

Original Research

DNA repair gene XPD Asp312Asn and XRCC4 G-1394T polymorphisms and the risk of autism spectrum disorder

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Abstract: Autism spectrum disorder (ASD) is a complex disorder, and its extreme heterogeneity further complicates our understanding of its biology. Epidemiological evidence from family and twin studies supports a strong genetic component in ASD etiology. Oxidative stress and abnormal DNA methylation have been implicated in the pathophysiology of ASD. Brain tissues from ASD cases showed higher levels of oxidative stress biomarkers than healthy controls in postmortem analysis. Association between oxidative stress and DNA damage has been well-known. Thus, we sought to investigate a potential link between DNA repair genes and ASD and analyze the role of XPD Asp312Asn and XRCC4 G-1394T gene polymorphisms for ASD in the Turkish population. Genotyping was conducted by PCR-RFLP based on 100 patients and 96 unrelated healthy controls. We, for the first time, demonstrated a positive association between XRCC4 gene variants and ASD risk. Frequencies of XRCC4-1394 T/G+G/G genotypes were higher in patients (34%) than the controls (18.7%). The statistical analysis revealed that the individuals who had XRCC4-1394 T/G+G/G genotype had an increased risk for ASD (OR = 2.23, 95% CI = 1.10-4.55). However, no significant association was found for XPD Asp312Asn polymorphism with the risk of ASD. Our findings suggest that XRCC4 G-1394T polymorphism might be associated with ASD pathogenesis.

Key words: DNA repair genes, XPD Asp312Asn, XRCC4 G-1394T, autism spectrum disorder, genetic polymorphisms.

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impairment in social interaction and the presence of repetitive behaviors and/or restricted interests. (1). Severity of ASD symptoms is variable and inversely correlates with adaptive functioning (2). The core symptoms of ASD usually appear before age 3 and the prevalence of ASD is strongly male-biased with the average male to female ratio around 5:1 (3). Recent studies have estimated the prevalence of ASD at 1 in 68 (4). ASD is a complex disorder, and its extreme heterogeneity further complicates our understanding of its biology. A wide range of genetic variation is involved in ASD, with interplays of gene-gene and gene-environment interactions. Epidemiological evidence from family and twin studies also supports a strong genetic component in ASD etiology. Studies of twin pairs, high-risk infant siblings, families, and populations have estimated concordance rates and segregation of the disorder within families. In the twin studies, the concordance rate of broad ASD phenotype in monozygotic twins is 70–90%, but 0–30% in dizygotic twins (5,6). Family studies indicate that the prevalence of ASD is about 25 times higher in siblings of the affected individuals than in the general population, and the rate is even higher for boys (7). It is currently believed that over 50% of the risk of developing ASD is attributed to genetic variation (8, 9).

Oxidative stress and abnormal DNA methylation have been implicated in the pathophysiology of ASD (10). There is increasing evidence that ASD pa-

tients present excessive reactive oxygen species (ROS) production and reduced methylation capacity. Brain tissues from ASD cases showed higher levels of oxidative stress biomarkers than healthy controls in postmortem analysis (11,12). Excess ROS may induce oxidative DNA damage, DNA strand breaks, base modifications, and chromosomal aberrations (13). Defects in the response to DNA damage, whether it is repair or signal transduction defects, underpin many human diseases, including cancer, immune dysfunction, radiosensitivity disorders, and neurodegenerative diseases. For repair of oxidative DNA damage, human cells have five DNA repair systems: Direct reversal repair (DRR), mismatch repair (MR), double-strand break repair (DSBR), base excision repair (BER), and nucleotide excision repair (NER) (14). Xeroderma pigmentosum group D (XPD), also named excision repair cross-complimentary group 2, is one of the most important genes in the NER pathway of the DNA repair system. XPD maps to chromosome 19q13.3 and is composed of 23 exons (15). The XPD Asp312Asn polymorphism is characterized by a G to A transition causing an aspartic acid (Asp) to asparagine amino acid (Asn) exchange at position 312 in exon 10. Previous studies have demonstrated that this polymor-

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Table 1. PCR and RFLP procedures and expected products of XPD Asp312Asn, and XRCC4 G-1394T genes.

Genes	Primers (forward and reverse)	PCR product	Restriction enzyme	Restriction Products
XPDAsp312Asn	5'-CTGTTGGTGGGTGCCCGTATC-3' 5'-TAATATCGGGGCTCACCTGC-3'	751bp	StyI (37 °C)	Asp/Asp: 507,244 Asp/Asn:507,474,244,33 Asn/Asn: 474,244,33
XRCC4 G-1394T	5'-AGAAGGGCAATCCACCTTTG-3' 5'-AGCATTAGCGCTTCTCGAG-3'	257bp	MboII (37 °C)	T/T: 257 T/G:257,165,92 G/G: 165,92

phism was associated with lower DNA repair capacity and higher level of DNA adducts (16). The gene encoding X-ray complementing group 4 (XRCC4) is one of the genes associated with the non-homologous end-joining (NHEJ) repair pathway, and the XRCC4 protein is believed to help repair the DNA DSBs. XRCC4 G-1394T polymorphism is characterized by the nucleotide change from G to T in the promoter region (17).

To further investigate the participation of NER and DSB repair mechanisms in ASD pathogenesis and to identify novel candidate DNA repair susceptibility genes, we investigated the association between XPD Asp312Asn and XRCC4 G-1394T gene polymorphisms and ASD risk in a Turkish Population.

Materials and Methods

Study population

The study sample comprised 100 subjects (mean age \pm S.D.: 6.11 \pm 2.16 years, age range: 2–10; male/female: 75/25) with ASD and 96 unrelated healthy control subjects (mean age \pm S.D.: 5.50 \pm 2.75 years, age range: 2–12; male/female: 60/36) with no personal or family history of psychiatric disorders. Subjects with ASD were recruited from the Department of Child and Adolescent Psychiatry, Istanbul University Cerrahpasa Medical Faculty outpatient clinic. All subjects had behavioural symptoms, and most of the subjects were receiving anti-psychotic medication at the time of recruitment. Each subject was given a diagnostic assessment based on clinical interviews using the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (1). Control subjects were randomly selected from the Istanbul University Cerrahpasa Medical Faculty, Department of Pediatrics. A total of 163 children were taken for healthy control subjects. Each control subject's clinical background and their family background were questioned. Routine biochemistry (complete blood count, biochemistry extensive tests) results have been asked from all groups. These scans were used to choose for healthy individuals in possible control group, and their physical examination were performed. DSM-5 criteria were performed by one psychiatrist based on consensus, utilization of cross-sectional interviews. Furthermore, 96 unrelated healthy control subjects were included into the study according to their both physical and mental conditions. There were no significant differences among the study and control groups in terms of mean age and sex distribution.

Inclusion and exclusion criteria

Inclusion criteria were a diagnosis of current DSM-5

ASD. Patients were excluded if their primary diagnosis was not ASD. Patients were also excluded from the study if they had a history of neurologic or medical disorder that would affect neuropsychologic function (i.e. seizures, head trauma, stroke, brain tumor, meningitis). In addition, control subjects were excluded if they had a diagnosis of any DSM-5 ASD.

Measurements, protocol, and procedure

All subjects were examined according to a standardized interview. Assessment was done in a semistructured sociodemographic form. We investigated their demographic data and medical and psychiatric history. None of the comparison subjects had a history of significant medical illness, head injury, neurological disorder, psychiatric disorder, and none had a family history of psychiatric disorder. The diagnosis was made using the DSM-5 ASD criteria. The subjects were then screened for ASD according to the Modified Checklist for Autism in Toddlers (M-CHAT). Healthy and unrelated volunteers without psychiatric disorders were selected as the control group. The control groups were also evaluated using the M-CHAT.

Polymorphism analysis

Genomic DNA was extracted from whole blood using Roche DNA purification kit (Roche Diagnostics GmbH, Mannheim, Germany). Polymerase chain reaction (PCR)/restriction fragment length polymorphism (RFLP) analysis was performed for the detection of the variations in these regions (18, 19). PCR was initially performed to determine the polymorphic regions using suitable primers. PCR products of XPD Asp312Asn (rs1799793), and XRCC4G-1394T (rs6869366) were further subjected to digestion with StyI, and MboII restriction enzymes, respectively (Table 1). The PCR products were visualized by electrophoresis through a 3% agarose gel. The relative size of the PCR products was determined through comparison of the migration of a 50–1000 bp DNA molecular weight ladder (Invitrogen, Grand Island, NY, USA). A permanent visual image was obtained using a UV illuminator (Figure 1 and Figure 2). All genotypes were read by 2 independent researchers. In the event of any conflicts, the genotypes were repeated.

Statistical analysis

Statistical analyses were performed using the SPSS software package (revision 13.0 SPSS Inc., Chicago, IL, USA). Data are expressed as means \pm standard deviation (SD). Demographic data were compared using Student's t-test and Chi-square test, as required. Chi-square

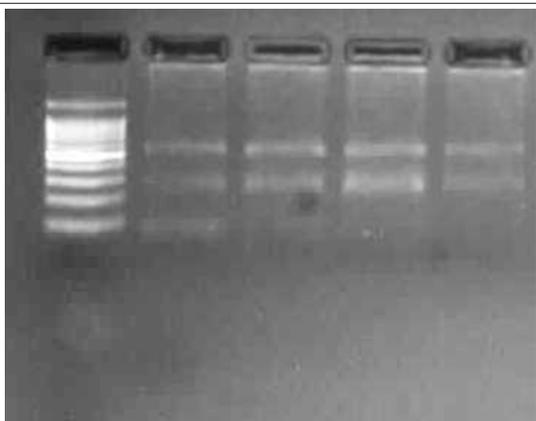


Figure 1. Genotypes of XPD Asp312Asn gene polymorphism.

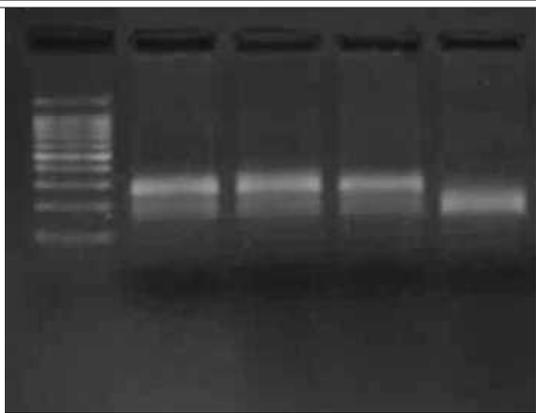


Figure 2. Genotypes of XRCC4 G-1394T gene polymorphism.

or Fischer's exact test (two-sided) was used to compare the gender distribution, test the association between the genotypes and alleles in relation to the cases and controls, and test for deviation of genotype distribution from Hardy–Weinberg equilibrium (HWE). The odds ratio (OR) and their 95% confidence intervals (CI) were calculated to estimate the strength of the association

between polymorphism genotype alleles in patients and controls. Values $P < 0.05$ were considered statistically significant.

Results

Controls and patients were matched for age and sex. Table 2 shows the characteristics of patient and control groups.

Table 3 summarizes the distributions of genotypes and alleles of XPD, and XRCC4 gene polymorphisms in patients with ASD and controls. The distributions of the XPD Asp312Asn and XRCC4G-1394T genotypes were in accordance with the Hardy–Weinberg equilibrium among the controls ($P = 0.74$, $P = 0.82$, respectively) and the cases ($P = 0.089$, $P = 0.94$, respectively). No statistically significant differences were observed in the allele or genotype frequencies of the XPD Asp312Asn polymorphisms between the controls and patients.

Although the allele frequencies were not different between the patients and healthy controls, there was a significant difference between frequencies for XRCC4-1394 TG+GG genotype in patients (%34) and healthy controls (%18.7) ($P = 0.02$). The statistical analysis revealed that the individuals who had XRCC4-1394 TG+G/G genotype had an increased risk for ASD (OR = 2.23, 95% CI = 1.10-4.55).

None of the coding polymorphisms were associated with initial symptoms, duration of disease, response to drugs, the subtypes of patients (data not shown).

Discussion

Oxidative stress is defined as damage to cellular tissue caused by free radicals such as ROS. Endogenous oxidative damage to proteins, lipids, and DNA has been implicated in the development of systemic diseases such

Table 2. Characteristics of patients with ASD and control groups.

	Controls	Patients	P value
Number, n	96	100	
SEX; n (male/female)	60/36	75/25	0.059
Age, years: mean \pm S.D.	5.57 \pm 2.68	6.11 \pm 2.16	0.096
Range	2-10	2-10	

Table 3. Distribution of XPD Asp312Asn, and XRCC4 G-1394T gene polymorphisms in patients with ASD and healthy control groups.

	Controls n (%)	Patients n (%)	P-value
XPD Asp312Asn			
Asp/Asp	37 (38.5)	48 (48)	
Asp/Asn	44 (45.8)	37 (37)	0.22
Asn/Asn	15 (15.6)	15 (15)	0.69
Asp	0.61	0.66	
Asn	0.38	0.33	0.96
XRCC4 G-1394T			
T/T	78 (81.2)	66 (66)	
T/G + G/G	18 (18.7)	34 (34)	0.02
T	0.90	0.80	
G	0.09	0.20	0.07

as cardiovascular disease (20), diabetes (21), and hypertension (22). Furthermore, oxidative stress has been implicated in several psychiatric disorders including schizophrenia (23), bipolar disorder (24), and Alzheimer's disease (25). Oxidative stress has also been reported in some individuals with ASD (26). Association between oxidative stress and DNA damage has been well-known (27). DNA repair gene polymorphisms have recently been implicated as notable contributors of pathogenesis in several mental disorders (28, 29). Thus, we hypothesized that impaired DNA repair mechanisms might enhance the risk for ASD because of genetically predetermined factors.

To our knowledge, this is the first case-control study in ASD patients investigating a possible association between the polymorphisms in the DNA repair genes involved in NER, and DSBR and the development of ASD. Decrease in NER or DSBR repairing activity might cause accumulation of oxidative damage, the situation becoming a vicious cycle in a cell or organ. These genetic variants might also affect proteins or other kinds of molecules involved in the whole DNA repairing system. In the present study, we investigated the association between XPD Asp312Asn and XRCC4 G-1394T gene polymorphisms and ASD risk in a Turkish Population.

NER pathway of the DNA repair systems is the primary mechanism for the removal of bulky adducts such as those caused by oxidative damage from DNA, and thus is an important part of the cellular defense against a large variety of structural unrelated DNA lesions. XPD is one of the most important genes in the NER pathway, and codes for a DNA helicase subunit of the core transcription factor IIIH, which is essential for NER, and plays a critical role in transcription-coupled NER pathway (30). Single nucleotide polymorphisms (SNPs) in the exons of XPD can diminish the helicase activity, resulting in defects in the NER pathway and reduced DNA repair capacity. Hence, it is biologically reasonable to hypothesize a potential relationship between the XPD Asp312Asn polymorphism and ASD risk. However, we did not find any significant differences for XPD genotypes and alleles frequencies between patients with ASD and controls in this study.

On the other hand, we, for the first time, demonstrated the positive association of XRCC4 gene variants with ASD risk. In our study, the frequencies of T/G+G/G genotypes in XRCC4, are more prevalent in patients than in controls. Eukaryotic cells have developed two pathways to repair DNA double strand breaks (DSBs); the homologous recombination (HR) and NHEJ pathways. XRCC4 gene, a key component of NHEJ pathway, DSBR (31). The DSBs have the most harmful and damaging effect on genome; leading to apoptosis or tumorigenesis (32). The information about functional aspects of XRCC4-SNP selected in the present study is quite limited. The XRCC4 G-1394T polymorphism is associated with children leukemia (33), breast (34), prostate (35), lung (36) and colorectal (37) cancers. Whether, XRCC4 G-1394T polymorphism modulates XRCC4 protein activity and expression is unknown so far. The polymorphisms in upstream promoter region are generally known to regulate gene expression. Though, this possible mechanism may partial-

ly explains modified ASD susceptibility with XRCC4 G-1394T polymorphism, the functional aspects remains to be elucidated in physiological conditions.

In conclusion, our findings have suggested that XRCC4 genetic variants may be a risk factor for ASD. However, the exact mechanisms by which XRCC4 G-1394T polymorphism increases ASD risk require further exploration. Further studies with larger sample groups are necessary to clarify the role of DNA repair genes and the development of ASD.

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