Molecular Genetics
Research Article – 16362

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Screening of three ERBB4 gene polymorphisms in a group of Turkish schizophrenia patients and controls

ERBB4 genindeki üç polimorfizmin bir Türk şizofreni hasta grubunda ve kontrollerde taranması

Abstract: Objective: The human ERBB4 gene (v-erb-a erithroblastic leukemia viral oncogene homology 4) codes for the ErbB4 receptor protein. One of the ligands of ErbB4 receptor is Neuregulin-1 and it is coded by NRG1 gene which is reported to be a susceptibility gene for schizophrenia. Since ErbB4 receptor is activated by binding of Neuregulin-1, ERBB4 gene is thought to be involved in development of schizophrenia (SZ) as well. Even though several association, expression and animal studies supported this idea and resulted with association between the disease and several single nucleotide polymorphisms (SNP) in the ERBB4 gene, independent studies done in different populations did not replicate that result. In the present study we aimed to get clues about involvement of three ERBB4 SNPs -which were found to be associated with schizophrenia in some other populations- in development of schizophrenia in a group of Turkish patients.

Methods: We screened 3 SNPs (rs707284, rs7598440 and rs839523) in a group of Turkish schizophrenia patients and a matched control group from Malatya-Turkey to test the presence of such an association. For SNP genotyping, we used a real-time PCR based method and we evaluated the results with chi-square test. We have analyzed the haplotypes constituted by those three SNPs for multiple locus associations as well as analyzing each for single SNP associations.

Results: After SNP genotyping and statistical tests comparing our case and control groups for allele, genotype and haplotype distributions, there was no significant difference between those two groups for three SNPs we screened.

Conclusion: We were not able to find a significant evidence for presence of an association between three SNPs located in the ERBB4 gene and schizophrenia in our patients.

Keywords: Association, schizophrenia, ERBB4, SNP, haplotype, Turkish patients

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Özet: Amaç: İnsan ERBB4 geni (v-erb-a erithroblastic leukemia viral oncogene homology 4) ErbB4 reseptör proteinini kodlarken. ErbB4 reseptörünün ligandlarından biri Neuregulin-1 olup şizofreni için yatkınlık geni olduğu rapor edilen NRG1 geni ile kodlanır. ErbB4 reseptörünün Neuregulin-1 bağlanması ile aktive olmasındaki rolünün olduğu düşünülmektedir. Bazı bağlantı (association), ifade ve hayvan çalışmalarının bu düşüncesi desteklemesi ve hastalık ile ERBB4 genindeki bazı tek nükleotid polimorfizmlerinin (SNP) bağlantılı olduğu göstermesine rağmen, bunlardan bağımsız olarak başka populasyonlarda yapılan çalışmaların sonuçları aynı yönde olmamıştır. Bu çalışmada biz de bazı ERBB4 SNP’lerinin bir Türk hasta grubunda şizofreninin ortaya çıkmasında rolü konusunda veriler elde etmeyi amaçladık.

Metod: Bu çalışmada Malatya yöresinden toplanan bir Türk şizofreni hasta grubunda ve uygun bir kontrol grubunda yukarıda belirtilen bağlantının olup olmadığını test etmek amacı ile belirtilen 3 SNP (rs707284, rs7598440...
ve rs839523) taraflanmıştır. SNP genotiplenmesi için gerçek zamanlı PCR temelli bir yöntem kullanılmış, sonuçlar ki-kare testi ile değerlendirilmiştir. Her bir SNP’nin aynı ayrı tek SNP bağlantısının yanı sıra bu üç SNP’in oluşturduğu haplotepler de değerlendirilerek çoku lokus bağlantıları da analiz edilmiştir.

Sonuç: Hastalıklarımızda ERBB4 geninde yer alan üç SNP ile şizofreni arasında bir bağlantının olduğuuna dair anlamlı bir delil elde edilememiştir.

Anahtar Kelimeler: Bağlantı; ERBB4; şizofreni, SNP, haplotip; Türk hastalar

Introduction

Schizophrenia (MIM181500) is a disabling mental disorder with a prevalence of 0.5–1% in the general population [1]. Positive symptoms, negative symptoms, and cognitive deficits are clinical characteristics of schizophrenia. Positive symptoms are delusion and hallucination and negative symptoms are social withdrawal, flat affect and anhedonia. Impaired synaptic connectivity is one of the over-arching pathologies of schizophrenia and it has been observed across numerous human post mortem, as well as functional and structural brain imaging studies [2]. Schizophrenia is accepted as a complex and multifactorial disease. Multiple susceptibility genes, epigenetics, stochastic and environmental factors contribute development of schizophrenia [3]. Genetic factors play a major role in the development of schizophrenia and the heritability of disease is estimated to be around 80% [4]. A number of candidate genes have been found associated with the disease [5]. The risk alleles include common SNVs (single nucleotide variation, SNPs), rare SNVs, copy number variations (CNVs) and de novo mutations (DNMs) [6]. Abnormal brain development is one of the factors suggested to be underlying schizophrenia. Several genes linked to the disease had protein products which play major roles in development of specific brain circuitries, especially in the cerebral cortex [7]. NRG1 gene which codes for neuregulin-1 protein has also been implicated as a susceptibility gene for schizophrenia after positional cloning [8] and screening of a large Scottish sample [9]. The association between NRG1 gene and schizophrenia was found in different populations [10–14]. This encouraged independent research groups to focus on ERBB4 gene that codes for the ErbB4 receptor which is activated after binding of several ligands including neuregulin-1.

ERBB4 (HER4/p180erbB4) is a transmembrane tyrosine kinase belongs to human epidermal growth factor receptor (EGFR) family. Structure of this 180 kD receptor is similar to the other three members of EGFR family and consists of an extracellular domain with four sub-domains (two receptor-L sub-domains and two GFR sub-domains), a hydrophobic transmembrane domain and a cytoplasmic region including a tyrosine kinase domain and a cytoplasmic tail domain. ERBB4 is predominantly expressed in normal heart, kidney, pituitary, parathyroid, spleen, testis, brain, cerebellum, and mammary gland [15], where it plays a role in development and differentiation. ERBB4 has four isoforms produced by alternative splicing and each isoform has different structure and function (JM-a, JM-b, CYT-1 and CYT-2) [16].

The ligands for ERBB4 include heparin-binding EGF-like growth factor, betacellulin, epiregulin, amphiregulin and neuregulins including neuregulin 1 [17–23]. Neuregulin 1 binding to the ErbB4 receptor has important roles in neuronal development especially in neuronal migration, postsynaptic maturation and dendritic morphogenesis [24–26]. Targets of ERBB4 include p85 subunit PI3K, GRB2, STAT5 and SHC. ERBB4 leads activation of downstream signaling cascades including Ras/mitogen activated protein kinase (MAPK) pathway, and the phosphoinositide 3-kinase (PI3-K)/Akt pathway after ligand binding [27]. In addition to the responses following ligand binding, ERBB4 has other activities carried out by its intracellular domain which can translocate to the nucleus where it acts in transcriptional regulation after it is released by proteolytic cleavage [28–30].

ERBB4 gene which consists of 28 exons is located on the chromosome 2 (2q33.3-q34) and spans 1.15 MB [31]. Screening of SNPs located in the ERBB4 gene resulted in either absence or presence of allele, genotype and haplotype associations with schizophrenia in different populations. In one of the first studies reporting the association between ERBB4 SNPs and schizophrenia, 19 SNPs were screened but only 3 SNPs (rs707284, rs7598440 and rs839523) were found to be associated with schizophrenia. The differences between the case and control groups for distributions of alternative alleles of each SNP were
relatively high, indicating the presence of single SNP associations. In addition to allelic and genotypic associations, a particular haplotype (G-G-A haplotype in the original article corresponding to our C-C-T haplotype) had an increased frequency in the patients (32.9% in controls and 51.5% in the patients). The difference between case and control groups was much higher when the homozygosity for the risk haplotype was compared (32.9% in the patients and 6.9% in the controls). In addition to these evidences supporting the presence of the genetic associations, gene expression analyses indicated that the CYT-1 and JM-1-a isoforms of ErbB4 were overexpressed in the patients’ brains [32]. This was supported by Law and colleagues reporting particular alleles of rs707284, rs7598440 and rs839523, and homozygosity of a particular haplotype constituted by these three SNPs (the risk haplotype reported in the reference 32) caused the CYT-1 isoform of ERBB4 mRNA to increase in dorsolateral prefrontal cortex (DLPFC). In the same study, these SNPs were also screened in two separate case-control groups from Caucasian and African American populations. Even though there was no increase in the frequency of risk haplotype in the Caucasian American patients when compared to the normal controls from the same population, a significant increase was observed in the African American patient group (31.5% in the patients and 16% in the controls) [33]. In addition to their association with schizophrenia, the SNPs mentioned above were also reported to be associated with working memory performance and stress-induced subclinical psychotic experiences (PE) and other cognitive intermediate phenotypes [34,35].

Even though ERBB4 has been suggested to be a susceptibility gene for schizophrenia in samples from Ashkenazi [32] and American (Caucasian and African) populations [33,35], that was not confirmed by the studies done in samples from Han Chinese [36,37] and Korean [37] populations. Besides these, 15 SNPs in the ERBB4 gene were screened in two large case and control groups recruited from the UK and Ireland but no allelic, genotypic or haplotype associations were found when only the SNPs in ERBB4 gene were analyzed. Interestingly, an epistatic interaction between ERBB4 SNP rs4673628 (IVS12-15 C>T) and Islandic haplotype of NRG1 gene found to be associated with the disease [38]. The genetic variations located in the ERBB4 gene associated with schizophrenia were all in the intronic regions. Sequencing of the exons of this gene in the patients did not reveal any mutations in the coding region [5,6,35,38]. Besides the SNP screening studies, three separate genome-wide association studies did not reveal an association between schizophrenia and ERBB4 gene [24]. The inconsistency seen in different populations subjected in the previously published papers may indicate more case-control studies to be done in the other populations would help to clarify the association between development of schizophrenia and three ERBB4 SNPs mentioned above. Especially the association with the risk haplotype which was investigated only in a few studies were done in small case-control groups from only a few populations [32,33]. This also indicates more evidences could be required from the other populations to clarify the contribution of the risk haplotype to development of schizophrenia. Especially the strong association with the homozygosity of risk haplotype seen in the Ashkenazi population [32] was not tested in the other populations and more evidences might help to confirm this association.

In the present study we have screened the SNPs rs707284, rs7598440 and rs839523 located in the ERBB4 gene in groups of schizophrenia patients and controls from a Turkish population living in Malatya-Turkey to find evidences to support presence or absence of an association between development of schizophrenia and particular alleles, genotypes or haplotypes. The genomic positions of the SNPs (Human Genome Assembly: GRCh38/hg38 - Dec 2013) are chr2:211951364 for rs707284, rs7598440 and chr2:211951364 for rs707284, rs7598440 and rs839523 respectively [31]. All three SNPs are biallelic with C or T alternative alleles (in some of the previous papers these alleles were called G and A respectively since the complementary DNA strand has been used as reference sequence). To our knowledge there is no publications in the literature reporting a case-control study in which the three SNPs subjected here were screened in the groups from any regions of Turkey. We had relatively small case and control groups and within our feasibilities we were able to screen a limited number of SNPs. Considering the differences of allele frequencies reported by Silberberg et al. [32], this sample size was estimated to be sufficient for detecting the expected difference between the case and control groups and capable enough to distinguish if there is a similar association.

We performed single SNP (single locus) and haplotype (multiple SNP or multiple locus) association analyses after SNP genotyping and allelic discrimination. We compared the case and control groups for distributions of alleles, genotypes and haplotypes. Our analyses did not reveal any evidence to support presence of an association. The absence of single SNP associations was similar to the groups from Asian, UK and Ireland populations [36-38] and the absence of a haplotype association was similar to a Caucasian American group [33]. But our study did not replicate the single SNP or haplotype associations found in Ashkenazi and African American Populations [32,33].
Materials and Methods

The present study was carried out in accordance with the Declaration of Helsinki guidelines. Ethical approval was granted by the local ethics committee. All participants gave written informed consent. Clinical diagnosis and evaluation of patients and controls was performed in the Department of Psychiatry of Inonu University Faculty of Medicine.

The case group included 92 unrelated schizophrenia patients (30 female and 62 male) between the ages of 18 and 64 years (average=36.9, SD: 11.6) from Malatya-Turkey. The Structured Clinical Interview for the DSM-IV (SCID-I) was used as criteria for diagnosis [39]. Patients in the case group have been followed at least for 3 years (average length of follow-up=15.2 years, SD=10, min. 3 years, max. 42 years). The average age of diagnosis was 24.4 years (SD=8.6, min: 9 years of age, max: 53 years of age).

All patients were evaluated by a senior psychiatrist (the second author). Patients diagnosed with one of schizophrenia subtypes and whose all four grandparents had Turkish ethnic origin were included in the cases group. Patients who diagnosed to have psychotic disorder due to a general medical condition, substance-induced psychotic disorder, and mood disorder with psychotic features, schizoaffective disorder, schizophrenia form disorder, schizotypal disorder, schizoid disorder, and paranoid personality disorder were excluded from this study.

The control group included 90 unrelated healthy people (47 female and 43 male) who are relatives of patients applied to Department of Cardiology of our hospital for cardiovascular diseases. All volunteers in the control group had Turkish ethnic origin. Healthy controls were also evaluated performing comprehensive diagnostic interviews by a senior psychiatrist (the second author) to confirm that they did not have any Axis I psychotic disorder or first-degree relatives with a psychotic disorder.

DNA isolation and genotyping

Genomic DNA was extracted from peripheral blood collected in EDTA coated tubes and coding system was applied to our blood and DNA samples in order to protect the confidentiality of the subjects. QIAamp DNA Blood Mini Kit (Qiagen, Germany) was used for DNA extraction according to manufacturer’s instructions. Genotyping of SNPs rs707284, rs7598440 and rs839523 were performed on a real-time PCR system (7500 Fast Real-time PCR system, Applied Biosystems, Foster City, California) using TaqMan® Universal PCR Master Mix (Catalog number: 4304437) and TaqMan® genotyping assays including probes and primers designed by Applied Biosystems (Catalog numbers: 4351379 for rs707284, 4351379 for rs7598440 and 4351379 for rs839523). Reactions were carried out according to manufacturer’s instructions in 10 µL of total volume, 5 µL of TaqMan® Universal PCR Master Mix, 0.5 µL of TaqMan® genotyping assay, 2.5 µL of water and 2 µL of genomic DNA (in 20 ng/ µL concentration). PCR conditions were 40 cycles of 95°C for 15 seconds and 60 °C for 1 minute after initial activation at 95°C for 10 minutes. An endpoint plate read was performed after PCR amplification for SNP detection after each reaction. Genotyping results were used for performing single SNP and multiple locus (haplotype) association analyses.

Statistical analysis

Single-SNP (Single Locus) association: The distribution of the allele and genotype frequencies are represented by count and percentage. Hardy–Weinberg equilibrium was tested using Pearson’s goodness-of-fit chi-squared test and Haploview v. 4.2 software [40]. The significance level for deviation from HWE was set at p-value less than 0.01 [24]. For determining the possible allelic or genotypic associations, Pearson’s chi-squared test was used to compare genotype and allele frequencies between case and control groups. The level of significance was set at p<0.05. Statistic power was calculated using the G*Power software [41].

Haplotype (Multiple Locus) association: Haploview v. 4.2 software was used for haplotype estimation [40]. Homozygousities for haplotypes (homozygous diplotypes) were determined directly filtering the genotype data in a spreadsheet software. The case and control groups were compared for the frequencies of each haplotype and each homozygous diplotype with chi-square test (Significance level: p<0.05).

Results

We recruited 92 schizophrenia patients and 90 healthy controls from Malatya-Turkey to test the association of three SNPs (rs7598440, rs839523 and rs707284) located in the ERBB4 gene with the disease. We compared our case and control groups for allele and genotype distributions of each SNP and distributions of haplotypes constituted by these three SNPs. To our knowledge there is no published
case-control studies done in the samples collected from any part of Turkey to investigate the association between schizophrenia and three SNPs mentioned above.

### Single-SNP (Single Locus) association

All SNPs were found to be in Hardy-Weinberg Equilibrium in both groups. The frequencies and distributions (counts) of alleles and genotypes for each SNP found in the patient and control groups are shown in Table 1. As seen in the columns showing p-values, after performing chi-square tests, we were not able to find a significant difference between the patient and control groups in allele or genotype frequencies and their distributions. Our sample had >80% power to detect a locus with an effect size=0.3 (significance level=0.05). The expected genotype frequencies according to Hardy-Weinberg Equilibrium are given in Table 2. The allelic discrimination plots showing the real-time PCR SNP genotyping results are available upon request.

### Haplotype (Multiple Locus) association

We performed a haplotype reconstruction and compared our patient and control groups for the frequencies of eight different haplotypes (named as HT-1 to HT-8) estimated by Haploview software [40]. Degrees of linkage disequilibrium (LD) between SNP pairs as $D'$ (normalized disequilibrium coefficient) and $r^2$ (correlation coefficient) values are given in Table 3. We have also analyzed the same LD

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**Table 1:** Distributions and frequencies of alleles and genotypes found in the patient and control groups for screened SNPs.

| SNP ID      | Allele | p-value (Allele) | Genotype | p-value (Genotype) | HW-p |
|-------------|--------|------------------|----------|--------------------|------|
| rs7598440   |        |                  |          |                    |      |
| Controls    | C      | 0.765            | CC       | 0.915              |      |
| Patients    | T      | 0.765            | CT       | 0.915              |      |
| rs839523    |        |                  |          |                    |      |
| Controls    | C      | 0.472            | CC       | 0.514              |      |
| Patients    | T      | 0.472            | CT       | 0.514              |      |
| rs707284    |        |                  |          |                    |      |
| Controls    | C      | 0.710            | CC       | 0.8                |      |
| Patients    | T      | 0.710            | CT       | 0.8                |      |

**Table 2:** Expected genotype frequencies according to Hardy-Weinberg Equilibrium.

| SNP ID      | Expected genotype frequencies |
|-------------|------------------------------|
| rs7598440   |                              |
| Controls    | CC 0.4624 CT 0.4352 TT 0.1024 |
| Patients    | CC 0.4356 CT 0.4488 TT 0.1156 |
| rs839523    |                              |
| Controls    | CC 0.4356 CT 0.4488 TT 0.1156 |
| Patients    | CC 0.39 CT 0.47 TT 0.14   |
| rs707284    |                              |
| Controls    | CC 0.3844 CT 0.4712 TT 0.1444 |
| Patients    | CC 0.36 CT 0.48 TT 0.16   |

**Table 3:** Chromosomal positions of SNPs and degrees of linkage disequilibrium (LD) between SNP pairs in our sample.

| Locus-1 SNP ID (Chromosomal Position)* | Locus-2 SNP ID (Chromosomal Position)* | $D'$ | $r^2$ |
|---------------------------------------|----------------------------------------|------|-------|
| rs7598440 (chr2:211928473)            | rs839523 (chr2:211951364)              | 0.969| 0.258 |
| rs7598440 (chr2:211928473)            | rs707284 (chr2:211974321)              | 0.971| 0.295 |
| rs839523 (chr2:211951364)             | rs707284 (chr2:211974321)              | 0.949| 0.791 |

*Human Genome Assembly: GRCh38/hg38 (Dec 2013).
block in different world populations using the data retrieved from HapMap database and compared the LD with the population we screened [42]. Table 4 shows the comparison of populations for D’ values.

The allele combinations of the SNPs constituting each haplotype, frequencies of estimated haplotypes and frequencies of homozygous diplotypes seen in the case and control groups and p-values of chi-square tests comparing the two groups are shown in Table 5. In accordance with our genotype and allele frequencies, there was no significant difference between our patient and control groups for haplotype distributions either.

Discussion

In this manuscript we present results of a case-control study which was done for testing the presence of an association between development of schizophrenia and three SNPs (rs707284, rs7598440 and rs839523) located in the ERBB4 gene in Turkish samples collected from the population living in Malatya-Turkey. Even though several publications suggested these SNPs are associated with schizophrenia in some populations, similar studies done in different populations did not replicate that result. The case-control studies done in new populations could help to clarify the genetic contribution of these three SNPs to development of schizophrenia. We carried out this case-control study in a population which was not screened for those SNPs before. Our genotype screening and association analyses did not reveal any evidence to support an association between the mentioned SNPs and development of schizophrenia at allele, genotype or haplotype level. To our knowledge this is the first study investigating the association of these SNPs with schizophrenia in the samples collected from a population living in any part of Turkey. Since our samples were collected from a limited geographical region our results may not reflect the entire Turkish Population.

ERBB4 gene is thought to be a susceptibility gene for schizophrenia (SZ). The association between schizophrenia and ERBB4 gene was first reported by Silberberg et al. [32]. It was screening of 19 SNPs in 59 cases and 130 matched control samples collected from an Ashkenazi Jewish population. Only three SNPs (rs707284, rs7598440 and rs839523) were found to be associated with schizophrenia.

Following this, more SNPs in the ERBB4 gene were screened in different populations including Caucasian and African American populations from the USA [33,35], Han Chinese and Korean populations [24,37], as well as

### Table 4: Comparison of linkage disequilibrium between our samples and populations in the HapMap database. (Data for the other populations were retrieved from HapMap database (www.hapmap.org) and D’ values were calculated with Haploview Software).

| Locus-1          | Locus-2          | Current study | TSI | CHB | CEU | ASW | LWK | MKK |
|------------------|------------------|---------------|-----|-----|-----|-----|-----|-----|
| rs7598440        | rs839523         | 0.97          | 0.96| 0.94| 0.90| 0.73| 0.13| 0.55|
| rs7598440        | rs707284         | 0.97          | 0.96| 0.94| 0.89| 0.78| 0.16| 0.56|
| rs839523         | rs707284         | 0.95          | 1   | 1   | 1   | 0.93| 1   | 0.98|

ASW: African ancestry in Southwest USA; CEU: Utah residents with Northern and Western European ancestry from the CEPH collection; CHB: Han Chinese in Beijing, China; LWK: Luhya in Webuye, Kenya; MKK: Maasai in Kinyawa, Kenya; TSI: Tuscan in Italy.

### Table 5: Distributions of 3-SNP and 2-SNP haplotypes and homozygous diplotypes in the patient and control groups.

| Haplotype | SNP | Estimated haplotype frequency | Homozygous diplotype frequency |
|-----------|-----|-------------------------------|-------------------------------|
|           | rs707284 | rs839523 | rs7598440 | Case | Control | P | Case | Control | P |
| Haplotype - 1 | T | T | C | 0.354 | 0.338 | 0.7488 | 0.174 | 0.167 | 0.896 |
| Haplotype - 2 | C | C | T | 0.327 | 0.321 | 0.9055 | 0.11 | 0.10 | 0.848 |
| Haplotype - 3 | C | C | C | 0.248 | 0.301 | 0.2568 | 0.043 | 0.11 | 0.087 |
| Haplotype - 4 | T | C | C | 0.043 | 0.039 | 0.8394 | – | – | – |
| Haplotype - 5 | C | T | C | 0.022 | 0.000 | 0.0504 | – | – | – |
| 2- SNP Haplotype | – | rs839523 | rs7598440 | Case | Control | P | Case | Control | P |
| Haplotype - 6 | – | T | C | 0.375 | 0.338 | 0.4542 | 0.173 | 0.167 | 0.9 |
| Haplotype - 7 | – | C | T | 0.327 | 0.321 | 0.9062 | 0.11 | 0.10 | 0.775 |
| Haplotype - 8 | – | C | C | 0.291 | 0.340 | 0.3171 | 0.065 | 0.144 | 0.08 |
UK and Ireland populations [38]. In addition to the results from single SNP association studies, three different haplotypes constituted by some of the SNPs in the ERBB4 gene were found to be associated with the disease in a family based study done in three different case and control groups from the USA by Nicodemus et al. One of the associated haplotypes was covering rs7598440 and rs839523 [35]. In a Han Chinese case-control study (227 patients and 223 controls) 13 SNPs in the ERBB4 gene including rs7598440 and rs839523 were screened, but only a different SNP (rs3748962) was found to be associated [24]. In a separate study rs707284 and rs839523 [34], and in another study rs707284, rs7598440 and rs839523 [37] were not found to be associated with schizophrenia in a different Han Chinese group and a group from Korea. The results from these three studies which did not confirm the association between three ERBB4 SNPs and schizophrenia in Asian groups were similar to the results we found in our study.

In the present study we screened 3 SNPs (rs707284, rs7598440 and rs839523) in a group of Turkish schizophrenia patients (n=90) and a matched control group (n=92) collected from Malatya which is a city located on the Eastern Anatolian Geographical Region of Turkey.

In the single-SNP analyses, patient and control groups were found similar for frequencies of alternative alleles (C and T) of all three SNPs. There were no significant differences between two groups for distributions of alleles, in other words, there was no evidence to support the presence of an allelic association. When we compare the genotype frequencies of each SNP between the case and control groups, we were not able to see any significant differences and no sufficient evidence was found to support a genotypic association either.

In addition to single locus association analyses, we also performed a haplotype reconstruction using Haploview v4.2 software and tested the presence of a haplotype association (multiple locus association). Degrees of linkage disequilibrium (LD) as D’ values (normalized disequilibrium coefficient) between the SNP pairs rs839523-rs707284, rs7598440-rs839523 and rs7598440-rs707284 we found in the population we studied were 0.95, 0.97 and 0.97 respectively. This LD pattern was similar to that of CEU, TSI and CHB populations, but different than LWK and MKK populations in the HapMap database. Linkage disequilibrium between these SNPs were also reported in two of the previously published papers. D’ values were reported as 0.87, 0.89 and 0.96 for Ashkenazi Population in which the SNPs were found to be associated with the disease [32]. The same LD block was also reported in samples from Han Chinese and Korean Populations which did not confirm the presence of an association. The D’ values between the same SNPs in that Han-Chinese and Korean sample were 0.99, 0.96 and 0.96 in the same order [37].

After haplotype reconstruction in our case and control groups, we have detected five different haplotypes covering 3-SNPs (3-SNP haplotypes), and three different haplotypes covering 2 SNPs (2-SNP haplotypes). Two of our 3-SNP haplotypes (rs707284-rs839523-rs7598440 T-T-C and C-C-T haplotypes, called HT-1 and HT-2 respectively) were same as the haplotypes reported by Silberberg et al. and the C-C-T haplotype (called as G-G-A and A-G-G haplotypes in the references 32 and 33 respectively) was reported to be a risk haplotype in Ashkenazi and African American samples even though that was not confirmed in the Caucasian American samples [32,33]. In addition to this genetic association, the homozygousity of risk haplotype was reported to give rise to the overexpression of CYT-1 isoform of ERBB4 mRNA in dorsolateral prefrontal cortex (DLPFC). The presence of risk haplotype only on one of the homologous chromosomes was reported not to be sufficient to cause this over expression. Like in the recessive allele behavior, samples with heterozygous diplotype carrying only one copy of risk haplotype did not have an increase in the expression of CYT-1 isoform [33].

Comparison of our patient and control groups for haplotype frequencies did not provide an evidence for an association with any haplotype and development of schizophrenia in the population from Malatya-Turkey. The frequencies of risk haplotype seen in our case and control groups were not significantly different (32.7% and 32.1% respectively) and close to the frequency reported by Silberberg et al. in their control group (32.9%), but we did not observe the significant increase reported in their patients. The homozygosity of risk haplotype (HT-2) in our patient group (11%) was not significantly more frequent than our control group (10%) either. The inconsistency in these results could be explained by the idea mentioned by Law et al. which is even though the risk haplotype alters the Erbb4 splicing patterns in the brain, the biological consequence of this alteration related to development of schizophrenia is not clear yet [33].

The presence of individuals who were homozygous for the risk haplotype in the control groups from Ashkenazi [32] and African American populations [33], and the absence of a significant difference for the frequency of this diplotype between the case and control groups in the present study and Caucasian Americans reported by Law et al. [33], may also indicate even if there is a connection between the changes in the expression of ERBB4 gene and the increased disease risk, the alternative splicing mechanism underlying the induction of particular isoforms could be altered by different genetic factors.
For instance, the alternative splicing caused by the risk haplotype might be affected by the presence of different alleles of the other polymorphisms in the ERBB4 gene, or epistatic effects of the polymorphisms in the other genes. In this case, the allele frequencies of the other polymorphisms which might change the effect of risk haplotype may be variable through different populations and this may help to explain not finding the similar association in our samples as well as the other populations which did not replicate the presence of association. Screening the other ERBB4 SNPs in the patients and controls who are homozygous for the risk haplotype may help uncovering the other SNP which can affect the alternative splicing of ERBB4 mRNA. Also genome wide SNP screening in such groups may help to find other genes contributing development of schizophrenia through this mechanism.

In conclusion, we were not able to find a significant evidence to accept the presence of an association between schizophrenia and rs707284, rs839523 and rs7598440 SNPs of the ERBB4 gene in the samples we have recruited from Malatya - Turkey. This result is consistent with the papers reporting lack of an association between all or two of these SNPs and schizophrenia in Han Chinese and Korean populations, but did not replicate the positive association reported in Ashkenazi population [32] and populations from the USA [35] and others. The first reason for this controversy might be caused by the heterogeneity seen in clinical diagnosis of disease and its clinical presentation. In addition, insufficient sample size or regional limitations could be other reasons for inconsistency. In this study we were able to screen considerably small case and control groups collected from only Malatya region of Turkey. Our sample size was predicted to be sufficient for detecting a disease association if the differences of the allele frequencies between the case and control groups were as strong as reported before [32]. But if the differences in the allele frequencies of three SNPs between our case and groups were smaller than the expected, and the association was not as strong as Ashkenazi population, this could be another reason for not finding an association. In this case, a larger sample which could be collected from different regions may help to find evidences for presence of an association with the SNPs we have screened. Also the differences between populations for risk alleles could be another reason for not finding an association. Even if there is a contribution of ERBB4 gene in development of schizophrenia in our patients, it may be associated with the other SNPs in the gene like seen in Han Chinese population reported by Lu et al. [24]. Screening of more SNPs in the ERBB4 gene may help to find results confirming the idea of ERBB4 gene to have risk alleles acting in development of schizophrenia. At last, existence of many other candidate genes and the possible epistatic interactions between them can also be other explanations for the absence of a disease association with the three SNPs which we have screened in our samples. Screening of more candidate genes or genome-wide association studies might help to explain the genetic components contributing to development of schizophrenia in the population we have screened in this study.

Acknowledgements: This study was supported by Inonu University Scientific Research Projects Unit (Project number: 2012/79). The Research Projects Unit had no role in the study design, methods, informant recruitment, data analysis, and preparation of the paper. There is no conflict of interest to report concerning this research. We thank all participants in our patient and control groups. We also thank Prof. Dr. Elif Yeşilada for providing us laboratory space and equipment.

Conflict of Interest: The authors have no conflict of interest.

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