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Genetic association analysis of *ERBB4* polymorphisms with the risk of schizophrenia susceptibility in a Jordanian population of Arab descent

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Background/aim: The *ERBB4* gene encodes a transmembrane tyrosine kinase and is considered to be one of the risk genes of schizophrenia. Although there is evidence of the roles of genes and the environment in the etiology of schizophrenia, a comprehensive biological and genetic background of the disease is still lacking. The aim of this study is to assess whether genetic variation in the human *ERBB4* gene is associated with vulnerability to schizophrenia in the Jordanian Arab population.

Materials and methods: A total of 185 inpatients with schizophrenia participated in this study and 195 healthy genetically homogeneous individuals were also used as controls. Two genetic variants, rs839523 (G/A, intron 2) and rs3748962 (A/G, exon 27), encompassing the *ERBB4* gene were genotyped using DNA sequencing.

Results: The results revealed a strong and statistically significant genetic association of rs839523 with schizophrenia (P = 0.002 for allele and P = 0.006 for genotype).

Conclusion: This study provides strong statistical evidence that there is an association between the *ERBB4* gene and schizophrenia in a Jordanian population of Arab descent.

Key words: *ERBB4*, schizophrenia, Jordanian Arab

1. Introduction

Schizophrenia (Sz) is a devastating psychiatric disorder that is considered to be one of the most incapacitating mental illnesses in modern medicine. This disorder generates a considerable global economic burden on national healthcare budgets due to the annual financial costs required to treat it (1). It affects approximately 1% of the worldwide population (2). In a clinical sense, Sz is characterized by positive symptoms (e.g., delusions, hallucinations), negative symptoms (e.g., anhedonia, avolition, social withdrawal, lack of personal hygiene), and cognitive impairments (e.g., working memory problems and poor attention) (3,4).

Although this disorder is caused by both the individual's genes and the environment, the biological background of this illness has been misleading (5). Sz has a complex genetic architecture with multiple genes that contribute to the etiology and the risk of this disease. Moreover, regardless of the huge amount of research made in the field, the molecular etiology of Sz is still in its early stages (6,7). Some of the proposed candidate genes of Sz are *DRD2*, *DRD3*, *DARPP-32*, *COMT*, *SLC1A6*, *SLC1A2*, *GRIN1*, *GRIN2A*, *GRIA1*, *NRG1*, *ERBB4*, *DTNBP1*, *DAAO*, *G72/30*, *GRM3*, *DISC1*, *RGS4*, *PRODH*, *DGCR6*, *ZDHHC8*, *DGCR2*, *CREB*, *IL-1B*, *IL-1RN*, *IL-10*, *IL-1B*, *TRAR4*, *PPP3CC*, and *AKT1* (8,9).

The *ERBB4* gene is located on human chromosome 2 (2q34) (10). It is one of four members of the erbB receptor tyrosine kinase (RTK) family. The main functions of this family are cell growth regulation, cell survival, maturation and apoptosis of the neurons, cell proliferation, and cell differentiation. The *erbB* family contains four genes: *erbB1*, *erbB2*, *erbB3*, and *ERBB4* (11,12). In terms of size, *ERBB4* is a large gene consisting of approximately 1.16 Mb that includes 28 exons encoding for type 1 transmembrane tyrosine kinase (10,13). There are four isoforms of ERBB4 that have been characterized due to alternative splicing of the ERBB4 transcript (12). Structurally, the ERBB4 receptor is composed of the following components: an extracellular ligand binding domain, an intracellular juxtamembrane (JM) region, a single transmembrane domain, a tyrosine kinase domain, and a carboxyterminal

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tail (14). The *ERBB4* gene is found to be expressed in the human heart, kidneys, neuronal tissues, renal tissues, and breasts and in most areas of the adult brain (15).

The role of *ERBB4* in the pathophysiology of Sz is evident at the biological level for many reasons (10,16,17). First, expression of *ERBB4* is detected in the developing and adult human brain neurons. Second, the NRG1-ERBB4 signaling pathway is involved in neurobiological operations comprising neurogenesis, synapsis formation and plasticity, neuronal migration, and axon guidance, all of which are hypothesized to be disturbed in Sz. The NRG1 protein acts as a ligand for the erbb4 receptor to form the NRG1-ERBB4 complex. After the binding, the ligand–receptor complex prompts dimerization and activates the tyrosine kinase receptor, resulting in phosphorylation of the receptor's cytoplasmic domain. Sequentially, this generates a docking site for adaptor proteins like Shc, Grb2, and the regulatory subunit of phosphoinositide-3-kinase (PI3-kinase), which in turn results in the activation of downstream signaling pathways such as the Ras-Raf-MAPK and the PI-3 kinase pathways that are involved in the mediation of various biological functions. For instance, in neurodevelopment, these pathways participate in neuronal migration, glial cell development, axon myelination and guidance, dendritic development, and neurotransmitter signaling (10,16–18). Third, mutant mice heterozygous for *ERBB4* show a Sz-related behavioral phenotype. Furthermore, the brains of schizophrenic patients are reported to have changes in *NRG1*-mediated *ERBB4* activation as well as changes in the gene expression of the *ERBB4* splice isoform (12).

Few genetic association studies have been conducted to reveal the role of *ERBB4* in Sz (10). However, Norton et al. reported that variants in *ERBB4* were associated with an increased risk of Sz (11). Two other studies conducted by Silberberg et al. and Law et al. independently replicated the findings of Norton et al., and they found that three intronic single nucleotide polymorphisms (SNPs), rs7598440, rs839523, and rs707284, are associated with the risk of Sz (12,19). Nicodemus et al. stated that a haplotype containing rs3748962-G has an association with Sz. Furthermore, the ratio of A/G of allelic expression of ERBB4 mRNA at this SNP was detected in a study conducted by Norton et al. (11) on brain mRNA expression in maternal and paternal chromosomes. This result further explained that the possibility of another cis-acting polymorphism also affects the expression of ERBB4. Therefore, either rs3745962 or another functional polymorphism in LD with rs3745962 could be associated with Sz. Unfortunately, the effect of rs3745962 on ERBB4 function has not been clarified yet.

In this study, we describe the allelic and genotypic distribution of SNPs in the *ERBB4* gene in Jordanian patients of Arab descent diagnosed with Sz, and we investigate the association between the *ERBB4* gene polymorphisms and Sz among Jordanian patients of Arab descent.

2. Materials and methods

2.1. Study samples

Patients' samples were collected from the Psychiatric Clinic of the National Center for Mental Health at Al Fuheis and the National Center for Mental Health at Al Karameh in Jordan. Male Arab patients (N = 185) diagnosed with Sz and meeting the criteria for Sz set by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (American Psychiatric Association, 1994), were included in this study, as well as 198 healthy male volunteers (control group) from an ethnically homogeneous Jordanian Arab population recruited from the blood bank at King Abdullah University Hospital. Approval to study these patients was granted by the Institutional Review Board of the Jordan University of Science and Technology (Ref No. RA/13/68/2013). Each participant gave signed informed consent.

2.2. DNA genotyping

Genomic DNA was extracted within 1 week of blood collection via the commercially available Puregene Blood Core Kit B (QIAGEN, Germany) according to the manufacturer's instructions**.** Yield (ng/µL) and purity (*A*260/280) of the extracted DNA were measured using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technology, USA). DNA was amplified using specific primers that map to the *ERBB4* gene, and the primers were designed using Primer 3 Software (http://primer3.ut.ee/)**.** Table 1 summarizes the primer sequences and the predicted product sizes of the polymerase chain reaction (PCR)-amplified products for the *ERBB4* gene.

The PCR reaction was optimized for the amplification protocol and an optimal annealing temperature of 60 °C was used (Applied Biosystems, USA). The amplification

Table 1. The primer sequences and the predicted product size of PCR amplified products for the *ERBB4* gene.

SNP	Forward primer 5′ → 3′	Reverse primer 5′ → 3′	Product size
rs839523	CCTCTTCTGCATTAGTTGGTCC	ACACCCTCCATCAACACATTT	401 bp
rs3748962	TGGCTTTGATATCCTTGTGGC	TGTGATCCAGTAAAGGCCTAGG	513 bp

protocol proceeded as follows in specific stages: stage 1 (initial activation step) of amplification was maintained at 95 °C for 10 min, followed by stage 2 of three steps of cycling (denaturation, annealing, extension) repeated 35 times, which were adjusted at 95 °C for 1 min for denaturation, 60 °C for 1 min for annealing, and 72 °C for 1 min for extension, respectively. Finally, stage 3 (the end of PCR cycling) was adjusted at 4 °C. The PCR product was visualized on 2% agarose gel (Promega Corporation, USA). Next, 5 µL of each PCR product and 5 µL of 100-bp ladders (Thermo Scientific, USA) were loaded into wells of 2% agarose gel and 3 µL of 10 mg/mL ethidium bromide (Bio Basic Inc., Canada) was added to the gel to stain the DNA band for visualization. The gel was run using 1X Tris-borate-EDTA (TBE) running buffer (prepared from 10X TBE: 108 g of Tris, 55 g of boric acid, and 40 mL of 0.5 M EDTA dissolved in up to 2 L distilled water) using an electrical current source of 150 V for 35 min. Gel documentation was done under a UV transilluminator supplied with a gel documentation system (Bio-Rad, USA). The PCR product size was measured by matching with known band sizes of the ladder. The PCR product was purified to remove the unwanted PCR component by using the EZ-10 Spin Column PCR Products Purification Kit (Bio Basic Inc.) according to the manufacturer's instructions. After purification of the PCR product, samples were ready for the sequencing reaction protocol. Briefly, 1 µL of Ready Reaction Mix was mixed with 4 µL of sequencing buffer, 5 µL of nuclease-free water, 2 µL of forward primer (single-sided sequencing reaction), and 2–3 µL of purified DNA in a PCR tube and vortexed. The sample was loaded on a thermocycler (Applied Biosystems) according to the following protocol: stage 1 (25 cycles), denaturation (96 °C for 10 s), annealing (50 °C for 5 s), and extension (60 °C for 4 min); and stage 2 (1 cycle), 4 °C for infinity.

The PCR sequencing products were cleaned to remove small molecules from the nucleic acids. This cleaning was performed according to the manufacturer's instructions with a special cleaning kit (NucleoSEQ, Germany). The sample was then either directly loaded on the genetic analyzer (3130x1, Applied Biosystems) using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) or stored at –20 °C.

2.3. Chromatogram analysis

DNA sequence chromatograms were visualized using Chromas Pro software v.1 (USA). The chromatograms were also compared to a reference sequence (Ensembl ID: ENSG00000178568) that was obtained from the Ensembl genome browser (http://www.ensembl.org/index.html) to search for abnormalities, deletions, insertions, substitutions, or heterozygosity.

2.4. Statistical analysis

A sequencing technique was used to genotype the two SNPs (rs839523 and rs3748962) within the *ERBB4* gene. In order to determine if the population fulfilled the Hardy–Weinberg equilibrium (HWE) equation ($p^2 + 2pq + q^2 = 1$), the genotyping frequency was calculated using the HWE calculator (20) involving analysis for ascertainment bias. SPSS 19.0 was used in statistical analysis. The chi-square test was used to determine the P-value of the allele and genotype association between Sz patients and healthy control individuals. Variations in allele and genotype frequencies between patients and controls were assessed using case-control genetic association analysis. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Sample characteristics

All 185 patients were diagnosed with Sz and met the criteria set by the DSM-IV. Patients were excluded if they displayed one of the following symptoms: diagnosis of bipolar disorder, schizoaffective disorder, major depression, and/or mental retardation. Patients with serious health and medical conditions were also excluded. Sample collection from patients was difficult due to the social stigma against mental health. Therefore, many families and patients refused to give blood samples or clinical information. A total of 195 control DNA samples were collected from unrelated matched healthy Jordanian individuals. Controls were excluded if they had a history of drug dependence, alcohol dependence, mental health problems, and/or psychosis. Detailed information on Sz patients and healthy individuals is summarized in Table 2. Clinical data were collected from the patient's medical records, as summarized in Table 3.

Table 2. Detailed information of cases and controls used in this study.

Parameters	Cases (schizophrenia)	Controls (healthy individuals)
Number of subjects	185	195
Age (years)*	45.9± 12.6	31.4± 8.1
Range of age	18–75	18–52
Sex	Male	Male

*Mean± standard deviation.

Table 3. Clinical data for schizophrenia patients.

Category		Subcategory	Value (n)	% or M ± SD*
Demographic data	Marital status	Married	26	13.3%
		Divorced	15	7.7%
		Single	154	79%
	Occupation	Unemployed	187	95.9%
		Employed	8	4.1%
General information	Type of schizophrenia	Acute	8	4.1%
		Chronic	187	95.9%
	Insight	Lacking	63	33.3%
		Partial	20	10.6%
		Full	106	56.1%
	Age at onset			25.6 ± 6.97
	Reason for admission	Relapse	46	23.7%
		Schizophrenia	100	51.6%
		Symptoms	15	7.7%
		Others	33	17%
Positive symptoms	Hallucinations	Yes	144	76.2%
		No	45	23.8%
	Delusion	Yes	152	81.3%
		No	35	18.7%
	Thought disorder	Yes	132	71%
		No	54	29%
	Change in behavior and thoughts	Yes	136	72.3%
		No	52	27.7%
	Movement disorder	Yes	74	38.9%
		No	116	61.1%
Negative symptoms	Loss of interest	Yes	153	79.3%
		No	40	20.7%
	Lack of concentration	Yes	124	64%
		No	70	36%
	Social withdrawal	Yes	136	70.5%
		No	57	29.5%
	Flattened emotions	Yes	126	66%
		No	65	34%
	Lack of care for appearance	Yes	150	76.9%
		No	45	23.1%
	Lack of personal hygiene	Yes	136	70.1%
		No	58	29.9%
	Change in sleeping pattern	Yes	106	56.1%
		No	83	43.9%
	Uncomfortable with people	Yes	138	71.1%
		No	56	28.9%
	Aggressive	Yes	89	46.4%
		No	103	53.6%

* Mean (M) ± standard deviation (SD).

3.2. Polymerase chain reaction and gel electrophoresis

Two SNPs were selected for genotyping and amplified within the *ERBB4* genomic sequence, located in intron 2 (rs839523) and exon 27 (rs3748962) as shown in Figure 1.

PCR analysis was conducted to show if the amplified fragments for each SNP had the expected sizes in all patients and controls and to verify the absence of contamination in PCR products. If no bands were visualized for any DNA sample on the gel, the DNA sample was reamplified again using different concentrations of either the primers or DNA.

Samples from 185 patients and 198 healthy individuals were amplified and sequenced successfully. All the fragments were loaded on 2% agarose gel and the size of each fragment was determined against a 100-bp ladder. The size of the amplified fragment product for rs839523 was 401 bp and 513 bp as shown in Figures 2 and 3, respectively.

3.3. DNA sequencing and genotyping analysis

After the PCR amplified products were purified, the purified DNA fragments were amplified again using sequencing PCR by forward primer. All the amplified fragments for the two SNPs (rs3748962, intron 1 and rs839523, exon 27) in the *ERBB4* gene were sequenced and the electropherogram sequences were blasted against their reference sequences (www.ensemble.org), and then the changes in bases of the SNPs were identified and tabulated in Excel sheet format.

The rs839523 SNP is located in intron 2 of the gene and changes G to A (Figure 4). Eighty-six patients were found to have the homozygous ancestral allele of GG, 74 patients had the heterozygous allele of GA, and 25 patients had the homozygous allele of AA for this polymorphism. The rs3748962 SNP is located in exon 27 of the gene and changes A to G (Figure 5). One hundred and six patients were found to have the homozygous ancestral allele of

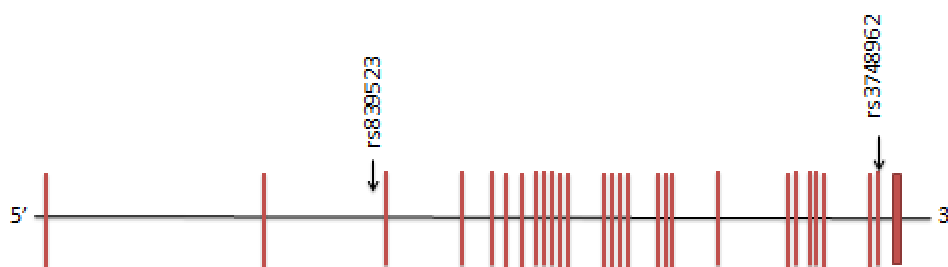

Figure 1. Schematic diagram of the location of SNPs of the *ERBB4* gene on chromosome 2q34 (red blocks represent coding exons).

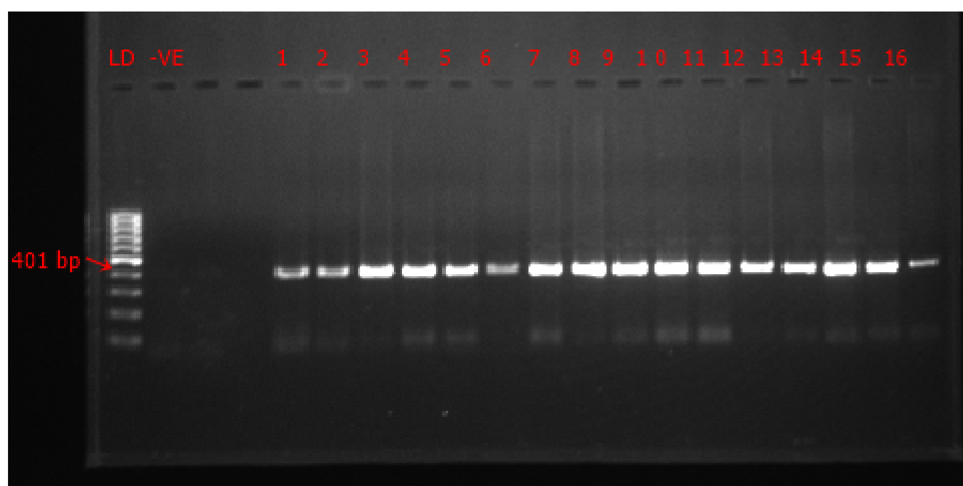

Figure 2. Agarose gel electrophoresis of amplified genomics DNA (*ERBB4*-INTRON1-PCR) from sixteen individuals: PCR product of *ERBB4* gene for intron 1 was separated using 2% agarose gel. The first lane (LD) from the left corresponds to the 100-bp DNA molecular ladder. Lane -VE represents the negative control for the PCR product, while lanes 1 to 16 represent the 401-bp amplified product of the DNA samples from Sz patients.

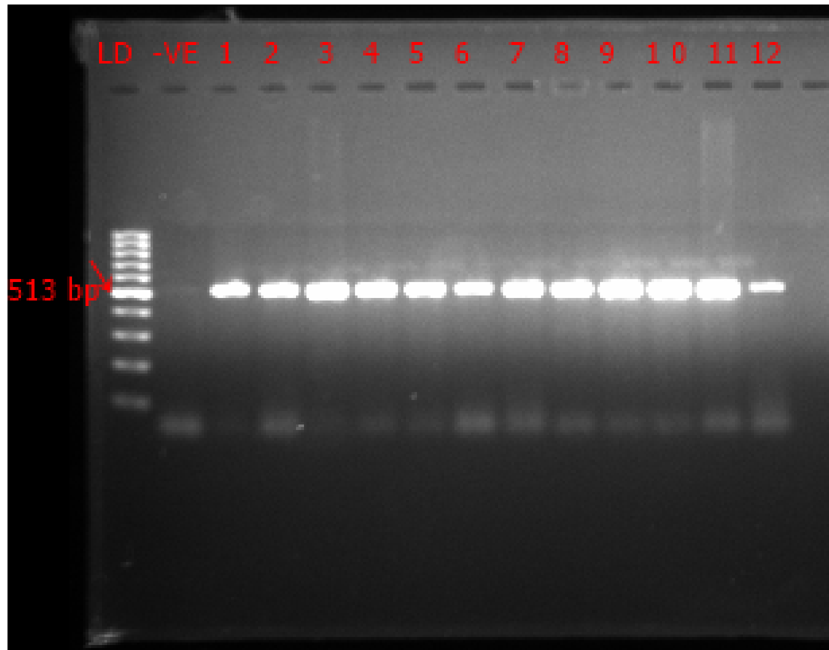

Figure 3. Agarose gel electrophoresis of amplified genomics DNA (*ERBB4*-EXON27-PCR) from twelve individuals: PCR product of *ERBB4* gene for exon 27 was separated using 2% agarose gel. The first lane (LD) from the left corresponds to the 100-bp DNA molecular ladder. Lane -VE represents the negative control for the PCR product, while lanes 1 to 12 represent the 513-bp amplified product of the DNA samples from Sz patients.

AA, 61 patients had the heterozygous allele of AG, and 18 patients had the homozygous allele of GG for this polymorphism.

3.4. Allelic and genotypic distribution of schizophrenia patients and healthy individuals

The distribution of the rs839523 and rs3748962 genotypes between the Sz patients and controls was assessed. The genotyping frequency of the rs839523 SNP was different between patients and controls. The GG genotype frequency was 46.5% in patients while it was 30.8% in controls. Furthermore, the G allele frequency was 66.5% and 55.8% in patients and controls, respectively. In contrast, the AA genotype and A allele frequencies were at 13.5% and 33.5% in patients, respectively, while in controls they were at 19.2% and 44.2% for the AA genotype and A allele frequencies, respectively. The heterozygous genotype showed a 40% frequency compared to 50% frequency in controls.

However, no major differences in genotypic and allelic frequencies of the rs3748962 SNP were detected between schizophrenic patients and controls. The AA genotype frequency was 57.3% in patients but it was 49.5% in controls. Furthermore, the heterozygous genotype frequency was 33% in patients while it was 44.4% in controls. The GG frequency was found to be 9.7% in cases compared to 6.1% in controls. The A allele frequencies were 73.8% and 71.7% in patients and controls, respectively. In addition, the G allele frequencies were 26.2% in schizophrenic patients and 28.3% in controls. Genotype and allele frequencies were evaluated for concordance with HWE. The rs839523 SNP in cases and controls were in HWE, but the rs3748962 SNP in cases was slightly out of HWE at P = 0.0446 (Table 4).

3.5. Genetic association of rs839523 and rs374896) SNPs with schizophrenia using different genetic models

The genotype and allele frequencies of the rs839523 SNP in the Sz cases differed significantly from those of the controls (P = 0.006 for genotype and P = 0.002 for allele). No significant differences were observed in the rs3748962 genotype and allele frequencies between Sz cases and controls (P = 0.05 for genotype and P = 0.52 for allele) (Table 5).

Genetic association analysis of the two SNPs genotyped in the Sz cases and controls was also conducted using dominant, additive, and recessive genetic models (Table 5). For rs839523, the heterozygous GA versus common homozygous GG and rare homozygous AA versus common homozygous GG categories were statistically significant (chi-square > 3.84, P < 0.05). In contrast, rare homozygous AA versus heterozygous GA showed no statistical significance.

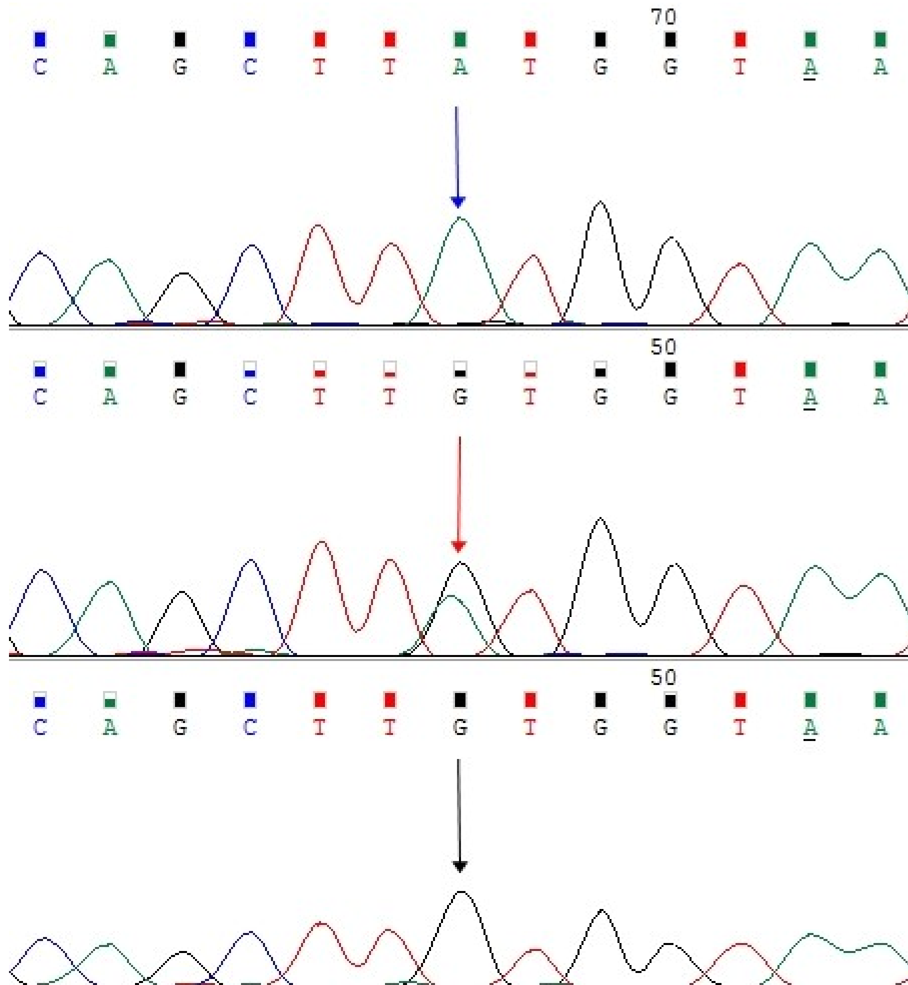

Figure 4. Representative DNA sequence chromatograms of the region including the rs839523 SNP in Sz patients. Vertical arrows indicate the position of the rs839523 (G > A) SNP. The blue arrow indicates the homozygous (AA) genotype, the red arrow indicates the heterozygous (AG) genotype, and the black arrow indicates the homozygous (GG) genotype for the ancestral allele in this site.

In contrast, for the other SNP, rs3748962, rare homozygous GG versus heterozygous AG and rare homozygous GG versus common homozygous AA were found to be statistically insignificant (P > 0.05). However, heterozygous AG versus common homozygous AA was significant (Table 5).

4. Discussion

Complex genetic diseases such as hypertension, diabetes, cancer, coronary heart diseases, and most psychiatric disorders (e.g., schizophrenia) follow a non-Mendelian mode of inheritance (21). The complexity in genetic architecture confers a critical burden in Sz research (22). However, genetic and functional analyses provide evidence of the association between the *ERBB4* gene and Sz. Therefore, various genetic epidemiology studies have proposed *ERBB4* as a candidate susceptibility gene for Sz. *ERBB4* is one of the *ErbB* family receptor genes, which has the most recognized functions in the central nervous system among these subfamilies (23). Because of the previous association of *ERBB4* with Sz in the literature, this study focused on the association of two SNPs of the *ERBB4* gene with Sz in a Jordanian population of Arab descent. To the best of our knowledge, this study is the first in the field of psychiatric genetics research in Jordan and in people of Arab descent in general.

Only male patients were recruited in this study for many reasons. Culture is the main factor responsible for the bias. For example, the stigma of mental health contributes to negative attitudes towards giving blood samples and medical information. Most of the interviewed females patients refused to give blood because of fear or distrust.

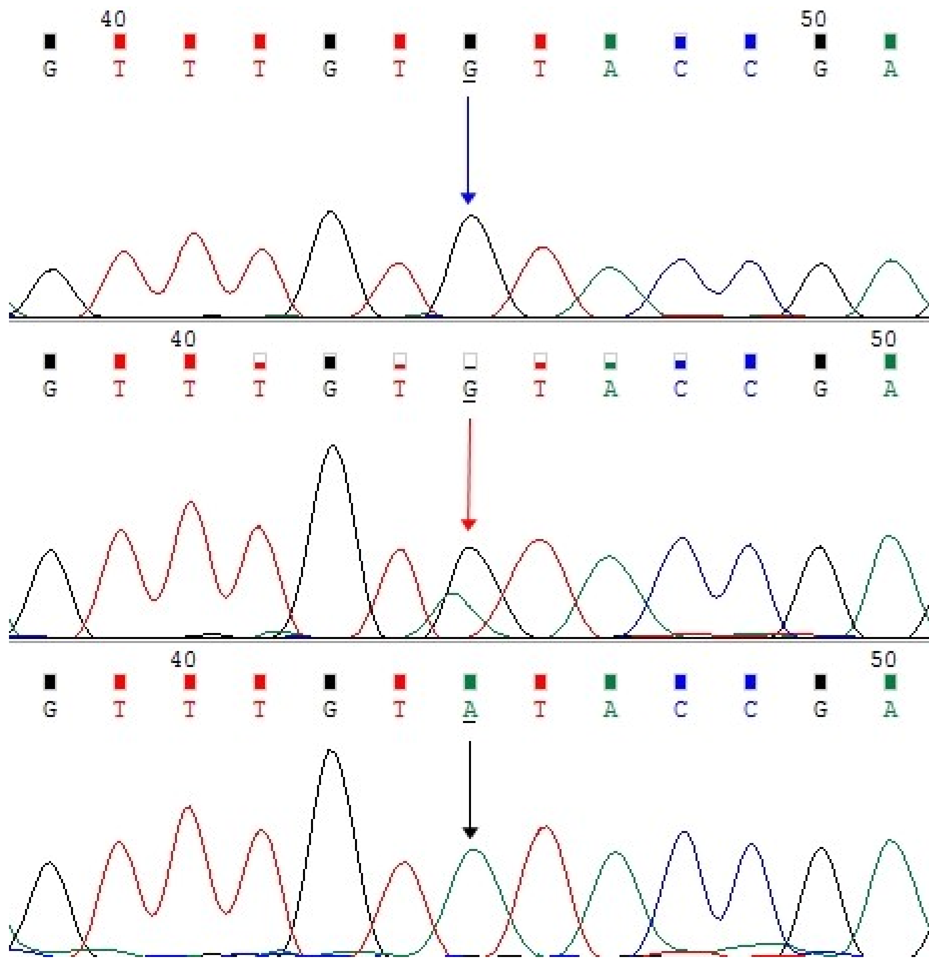

Figure 5. Representative DNA sequence chromatograms of the region including the rs3748962 SNP in Sz patients. Vertical arrows indicate the position of the rs3748962 (A > G) SNP. The blue arrow indicates the homozygous (GG) genotype, the red arrow indicates the heterozygous (AG) genotype, and the black arrow indicates the homozygous (AA) genotype for the ancestral allele in this site.

Table 4. *ERBB4* SNPs, their minor allele frequencies, and HWE P-values in cases and controls.

Gene	SNP _ID	Cases (n = 185)			Controls (n = 198)		
		MA[1]	MAF[2]	HWE[3] P-value	MA[1]	MAF[2]	HWE[3] P-value
ERBB4	rs839523	A	33.51%	0.1636	A	44.19%	0.8474
	rs3748962	G	26.22%	0.0446	G	28.28%	0.1787

[1]MA: minor allele. [2]MAF: minor allele frequency. [3]HWE: Hardy–Weinberg equilibrium.

The currently used diagnostic criterion in Jordan is the DSM-IV. This criterion is used in most of the psychiatric hospitals in the country, which is the reason why the DSM-IV was used in this research.

In this study, a genetic association was found between the rs839523 genotype and male Sz patients (P = 0.006). In contrast, the rs3748962 genotype showed no statistically significant association with the disease (P = 0.05). Analyses of genetic association using different genetic models (dominant, additive, and recessive) were conducted to confirm the association of the variant's genotypes between Sz cases and controls. The results of the genetic models also

Table 5. Alleles and genotypes distributions of the rs839523 and rs3748962 polymorphisms in the *ERBB4* gene in schizophrenia patients and controls and genetic association analysis using different genetic models.

Polymorphisms	Patients (n = 185)	Controls (n = 198)	Chi-square*	P-value	OR (95% CI)
rs839523					
GG	86 (46.5%)	61 (30.8%)	10.117	0.006	-
GA	74 (40%)	99 (50%)			
AA	25 (13.5%)	38 (19.2 %)			
Het (GA) vs. common hz (GG)	-	-	7.860	<0.025	0.53 (0.34–0.83)
Rare hz (AA) vs. het (GA)	-	-	0.180	>0.025	0.88 (0.49–1.58)
Common hz (GG) vs. rare hz (AA)	-	-	6.270	<0.025	0.47 (0.26–0.85)
Alleles					
G	246 (66.5%)	221 (55.8%)	9.165	0.002	-
A	124 (33.5%)	175 (44.2%)			
rs3748962 (Val1065Val)					
AA	106 (57.3%)	98 (49.5%)	5.972	0.050	-
AG	61 (33%)	88 (44.4%)			
GG	18 (9.7%)	12 (6.1%)			
Het (AG) vs. common hz (AA)	-	-	4.200	<0.025	0.64 (0.42–0.98)
Rare hz (GG) vs. het (AG)	-	-	3.680	>0.025	2.16 (0.97–4.82)
Rare hz (GG) vs. common Hz (AA)	-	-	0.680	>0.025	1.39 (0.64–3.03)
Alleles					
A	273 (73.8%)	284 (71.7%)	0.412	0.520	-
G	97 (26.2%)	112 (28.3%)			

P < 0.05 is significant for allelic and genotypic associations (in bold).
*For significant association, the chi-square value should be >3.84 with P < 0.025 for the genetic association model test (in bold).
CI: confidence interval.

Table 6. Frequency of the rs839523 SNP in different populations compared with the Jordanian population.

Population	rs839523 genotype frequencies, n (%)						Association	Reference
	Cases			Controls				
	A/A	A/G	G/G	A/A	A/G	G/G		
Bulgaria	23 (12.4%)	84 (45.4%)	78 (42.2%)	14 (7.7%)	74 (40.4%)	95 (51.9%)	No	30
China	170 (16.2%)	480 (45.8%)	398 (38%)	186 (17%)	523 (47.7%)	388 (35.4%)	No	13
Israel	11 (18.6%)	15 (25.4%)	33 (55.9%)	23 (17.7%)	76 (58.5%)	31 (23.8%)	Yes	19
Japan	62 (14.9%)	205 (49.4%)	148 (35.7%)	73 (14.2%)	265 (51.5%)	177 (34.4%)	No	26
Jordan	25 (13.5%)	74 (40%)	86 (46.5%)	38 (19.2%)	99 (50%)	61 (30.8)	Yes	Current study
Taiwan	46 (20.3%)	111 (48.9%)	70 (30.8%)	39 (17.5%)	96 (43%)	88 (39.5%)	No	25
UK	27 (6.8%)	153 (38.5%)	217 (54.7%)	100 (7.5%)	533 (39.7%)	708 (52.8%)	No	16

confirm the association between the rs839523 SNP and Sz. The association was found in the heterozygous AG versus common homozygous GG model and in rare homozygous AA versus common homozygous GG (chi-square > 3.84 and $P < 0.05$ for both models). On the other hand, none of the genetic models showed a significant association with the rs3748962 SNP.

Despite the convincing evidence of the involvement of *ERBB4* in the progression of Sz, comparatively few genetic association studies have been performed in different populations, including Ashkenazi Jews, Americans (Caucasians and African-Americans), and Han Chinese (19,24,25). Comparing the genotypic frequency results in this study with other populations provides an insight into the differences between populations and ethnic groups in terms of disease risk. This comparison of the rs839523 polymorphism results of this study with other populations (Table 6) revealed an inconsistency in frequencies of each genotype. For example, Silberberg et al. reported the first genetic association study between different *ERBB4* SNPs and Sz in Ashkenazi Jews. They found a highly significant genotypic and allelic association of the rs839523 SNP ($P = 0.000021$ and $P = 0.0045$, respectively) and Sz disorder (19). In their study, the GG genotype frequency was 55.9% in Sz cases and it was only 23.8% in controls, while in our study the GG genotype showed a frequency of 46.5% in Sz cases and 30.8% in controls. In Asian populations, a relatively similar frequency result is found between different genetic association studies. For example, the GG genotype frequency in Sz cases was 38% in China (13), 30.8% in Taiwan (25), and 35.7% in Japan (26), and those studies reported no association between this SNP and Sz. Our results are consistent with the results shown in Ashkenazi Jews (19), but they are not consistent with the results reported in Korean (10), Han Chinese in China (13), Taiwanese (25), and Japanese (26) populations.

In the case of rs3748962 (Val1065Val), our result is consistent with the results found in the UK population ($P = 0.94885$) and in Ashkenazi Jews (16,19), but not consistent with those shown in Taiwanese ($P = 0.0027$ for genotype and $P = 0.0007$ for allele) or Korean populations (10,25). The discrepancy between results is due to the differences between ethnicities and the population stratification in the different studied populations. The variations in genotype and allele frequencies between several studies are also related to the lack of replication in different populations. However, the risk of population stratification in this study is unlikely because the Jordanian population is considered to be a genetically homogeneous population. Jordanians are descended from villagers and a Bedouin lineage originating in the Arabian Peninsula (27). Notably, the majority of the population is Arab (nearly 98%), and the remaining 2% is divided equally into Armenians and Circassians. Minor ethnic factions include the Kurds, Druze, and Chechens (28).

The possible explanation of the association of rs839523 with the risk of Sz might be related to a change of expression of the CYT-1 isoform in the brain. Silberberg et al. reported that three SNPs (rs707284, rs839523, rs7598440) in *ERBB4* exhibited highly significant differences between Sz cases and controls. They also were from one linkage disequilibrium block with significant P-values for allele frequencies ($P = 0.013$, 0.0045, and 0.0049, respectively), genotype frequencies ($P = 0.00013$, 0.000021, and 0.00018, respectively), and risk haplotype frequency ($P = 0.00044$). This haplotype is tightly linked to the overexpression of a splice variant encompassing exon 26 (CYT-1). CYT-1 is found to be overexpressed in the postmortem dorsolateral prefrontal cortex of Sz cases ($P = 0.047$). The CYT-1 domain acts as a phosphoinositide 3-kinase (PI3-K) binding site and leads to the activation of the PI3-K/Akt pathway. *Akt1* might contribute to the pathophysiology of Sz as several studies reported a positive association with the disorder (19).

Law et al. replicated the study of Silberberg et al. and found an association between these three SNPs and an elevated level of expression of the CYT-1 isoform in the patient's brain (12). Another study using a large sample of Sz families and controls found an association of the rs7598440, rs839541, and rs839523 SNPs surrounding exon 3 of *ERBB4* with Sz, and this finding was confirmed in African-American families (24).

The divergence in the results between different published studies might be due to population stratification and ethnic differences. For example, the rs3748962 SNP in cases was found to deviate slightly from HWE. This deviation could be a result of population homogeneity, since Jordan is considered as an isolated population. It is also possible that the presence of another cis-acting polymorphism affects *ERBB4* expression in patients or that other SNPs or genes may compensate for the deficiency in the function of ERBB4. The lack of association of the *ERBB4* gene in some studies is probably due to the dysfunction of other pathways and genes (e.g., *NRG1*, *GLU*, and *GABA*) that are involved in the compensation of gene expression. The NRG1-ERBB4 dysregulation might compensate for the changes in other pathways that use the ERBB4 receptor to mediate signaling (29). For example, when NRG2 or NRG3 is downregulated, the NRG1-ERBB4 signaling pathway could be activated. Another reason is that the NRG1-ERBB4 pathway is a part of a large molecular complex that has many proteins linked to various signaling pathways. Banerjee et al. suggested that these pathways might communicate and coordinate with each other. As ERBB4 is a part of that complex, it may influence other pathways or be affected by those that have a role in Sz. ERBB4 and

glutamatergic receptors are also anchored to the complex and concentrated in the postsynaptic density. ERBB4 can affect and change the glutamatergic receptor function and, in return, the glutamatergic receptors influence ERBB4 functions. Glutamatergic receptor hypofunction is found to be associated with Sz (29).

Genotyping error in this study is unlikely to have occurred as a high-throughput sequencing technique was used, and resequencing of the samples was also performed in samples that had high noisy peaks or failed to display a result on the genetic analyzer. Another issue might arise regarding sex differences. However, this confounding factor was excluded in this study since all the patients included in the study were male. Finally, the genotyping process for the patients and controls was performed during the same period and under the same conditions.

In conclusion, this study is the first genetic analysis of the *ERBB4* gene in Jordanian schizophrenic patients. No previous studies have been conducted on *ERBB4* and its association with Sz in Arab nations. Herein, we relied on a sensitive sequencing technique for the genotyping process. The results proved that the intronic variant rs839523 was strongly associated with the risk of Sz in the Jordanian population (P = 0.006 for genotype and P = 0.002 for allele). In contrast, the exonic variant rs3748962 (Val1065Val) showed no significant association (P = 0.05 for genotype and P = 0.52 for allele) with this disorder in our Jordanian sample of Arab descent. Findings from this study support a genetic association of *ERBB4* with Sz disorder. However, this study needs to be replicated in other ethnicities and specifically in Arabs in order to confirm these results. It is worth it to broaden this kind of research in the psychiatric field in order to improve the treatment and quality of health of patients.

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