2001)

Other explanations include protective or predisposing variables in men and women that may make them more (or less) susceptible to the disease at certain stages of their lives. Estradiol, for example, has been shown to be useful in treating SCZ when combined with antipsychotic medication, suggesting that estrogen may be a protective factor for women. (Kulkarni *et al.*, 2001)

The World Health Organization observed that SCZ prevalence and incidence were essentially similar over the world in 2000, with age-standardized prevalence per 100,000 ranging from 343 in Africa to 544 in Japan and Oceania for men and 378 in Africa to 527 in Southeastern Europe for women. (James *et al.*, 2018)

However, SCZ has the greatest influence in Oceania, the Middle East, and East Asia, while it has a minor impact in Australia, Japan, the United States, and most of Europe. Despite their proximity, Indonesia's DALY rate (Disability-Adjusted Life-Year) for SCZ is approximately double that of Australia (the nation with the highest and lowest respective DALY rates). Differences in medical treatment availability may explain differences in DALY rates and prevalence: years lived with mental disorders have much higher DALY values when unmedicated than when medicated. (James *et al.*, 2018)

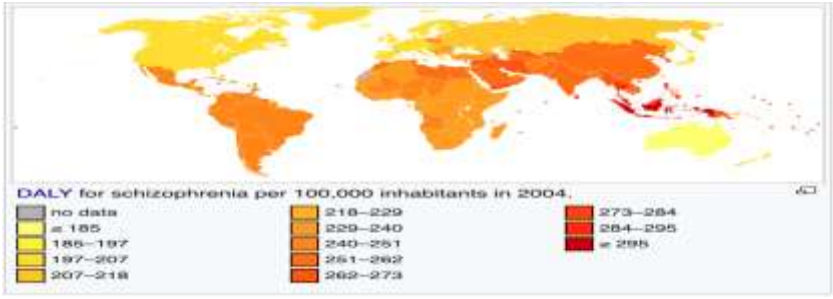
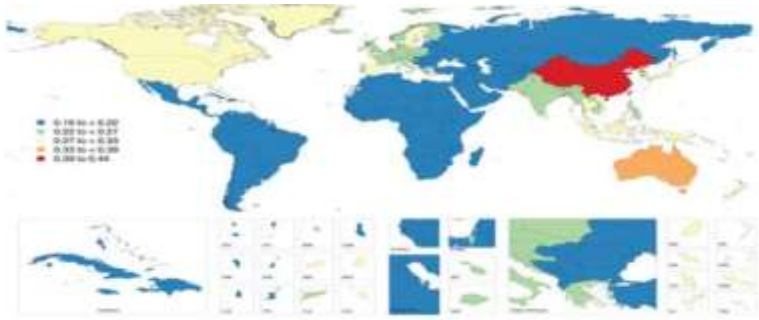


Fig.1: DALY for SCZ per 100.000 inhabitants in 2004 (James *et al.*, 2018)



Immigrant groups in Western Europe are more prone to be diagnosed with SCZ. Immigrants of African descent account for the majority of the rising SCZ diagnoses. (Bresnahan *et al.*, 2007) Those of Afro-Caribbean heritage and black African descent have the greatest incidence of SCZ diagnosis. (Bresnahan *et al.*, 2007) In the United States, African Americans are three times more likely than whites to be diagnosed with SCZ. When the socioeconomic situation is taken into account, they are two times more likely. (Bresnahan *et al.*, 2007) Those diagnosed with SCZ in underdeveloped nations, on the other hand, have a better prognosis and outcome than those in developed countries. (Bae and Brekke, 2002) These countries' better outcomes may be due to their emphasis on harmonious interpersonal connections. (Bae and Brekke, 2002)

Fig.2: Map of age-standardized prevalence by country, 2016.(*Charlson et al., 2018)*

DIAGNOSIS OF SCZ

The symptoms of schizophrenia are closely tied to the diagnosis. These symptoms, on the other hand, might appear in a variety of neurological conditions. As a result, proper diagnosis is critical in the diagnosis and treatment of SCZ. The diagnosis of SCZ is determined by two major international documents:

I. The Diagnostic and Statistical Manual of Mental Disorders (DSM-V) of the American Psychiatric Association.
II. the World Health Organization's International Statistical Classification of Diseases and Related Health Problems (the ICD-10). (van Os and Kapur, 2009)

Two or more of the following positive or negative symptoms with a duration of one month, according to DSM-V (First, 2013), are diagnostic criteria for SCZ:

I. Delusions.
II. hallucinations.
III. disordered speech.
IV. disorganized or catatonic conduct.
V. negative symptoms.

Other factors include a lower level of functioning in areas such as job, self-care, and interpersonal relationships and ongoing indicators of disruption (at least 6 months) not

caused by pharmacological drugs. Finally, schizoaffective disorder, as well as depressive or bipolar illnesses, must be ruled out.

Other kinds of psychosis with similar symptoms, such as bipolar disorder, post-traumatic stress disorder, autistic spectrum disorder, and schizoaffective disorder, should be ruled out using the differential diagnosis. The primary distinction between SCZ and other psychotic disorders is frequently the length of symptoms.

Delusions and hallucinations generated by a short psychotic illness, for example, endure at least 1 day but no more than 1 month. However, it is required to conduct a more thorough investigation of the symptoms. Patients should be monitored for a long period of time, with family members providing further historical analysis. (Holder and Wayhs, 2014)

People with SCZ are people who have odd delusions and undesirable symptoms for an extended period. Bipolar disorder or psychotic depression is identified in people who have multiple unfavorable symptoms, such as high levels of sadness and mania.(van Os and Kapur, 2009)

However, there are some discrepancies between patients with diseases even when all thorough criteria are employed in diagnosis. As a result, it's vital to think of new solutions to solve problems.

The field of diagnosis It is now widely accepted that SCZ is a mental illness.is connected to dopamine and glutamate disorders transmission. Functional magnetic resonance imaging (fMRI) is a measuring technique for regional cerebral blood flow.

low that reveals aberrant activity in some parts of the brain (The cortex, hippocampus, striatum, thalamus, and cerebellum) in the human brain patients with SCZ. (McGuire *et al.*, 2008)

These anomalies, however, are not seen in all cases. PET and single-photon emission tomography (SPET) employ radioactive ligands to observe neurotransmitter distribution, synthesis, and release, while magnetic resonance spectroscopy (MRS) may be used to assess the concentration of chemicals within the brain. Presynaptic striatal dopamine production and release are boosted using these approaches. SPET studies revealed decreased NMDAR binding in the hippocampus, whereas MRS studies revealed increased glutamine in the medial frontal cortex, confirming glutamate release in SCZ patients. Despite the potential for neuroimaging to be used in SCZ diagnosis, these approaches have significant drawbacks, including variability in symptom profiles and molecular abnormalities, restricted availability, and expensive diagnostic costs. (McGuire *et al.*, 2008)

ETIOLOGY AND RISK FACTORS OF SCZ

A variety of genetic and environmental variables influence the outcome development of SCZ. The prevalent model of SCZ is that it is a distinct neurodevelopmental illness with no clear cause or border (i.e. arises from multiple mechanisms).(Notaras *et al.*, 2021)

SCZ is hypothesized to develop because of intricate gene-environment interactions, including susceptibility factors.(Mullin *et al.*, 2013; Hayes and Kyriakopoulos, 2018)

Defects in the brain circuits that impact sensory input and cognitive functioning are caused by a mix of hereditary and environmental factors. (George *et al.*, 2017) This idea has been widely accepted; however, it is impossible to establish due to ethical constraints. Due to cell-by-cell encoding of SCZ -related neuropathology, Inhuman tissue grown from patient stem cells, the first definitive proof that SCZ is caused by multiple biological changes in the brain was recently established, revealing that the disease is "even more complex than currently accepted."(Notaras *et al.*, 2021)

Without the addition of environmental risk factors, a genetic predisposition does not usually lead to SCZ. (Davis *et al.*, 2016; Perkovic *et al.*, 2017) Pregnancy problems, prenatal stress and nutrition, and bad childhood experiences are all examples of environmental risk factors. A risk factor in the environment might function alone or in conjunction with others. (Stilo and Murray, 2019)

Although SCZ is highly heritable, many persons who appear to inherit SCZ -related genes do not acquire the illness. (Carlson, 2014) According to research, SCZ is a polygenic condition with a multifactorial genetic susceptibility produced by interactions between numerous genes and environmental risk factors. (Hayes and Kyriakopoulos, 2018)

Seven genes were also identified in a 2003 assessment of linkage studies as being likely to enhance the probability of a subsequent diagnosis of the condition. (Harrison and Owen, 2003) According to two reviews (Owen, Craddock and O'Donovan, 2005; Riley and Kendler, 2006), the evidence was highest for two genes known as dysbindin (DTNBP1) and neuregulin (NRG1), while some additional COMT, RGS4, PPP3CC, ZDHHC8, DISC1, and AKT1 genes provided some promising early findings. In 2008, the largest and most comprehensive genetic study of its kind, which included testing of hundreds of single-nucleotide polymorphisms (SNPs) in nearly 1,900 people with SCZ or schizoaffective disorder and 2,000 control subjects, found no evidence of a significant link between SCZ and any of the 14 potential genes already identified (RGS4, DISC1, DTNBP1, STX7, TAAR6, PPP3CC, NRG1, DRD2, HTR2A, DAOA, AKT1, CHRNA7, COMT, and ARVCF). The statistical distributions showed that the variance was due to chance. Although tiny effects could not be ruled out, the authors concluded that the data made Common SNPs in these genes are unlikely to be the cause. A significant amount of the hereditary risk for SCZ. (Hamilton, 2008; Sanders *et al.*, 2008)

In 2011, this group discovered 129 single-nucleotide polymorphisms (SNPs) strongly related to SCZ in the major histocompatibility complex region of the genome using a meta-analysis of genome-wide association studies. (Ripke *et al.*, 2011b)

This dataset was enlarged in 2013, resulting in the identification of a total of 13 potential loci for the condition and the identification of calcium signaling as a key role in the disease.(Ripke *et al.*, 2013)

In 2014, this partnership published the largest-ever meta-analysis of GWAS data (36,989 cases and 113,075 controls) in Nature, revealing 108 SCZ -associated genetic regions, including 83 previously unknown. (Ripke *et al.*, 2014)

These candidate genes, taken together, suggested that neurotransmission and immunology played major roles in the condition.

Varied clinical categories of SCZ displayed a different pattern of SNP changes, demonstrating the disease's heterogeneity. (Arnedo *et al.*, 2015)

The C4A gene was linked to SCZ risk in 2016 research. C4A has been discovered to have a role in synaptic pruning, and greater C4A expression is linked to fewer dendritic spines and a higher risk of SCZ. (*Schizophrenia's strongest known genetic risk deconstructed | National Institutes of Health (NIH)*, no date)

A 2008 study looked at copy number variation CNVs in neurexins and showed that exon-affecting deletions in the NRXN1 gene increased the risk of SCZ in 2,977 SCZ patients and 33,746 controls from seven European countries. (Rujescu *et al.*, 2009)

There are some other factors like Dopamine antagonists, particularly dopamine D2 receptor antagonists, restore elevated dopaminergic activity in SCZ patients. (Yang and Tsai, 2017)

Glutamate is the most frequent neurotransmitter in the brain and one of the key excitatory neurotransmitters. (Moghaddam and Javitt, 2012) NMDA receptor function may be influenced by disruptions in glutamatergic neurotransmission that can lead to symptoms comparable to those seen in SCZ patients.

Stress-induced overproduction of serotonin in the dorsal raphe nucleus may blame for altered cortical neuron activity in SCZ. (Eggers, 2012)

The most frequent inhibitory neurotransmitter in the central nervous system is gamma-aminobutyric acid (GABA) (Benes, 2015) GABAergic neuron activity is required for perception, memory, learning, and cognition. (Tso *et al.*, 2015) One of the primary aspects in the pathophysiology of SCZ is an imbalance between excitation and inhibition in the cerebral cortex.

TREATMENT OF SCZ

Because the pathogenesis of SCZ is not fully known, treating people with the illness is difficult. SCZ is best treated at specialty clinics. The main goals of SCZ therapy are to reduce the occurrence and severity of psychotic exacerbations, improve positive and negative symptoms, and improve patients' functional ability and quality of life. Identifying patients in the early stages of psychosis and providing the appropriate therapy is consequently a top responsibility for neuropsychiatric practitioners. Clinicians deal with the diagnosis in several situations, including the first presentation, providing support to family members, evaluating concomitant medical disease, managing drugs and their negative effects, and providing primary care when specialist choices are unavailable.

However, little research has focused on understanding and treating SCZ patients. (Yousaf, 1997) Mental health care in this region is highly reliant on the use of pharmaceuticals (antipsychotic drugs) as well as medical and physical therapies, including electro-convulsive therapy (ECT). In general, antipsychotic medications are used to treat SCZ and address neurotransmitter abnormalities (Stone, Morrison and Pilowsky, 2007). Such medications have been shown to inhibit the behavioral effects

of phencyclidine (PCP) and several neurotransmitter receptors, including N-methyl-D-aspartate receptor (NMDA)-glutamate ion channel, resulting in a reduction in both positive and negative symptoms.(Lodge and Mercier, 2015) Long-term antipsychotic therapy can cause dopamine super sensitivity psychosis in SCZ, and delusional procreation syndrome (DPS) may have a major role in the development of SCZ treatment recalcitrance. (Seeman *et al.*, 1976; Yamanaka *et al.*, 2016)

Furthermore, clozapine, a multi-receptor atypical antipsychotic, is approved for the treatment of resistant SCZ; however, despite its demonstrated efficacy in the treatment of resistant SCZ, some populations, such as the elderly and adolescents, may be particularly vulnerable to clozapine side effects. (De Berardis *et al.*, 2018)

Antipsychotics are used to treat psychosis and manage the disorder's symptoms, allowing patients to function in everyday life. (Suhail and Chaudhry, 2004)

SCZ is a complicated multi-factor condition that, based on present knowledge, is unlikely to be treated with a single-target therapy.

Neurexin family

Neurexins (NRXNs) are presynaptic transmembrane proteins that are mostly found on the cell surface of neurons and are essential for synaptic transmission. (Ullrich, Ushkaryov and Südhof, 1995; Südhof, 2017). There are three neurexin genes in animals (NRXN1, NRXN2, and NRXN3) (Ushkaryov *et al.*, 1992)

Each gene produces two main protein isoforms with a shared C terminus: the longer -neurexin and the shorter -neurexin (Tabuchi and Südhof, 2002). The larger neurexin isoform, which is located upstream of exon 1, produces a protein with the following features: (i) a signal peptide (SP) at the N-terminus; (ii) six laminin/neurexin/sex-hormone-binding globulin (LNS) domains.

(iii) three epidermal growth factor (EGF) like regions in the middle; (iv) a carbohydrate attachment (fig 3).

The shorter neurexins have a promoter downstream of exon 18 for NRXN1 and exon 17 for NRXN2 and NRXN3 that produces a protein with the same sequence as an isoform beginning at the sixth LNS domain but no EGF-like repeats (Missler and Südhof, 1998). Extensive alternative splicing, a hallmark of these genes, gives rise to thousands of isoforms at the 6 alternative splicing sites (SS1-6 in NRXN1/3 and SS1-5 in NRXN2) in the a-isoform, two of which are shared by the -isoform (Ullrich, Ushkaryov and Südhof, 1995; Missler and Südhof, 1998; Treutlein *et al.*, 2014) (fig 3).

Because of many isoforms, this gene is extremely difficult to research, as seen by the phenotypic variation seen in both people and mice models shown below.

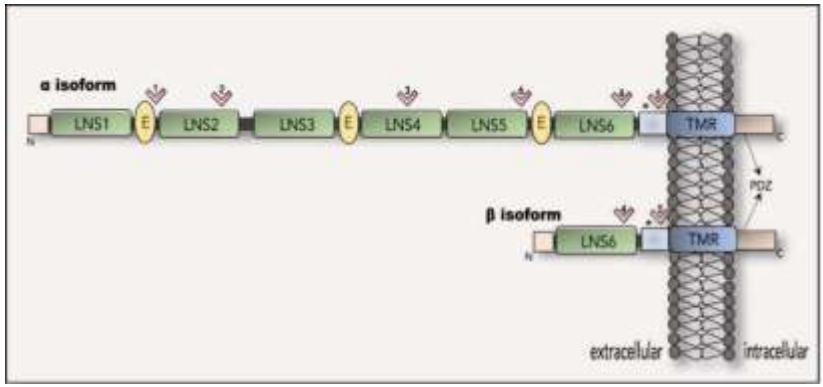


Figure 3: Domain organization of α-neurexins and β-neurexins

The α isoform has six LNS (laminin/neurexin/sex hormones binding protein domains) and three EGF (epidermal growth factor) domains, whereas the isoform has just one LNS domain. The six possible splice sites of NRXN1 and NRXN3 (only 5 in NRXN2), with two commons in the β isoform, are shown by red arrows, as well as a highly glycosylated region (*), transmembrane region (TMR), and PDZ domain-binding motif at the C terminal end.

Association between Neurexin and SCZ

Kirov and colleagues (Kirov *et al.*, 2008) were the first to report a function for NRXN1 in SCZ. They examined the genomes of 93 SCZ patients for CNVs and found a 250Kb deletion in the

5 end of NRXN1 in an afflicted sibling pair and no loss in 372 controls. A 115Kb deletion at the 3 end of NRXN1 was discovered in an afflicted twin pair (2/233 cases; 0/268 controls) in a subsequent publication (Walsh *et al.*, 2008).

One SCZ patient was found to have a 389Kb deletion in exon 1 of NRXN1, and four other SCZ patients were found to have deletions in exons and introns of NRXN1a (Vrijenhoek *et al.*, 2008).

Guilmette et al.(Guilmatre *et al.*, 2009) investigated 236 SCZ patients and 236 controls and discovered two deletions, one affecting exon 1-2 of NRXN1a and the other affecting both NRXN1 and. Rujescu et al. (Rujescu *et al.*, 2009) looked at CNVs in 2977 European ancestry SCZ patients and 33,746 controls on a much bigger scale. They found an excess of CNVs in NRXN1 in SCZ (cases: 0.47 percent, controls: 0.15 percent) and focused on exon disruptive deletions or duplications, concluding that the CNVs found must influence disease propensity. They discovered 12 deletions and two duplications in the NRXN1 gene or its promoter region; however, no novel CNVs were discovered in NRXN2 or NRXN3.

Gauthier et al. detected a de novo 24 heterozygous frameshift mutation in exon 22 of NRXN1, altering the isoform, in a female with disordered SCZ. A premature stop codon resulted from the 4-nucleotide insertion, resulting in a truncated protein lacking the transmembrane region and cytoplasmic tail (Gauthier *et al.*, 2011). Similar to the NRXN2 study published earlier by the same authors, immunofluorescence tests and

western blot analysis on neuronal cell cultures demonstrated intracellular accumulation of a partially non-functional NRXN1 protein resulting in a failure to establish synapses. Marshall and colleagues published the biggest SCZ cohort to date in a landmark 2017 research (Marshall *et al.*, 2017). (21,094 cases; 20,227 controls). A genome-wide significant association signal was discovered at eight loci using a gene-based association test. The lone single-gene locus discovered, NRXN1 has one of the highest odds ratios (OR: 14.4; cases: 35; controls: 3) of all reported loci. This odds ratio matches the odds ratio (OR 9.74: cases 67; controls 15) of a genome-wide CNV analysis of NRXN1 deletions from (Hu *et al.*, 2019) of studies involving samples from the United States(Need *et al.*, 2009; Levinson *et al.*, 2012; Todarello *et al.*, 2014) , Japan (Ikeda *et al.*, 2010), China (Li *et al.*, 2016), and many European countries.

This supports the substantial link between NRXN1 deletions and an increased likelihood of developing SCZ in individuals.

There has been no research linking NRXN2 to SCZ to date. The first investigation to link NRXN3 to SCZ was conducted in a Chinese Han community (1,214 SCZ cases; 1,517 controls). Three of the seven genotyped SNPs related to SCZ (rs7157669, rs724373, and rs7154021) were found in intron 1 and 2 of the gene (Hu *et al.*, 2013)

MATERIALS AND METHODS

ETHICS STATEMENT

Subjects provided written informed consent, and the Institutional Biomedical Ethics Committee at Umm Al-Qura University approved the study protocol for human research in accordance with the recommendations of the National Committee of Biomedical Ethics (http://bioethics.kacst.edu.sa/About.aspx?lang=en-US). Enrolled Saudi simplex individuals diagnosed with SCZ from neuropsychiatric clinics throughout Saudi Arabia's western region (including Jeddah, Mecca, and Taif), as well as healthy controls with no clinical history of mental disorders, behavioral illnesses, or epilepsies.

STUDY DESIGN

Case-Control study enrolled Saudi simplex individuals diagnosed with SCZ from neuropsychiatric clinics throughout Saudi Arabia's western region (including Jeddah, Mecca, and Taif), as well as healthy controls with no clinical history of mental disorders, behavioral illnesses, or epilepsies.

CHARACTERISTICS OF THE STUDY POPULATION

The study was conducted among Saudi male patients diagnosed with schizophrenia (SCZ) and healthy controls. Patients aged 23-68 years who presented at the Outpatient Psychiatry Clinics at governmental and private hospitals in Saudi Arabia, over a six-

month period were considered for inclusion. The mean age was 26 years at onset and 38.0 years at examination.

The percentage of patients with insidious-onset schizophrenia (62%) was greater than the percentage of patients with acute-onset schizophrenia (38 percent). Antipsychotics were often used by patients to treat behavioural and psychological symptoms of schizophrenia. Individual interviews, clinical observation, medical records (hospital and outpatient clinic case notes), and family information were used to confirm the diagnosis of SCZ according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria (American Psychiatric Associations, 1994). The patients were also exposed to a 45- to 50-minute interview using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) to determine the severity of their schizophrenia"Positive symptoms" denoted an overabundance or distortion of normal functions (e.g., hallucinations, delusions), whereas "negative symptoms" denoted a decrease or loss of normal functions. 30 symptoms (7 positive symptoms, 7 negative symptoms, and 16 symptoms of general psychopathology) were scored on a scale of 1 to 7 during the interview. For a schizophrenic patient, the overall PANSS score (with a maximum of 210) could not be lower than 30. The mean total PANSS score was 92.8 (SD, 13.56), positive psychotic symptoms were 23.1 (SD, 7.91), negative psychotic symptoms were 22.7 (SD, 4.16), and overall psychopathology was 47.8 (SD, 7.79).

People having a history of traumatic brain injury, neurologic diseases, mental retardation, epilepsy, or drug addiction (excluding nicotine) were not allowed to participate. Healthy volunteers who did not fulfill any of the exclusion criteria and had no current, previous, or family history (first-degree relatives) of mental disorder made up the control group.

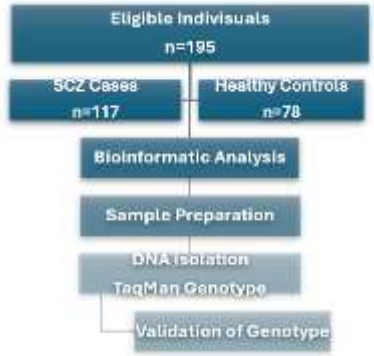


Figure 4. Flow chart of eligible subjects and methodology

SAMPLES PREPARATION

In an EDTA tube, five ml of peripheral blood was taken from One hundred seventeen patients SCZ patients and Seventy-Eight healthy controls. The manufacturer recommends using the QIAamp DNA blood kit to extract genomic DNA from peripheral blood (200 µL) (Qiagen, Hilden, GmbH, Germany).

METHODOLOGY

DNA EXTRACTION

I. Pipet 20 µl QIAGEN proteinase K into the bottom of a 1.5 ml microcentrifuge tube.

II. Add 200 µl sample to the microcentrifuge tube.

III. Add 200 µl Buffer AL to the sample. Mix by vortex for 15 s.

The sample and Buffer AL must be mixed thoroughly to yield a homogeneous solution to ensure efficient lysis.

IV. Incubate at 56°C for 30 min.

V. Briefly centrifuge the microcentrifuge tube to remove drops from the inside of the lid.

VI. Add 200-µl ethanol (96–100%) to the sample, and mix again by vortex for 15 s. After mixing, briefly centrifuge the microcentrifuge tube to remove drops from the inside of the lid.

VII. Carefully apply the mixture from step 6 to the spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 2 min. Place the spin column in a clean 2 ml collection tube, and discard the tube containing the filtrate.

VIII. Carefully open the spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the spin column in a clean 2 ml collection tube, and discard the collection tube containing the filtrate.

IX. Carefully open the spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.

X. Recommended: Place the spin column in a new collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.

This step helps to eliminate the chance of possible Buffer AW2 carryover.

XI. Place the spin column in a clean 1.5 ml microcentrifuge tube and discard the filtrate collection tube. Carefully open the QIAamp Mini spin column and

add 200 µl Buffer AE or distilled water. Incubate at room temperature (15–25°C) for 1 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min.

DNA QUALITATIVE AND QUANTITATED VALIDATION

We were eager to run quantitative and qualitative quality testing on the extracted DNA using two methods: Gel Electrophoresis and NanoDrop, after the DNA extraction technique to assure DNA purity, concentration, and quality, as they are some of the most critical aspects determining the experiment's success and reliance on the results.

GEL ELECTROPHORESIS

To check DNA integrity, we pipet 5 ul of DNA from each sample to be separated on a 1 percent agarose gel electrophoresis with 2 ul loading dye. In a gel containing 60 ul appropriate ethidium bromide, the DNA was electrophoresed for 1 hour at 100 volts. The gel was photographed using a UV transilluminator and a Syngene Gel Documentation System G-BOX F3 (England, Cambridge). GeneSys application-driven picture capture software manages the system, which contains an infinite number of copies of GeneTools analysis software.

Gel Preparation:

I. Flask with 100 mL of 1X TBE buffer.
II. 1 g of agarose was added to the flask.
III. The flask was placed in the microwave.
IV. The flask was cooled down.
V. 60 ul of ethidium bromide was added.
VI. Flask mixture poured into a mold with combs inserted to make walls.
VII. 60 ul of ethidium bromide was added.
VIII. The gel was placed in the submarine and filled with buffer.

NANODROP TECHNEQUE

NanoDrop is a mathematical technique for determining the concentration and purity of DNA after it has been extracted. The samples were calculated using NanoDrop 2000 Spectrophotometers (Thermo Fisher Scientific, USA) and NanoDrop 2000 operating software version 1.6. Protein concentrate is calculated at 280 nm, whereas DNA concentration is calculated at 260 nm.

Calculation Steps:

I. Blank with 1 ul of elution buffer for calibration.
II. 1 ul of DNA was added to the NanoDrop match.
III. Close the capping, then measure.

TaqMAN GENOTYPING ANALYSIS

We genotyped people for the chosen SNPs of the NRXN3 gene using TaqMan genotyping assays (Thermo Fisher Scientific, USA) on a 7500 Fast-Dx Real-Time Polymerase Chain Reaction (PCR) System (Applied Biosystems, Life Technologies Inc., USA). Probe test kits were provided by Integrated Gulf Biosystems (ABI agency,

Jeddah, SA). The SNPs' assay IDs were rs12879016 (C___31754644_10) and rs2270964 (C___15959111_20) (table3)

The tests included all the DNA samples. All samples were genotyped twice, and the results were 100% consistent.

Preparing the master mix and genomic DNA with pooling technique:

I. 10 ul of TaqMan genotyping master mix 2X was added to each sample.
II. 0.5 ul of TaqMan genotyping assay mix 40X was added to each sample.
III. 7.5 ul of nuclease-free water was added to each sample.
IV. 2 ul of genomic DNA.

Preparing the reaction plate for PCR:

I. The master mix and genomic DNA sample was pipetted into a MicroAmp Fast Optical 96-Well Reaction Plate from (Applied Biosystems, Life Technologies Inc., USA).
II. The plate was covered with MicroAmp Optical Adhesive Film (Applied Biosystems, Life Technologies Inc., USA).
III. The plate was centrifuged briefly to spin down the contents, and bubbles were eliminated.
IV. The plate was inserted into the machine to perform the PCR.

Table2: Examined SNP markers

Marker name	Assy ID	NCBI SNP reference	SNP type
NRXN3	C___31754644_10	rs12879016	UTR3 Substitution
	Context Sequence: ATGAAGTGTGTCCTCTGGAGGGTCA**[G/T]**ATATACAATTTCTTTTGTACAGATG		
	C___15959111_20	rs2270964	INTRON Transversion Substitution
	Context Sequence: CAGATCGAGCGTGGCTGTGAAGGTA**[A/C]**AACCTATTTTTTTCTTGTTAAGCTA		

BIOINFORMATICS ANALYSIS

Bioinformatics contributes to the finding of inherited genetic variants such as SNPs, as well as understanding how these changes affect protein function, gene regulation, and expression. It can also help to better assess a person's illness risk and/or responsiveness to prospective treatments. Bioinformatics greatly influences the statistical analysis of SNP data and the identification of signature SNPs for a specific haplotype block. Haplotyping, Linkage Disequilibrium tests, and public data repository tools are some of the various bioinformatic approaches utilized to undertake linkage analysis. Using in *silico* methods, we investigated the impact of various variations on their functional proteins. Table.2 shows the two SNPs used for this investigation. Mutations, splicing sites, and connection to miRNA binding sites may all be predicted using this method. We used Sorting Intolerant From Tolerate (SIFT) (http://sift.jcvi.org/),

PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/),

MutationTaster (http://mutationtaster.org/),

and Align GVGD (http://agvgd.hci.utah.edu). Splicing variations were examined using Human Splice Finder (http://www.umd.be/HSF3/HSF.html)

and Exon Splice Finder (http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?Process=home).

NRXN1-NRXN3 PROTEIN INTERACTION

The Search Tool for Retrieval of Interacting Genes (STRING) database (https://string-db.org) was also used to predict functional interactions between proteins

STATISTICAL ANALYSIS

Both healthy controls and cases with SCZ were tested for exact Hardy-Weinberg equilibrium (HWE) using the $\chi 2$ test, and a P-value < 0.05 was considered a departure from HWE. We conducted the statistical analysis for the examined SNPs considering the models of inheritance—the codominant, dominant, recessive, overdominant, and additive models— using the SNPStats software (https://www.snpstats.net). Logistic regressions for genotypic distributions and allelic frequencies for SCZ cases and controls were measured in terms of odds ratios (ORs) and 95% confidence intervals (CIs). The less Akaike information criterion (AIC) value that corresponded to the minimally expected entropy was adopted to assess the best model of inheritance. The t-test and the chi-square test were utilized to evaluate demographic and clinical characteristics including age, gender, IQ, and CARS score (https://www.medcalc.org). A two-tailed $P \leq 0.05$ was considered statistically significant.

RESULT

For the study, 117 eligible males with schizophrenia and 78 healthy non-psychotic controls were enrolled. **Table 3** showed selected socio-demographic and clinical characteristics. Twenty-five additional eligible males with schizophrenia did not enrol because they refused to be clinically investigated, they could not be traced, or their clinical profiles were incomplete.

Table 3. Sociodemographic and clinical information of schizophrenia males.

Parameter	**Schizophrenia cases (N= 117)**	***t* (95% confidence interval)**
No. (%) with family history (+)[a,b]	24 (20.5)[c]	146.8 (0.2-0.4)[d]
Age at onset (years)	25.8 ± 3.93 (36-16)	57.2 (25.1-26.6)[d]
Age at examination (range, years)[e]	38.0 ± 7.2 (68-23)	49.6 (36.7-39.3)d
Status:		
Single	66 (56.4)	25.5 (46.9-65.5)[d]
Married	27 (23.1)	8.98 (15.8-31.8)[d]
divorced	24 (20.5)	7.69 (13.6-29.0)[d]
Occupation:		
Jobless	63 (53.8)	24.2 (44.3-63.1)[d]
Military	36 (30.8)	12.8 (22.6-40.0)[d]
Others	18 (15.4)	5.2 (9.4-23.20)[d]
PANSS score:		
Total	92.8 ± 13.56	74.7 (90.3-95.3)[d]
Positive	23.1 ± 4.52	56.1 (22.3-23.9)[d]
Negative	22.7 ± 4.16	59.1 (21.9-23.5)[d]
General psychopathology	47.8 ± 7.79	67.1 (46.4-49.2)[d]

PANSS, Positive and Negative Syndrome Scale; SD, standard deviation.

[a] Number of patients, with percentages in parentheses,

[b] Family history was considered positive (+) if there was more than one case having schizophrenia in the same family and negative (−) if the case was sporadic,

[c] z-value's test, [d]Student's *t*-test. Values are mean ± standard deviation,

[d] Very highly significant difference ($P < 0.0001$).

HARDY-WEINBERG EQUILIBRIUM OF SNPS

All controls were consistent with the Hardy-Weinberg Equilibrium (HWE) at the examined SNPs: *NRXN3* rs2270964 ($\chi^2 = 0.54$; $P = 0.462$), and *NRXN3* rs12879016 ($\chi^2 = 0.542$; $P = 0.462$). In contrast, our cases showed deviations in cases with SCZ for rs2270964 ($\chi^2 = 49.9$; $P = 0.000$), and *NRXN3* rs12879016 ($\chi^2 = 4.61$; $P = 0.032$).

ALLELE FREQUENCIES OF *NRXN3* GENETIC LOCI

Table 4 shows the allele frequencies of the *NRXN3* gene (rs2270964C>A and rs12879016G>T) polymorphic loci. The ORs of the allelic variants were 0.48 (95% CI 0.20-1.17; $P = 0.106$) for *NRXN3* rs2270964C>A and 0.94 (95% CI 0.61-1.46; $P =$

0.787) for *NRXN3* rs12879016G>T (Table 2). In the rs2270964C>A SNP, the cases with the variant A-allele were smaller than controls (4% versus 8%, respectively). Similarly, the G-allele is slightly lower in cases than in controls (29% versus 31%, respectively) in rs12879016G>T SNP. Consequently, both the rs2270964C>A and rs12879016G>T SNPs are statistically not significant with SCZ (P > 0.05).

GENOTYPIC DISTRIBUTION OF *NRXN3* GENETIC LOCI

The best interactive model of inheritance was selected based on the lowest *AIC* values. Thus, the best interactive statistical model was overdominant for the *NRXN3* rs2270964C>A SNP with a statistical significance (OR= 0.14, 95% CI 0.02-1.38; *P* = 0.047). Thus, the cases with SCZ (94.9%) were carrying the wild-type C/C-genotype compared to controls (84.6%). The heterozygous genotype rs2270964 C/A was overexpressed in control compared to cases (15.4% versus 2.6%). This observation might explain that even one copy of the allelic A-variant in the rs2270964C>A SNP had no effect on the risk of the SCZ. But one copy A-allele variant might show a protective effect on individuals. The homozygous variant genotype carrying the A-allele was absent in control but rather with the cases with SCZ (0.0% versus 2.6%).

Table 4. Genotype distributions and allele frequencies of selected SNPs in SCZ cases and controls (adjusted by age).

Genetic Model	Interactive Genotype	Control *n* = 78	Cases *n* = 117	Logistic regression		
				OR (95% CI)	***P*-value**	**AIC**
NRXN3 rs2270964C>A: A= variant						
Codominant	C/C C/A A/A	66 (84.6) 12 (15.4) 0 (0.0)	111 (94.9) 3 (2.6) 3 (2.6)	1 0.2 (0.04 0.55) 4.2 (0.2-82.1)	0.1 0.35	92.0
Dominant	C/C A/A-C/A	66 (84.6) 12 (15.4)	111 (94.9) 6 (5.1)	1 0.3 (0.05-1.76)	0.17	92.6
Recessive	C/C-C/A A/A	78 (100) 0 (0.0)	114 (97.9) 3 (5.1)	1 4.8 (0.2-94.2)	0.31	92.3
Overdominant	C/C-A/A C/A	66 (84.6) 12 (15.4)	114 (97.4) 3 (2.6)	1 0.1 (0.02-1.38)	**0.047**	87.9
Log-additive	---	---	---	0.6 (0.14-2.25)	0.40	90.8
Allele: 	C A	144 (0.9) 12 (0.08)	225 (0.96) 9 (0.04)	1 0.5 (0.20-1.17)	0.106	NA

NRXN3 rs12879016G>T: G= variant						
Codominant	T/T G/T G/G	36 (46.1) 36 (46.1) 6 (7.7)	63 (53.9) 39 (33.3) 15 (12.8)	1 0.6 (0.34-1.14) 1.4 (0.51-4.00)	0.124 0.498	50.9 52.0
Dominant	T/T G/T-G/G	36 (46.1) 42 (53.9)	63 (53.9) 54 (46.1)	1 0.7 (0.41-1.31)	0.293	50.5
Recessive	T/T-G/T G/G	72 (92.3) 6 (7.7)	102 (87.2) 15 (12.8)	1 1.8 (0.65-4.77)	0.263	46.3
Overdominant	T/T-G/G G/T	42 (53.9) 36 (46.1)	78 (66.7) 39 (33.3)	1 0.9 (0.32-1.05)	0.073	<u>45.5</u>
log-additive	---	---	---	1.4 (0.42-4.50)	0.075	45.6
Allele:	T G	108(0.7) 48 (0.31)	165 (0.71) 69 (0.29)	1 0.9 (0.61-1.46)	0.787	NA

SCZ, Schizophrenia disorder; SNP, single nucleotide polymorphism; OR, odds ratio; CI: confidence interval.

[a]Number of subjects, with percentages in parentheses, [b]*P*-values were evaluated from logistic regression analysis after adjusting for age. Bold numbers indicate statistically significant *P*-value ($P < 0.05$), [c]*AIC* values refer to the model with the less *AIC* value that corresponds to the minimal expected entropy. Underlined numbers represent the best model of inheritance with the less AIC value.

HAPLOTYPE ANALYSIS AND LINKAGE DISEQUILIBRIUM

The results of the case-control haplotype analysis and comparisons of individual haplotypes between groups are displayed in (**Table 5**.) Among the three possible haplotypes of rs2270964C>A and rs12879016G>T polymorphic loci, the A-T haplotype was found to have an overall frequency of 5.4%. However, no significant difference was found between SCZ cases and control group (P = 0.53). The case-control haplotype analysis showed no significant different in the haplotype distribution between cases and controls (P = 0.65). Also, there was no significant linkage disequilibrium between the two SNPs (P = 0.0761). (Table 6).

Table 5. Haplotype association with response (n = 195, adjusted by age)

Haplotype	NRXN3 rs2270964C>A	NRXN3 rs12879016G>T	Frequency	OR (95%, CI)	P-value
1	C	T	0.6462	1	---
2	C	G	0.3	0.86 (0.4-1.8)	0.69
3	A	T	0.0538	0.51 (0.1-2.2)	0.53

Global haplotype association *P*-value = 0.65

In addition, linkage disequilibrium (LD) between the *NRXN3* rs2270964C>A and *NRXN3* rs12879016G>T SNPs showed relatively weak LD (P = 0.0761) (**Table 4**).

Table 6. Linkage disequilibrium analysis of the *NRXN3* rs7154021T>C and *NRXN3* rs724373T>C SNPs in schizophrenia sample group.

LD measure
D (linkage disequilibrium) = -0.0161
D` (linkage disequilibrium coefficient = 0.9961
r (correlation coefficient) = -0.1556
r^2 (square of the correlation coefficient between two allelic variants) = 0.0241
P-value = 0.0761

Bold numbers indicate statistically significant P value ($P < 0.05$).

IN-SILICO ANALYSIS OF SNPS

The Variant Effect Predictor-genome browser (https://www.ensembl.org/) showed that the intronic variant NRXN3 rs2270964 does not have deleterious effects on the genome; since the CADD score was very low (CADD raw= 0.93). Also, no clinical significance was found on ClinVar. The SpliceAI tool predicts acceptor gain of 23 positions and a potential donor loss of 10 positions. This means that the SNP lies near a donor site and could be involved in the alternative splicing of NRXN3 transcripts. Similarly, the 3`UTR variant *NRXN3* rs12879016 did not have any effects on the genome as shown by the CADD score (CADD raw = 1.45). SpliceAI tool did not show any effects on the acceptor or donor.

DISCUSSION

This is the first case-control study to investigate the association between the rs2270964C>A and rs12879016G>T SNPs in the *neurexin* gene and schizophrenia (SCZ) in the Saudi male community.

Overall, our results found that the two allelic variants of the rs2270964C>A SNP, and rs12879016G>T SNPs had not obvious susceptibility to SCZ among Saudi males. However, these biomarkers might paly roles as significant protective biomarkers. Moreover, molecular observations might explain that even one copy of the allelic A-variant in the rs2270964C>A SNP had no effect on the risk of the SCZ, but a potential protective effect on individuals.

Our results showed that having C/A genotype was significantly different (P = 0.047) between controls and cases, where 15% of the controls were carrying this genotype compared to 2.6% of cases, which suggests a protective behaviour.

Based on literature, there is no research made on the two SNPs in this study and their association with SCZ in different population. In a Chinese population, (Wang *et al.*, 2018) have reported a potential susceptibility of the rs12879016G>T SNP to SCZ (P = 0.023) but could not have found any significant association between the rs2270964C>A SNP and autism spectrum disorder (ASD) (Wang *et al.*, 2018) Moreover, (Wang *et al.*, 2018) also described that the individuals carrying the G-allele (G/G-G/T genotypes) of the rs12879016 SNP had less risk of SCZ than individuals carrying the homozygous T allele(Wang *et al.*, 2018). To an extent, we showed similar results where the G/G genotype was more prevalent in SCZ cases compared to the control group, 12.8% and 7.7%, respectively.

According to ALFA project (https://www.ncbi.nlm.nih.gov/snp/rs2270964#frequency_tab) our results regarding the allele frequency of rs2270964C>A SNP in Saudi males with SCZ is consistent with most ethnic populations. But Asian populations recorded significant allelic differences ranging from 60% for the C-allele (reference), and 40% for the A-allele (alternative) (**Table 7**).

Table 7. Allele frequency of the reference and alternative alleles for the *NRXN3* rs2270964C>A

Population (Ethnicity)	Sample size	C-Allele (Reference)	A-Allele (Alternate)
Saudi Arabia (This study)	**117 (78)***	**0.96**	**0.04**
Total	165570	0.981361	0.018639
European	132100	0.986185	0.013815
African	8072	0.9836	0.0164
African American	7814	0.9834	0.0166
Asian	468	0.577	0.423
East Asian	332	0.602	0.398
South Asian	4994	0.8915	0.1085
Latin American	1144	0.9834	0.0166

*Patients (controls)

According to ALFA project (https://www.ncbi.nlm.nih.gov/snp/rs12879016#frequency_tab) our results regarding the allele frequency of *NRXN3* rs12879016G>T SNP in Saudi males with SCZ is

consistent with the European, Asian, East Asian and South Asian. But African and African American groups seem to have different allele frequencies (**Table 8**).

Table 8. Allele frequency of ref and alt alleles for *NRXN3* rs12879016G>T.

Population (Ethnicity)	Sample size	G-Allele (Reference)	T-Allele (Alternate)
Saudi Arabia (This study)	**117 (78)***	**0.29**	**0.71**
Total	149868	0.231717	0.768283
European	126480	0.208167	0.791833
African	9306	0.5933	0.4067
African American	8980	0.5896	0.4104
Asian	630	0.283	0.717
East Asian	498	0.265	0.735
South Asian	184	0.261	0.739
Latin American	750	0.351	0.649

*Patients (Controls)

Neurexins are expressed in various areas of the brain and they function as cell adhesion molecules in the nervous system at inhibitory and excitatory synapses, which appears to be limited to neurons (Reichelt, Rodgers and Clapcote, 2012; Hu *et al.*, 2013). Despite that the mechanism by which neurexins may modulate schizophrenia is yet to be described, it is likely that abnormalities in the neurexin-mediated sign{Bibliography}alling play a role in the disease etiology (Gupta *et al.*, 2009) A brief report suggested that NRXN3 polymorphisms could play a role in schizophrenia as studied in Disc1-schizophrenia mouse model (Brown *et al.*, 2011)

There are several studies that associated NRXN1 polymorphisms with SCZ, where the first study being published in 2008 by Kirov and colleagues (Kirov *et al.*, 2008) Subsequent reports identified multiple deletions in the NRXN1 gene in SCZ patients, which adversely affected the function of the protein (Vrijenhoek *et al.*, 2008; Walsh *et al.*, 2008). After that several studies have investigated NRXN1 CNVs with SCZ and found strong associations of NRXN1 CNVs with increased risk patients developing SCZ (Need *et al.*, 2009; Ikeda *et al.*, 2010; Levinson *et al.*, 2012; Rees *et al.*, 2014; Todarello *et al.*, 2014; Lew *et al.*, 2018; Hu *et al.*, 2019) To date, there were no studies that associated the NRXN2 with SCZ risk. The first study to report NRXN3 association with SCZ was in the Chinese Han population, where they studied seven SNPs in the NRXN3 gene. Of those seven SNPs, three SNPs (rs7157669, rs7154021 and rs724373) were associated with SCZ risk.

wSTUDY LIMITATION

According to our study we face some limitations. We need to have more subjects to be contribute to the future studies since it was hard to find more confirmed cases, beside that we need to cooperate with other Saudi region hospital to have general representing

of Saudi population. In Covid 19 the pandemic situation make it difficult to have more candidates.

One of the issues we face that we couldn't bring female cases for our study since they refuse to participate with us because of some restrictions and habits among Saudi families

CONCLUSION

The association between NRXN3 gene and schizophrenia still needs to be supported or refuted by other studies. In this study, there was no evidence of NRXN3 polymorphisms and increased risk of SCZ. The rs2270964 C/A genotype was more prevalent in controls than cases suggesting a protective effect. With that being, NRXN gene family need further studies as they might be promising candidate genes to several neurodevelopmental disorders.

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