Could be NCOA5a Novel Candidate Gene Playing a Role in MS Disease Susceptibility?

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Abstract

Background

Multiple sclerosis (MS) is an inflammatory immune-mediated demyelinating disease which characterized a challenging and disabling condition. It is known that environmental and genetic factors play a role in directing the disease state. Recent studies have shown that nuclear cofactor genes may play a role in the MS pathogenesis. NCOA5 is a nuclear receptor coactivator independent of AF2 that modulate ERa-mediated transcription. NCOA5 gene is also involved in the pathogenesis of various diseases such as psoriasis, Behçet's disease and cancer.

Methods and Results

We were investigated the relationship between the NCOA5 gene rs2903908 polymorphism and MS disease on 157 unrelated MS patients and 160 healthy controls by RT-PCR. The frequency of CC, CT, and TT genotypes was 19.87%, 37.82%, and 42.31% for the MS group while 5.63%, 43.75%, and 50.62% control group, respectively. In the obtained results, CC genotype and C allele were found to be significantly higher in the patient group ($p = 0.0002$ and 0.003, respectively). In particular, the fact that the CC genotype was found to be significantly higher in the patient group compared to the control group ($p = 0.0002$) and that it had a statistically significantly higher OR value (OR,95%CI = 4.16, 1.91–9.05) suggests that the C allele may recessively predispose to the MS disease for this polymorphism.

Conclusions

These results suggest for the first time in the literature that, the NCOA5 gene may have an effect on the occurrence MS disease through different molecular pathways which discussed in the manuscript.

Introduction

Multiple Sclerosis (MS) is a composite and complex disease; it is a relapsing, autoimmune condition with inflammatory and neurodegenerative characteristics in central nervous system (CNS). MS predominantly affects individuals in their early adult life. Some of the fundamental questions relating to pathogenesis and susceptibility of the disease are explained, but some questions still remain to be answered. Particularly the possible contribution of genetic structure in MS disease is still not fully elucidated. MS results in demyelization and varying degrees of axon dmage that often leads to severe physical or cognitive incapacitation, thus impacting the quality of life both functionally and financially [1]. The present data indicate that cause of MS is multifactorial and includes genetic predisposition in synchronize with environmental factors [2]. Familial analyses and epidemiological studies suggest that genetic factors may be more predominant [3, 4]. In recent years, 236 independent genetic variants have been reported to be associated with an increased risk of MS, but none of them have been shown to be associated with MS disease alone [5]. Although the first identified genetic regions were polymorphisms in HLA gene clusters within the MHC region [6–8], later genome-wide association studies (GWAS) showed

that non-MHC loci may also be associated with MS. Some of these loci (eg, TAGAP, TYK2, IL-6) are gene regions that are also associated with some other autoimmune diseases [9–13]. These studies indicate that influence of nuclear receptor coactivators may have a critical influence in the pathophysiology of MS due to their capacity to enhance the expression of pro-inflammatory mediators, especially interleukin-6 (IL-6) [14–16].

The nuclear receptor coactivator 5 (NCOA5) gene, also known as Coactivator Independent of AF2 (CIA), is located in the chromosome 20q.12 – q13.12, a 400-kb surrounding region of CD40, a member of the TNF-receptor superfamily, which is also associated with MS [17–19]. The NCOA5 gene encodes a coregulator for the orphan nuclear receptor NR1D2 and the alpha and beta estrogen receptors. The protein is localized in nucleus and has coactivator and corepressor functions. Like other nuclear receptor coactivator genes, NCOA5 deeply involves in a distinctive range of autoimmune, inflammatory and neoplastic diseases. Its insufficiency results in elevated expression and increased levels of IL-6 [17–20]. Although IL-6 immunoreactivity is involved in the pathogenesis of MS lesions, no correlation between the NCOA5 gene and MS has been described. In a recent study, it was shown that the epididymis epithelial cells of haploinsufficient NCOA5 mutant ($NCOA5^{+/-}$) heterozygous mice had a marked increase in IL-6 expression compared to normal mice, resulting in infertility in male mice. Thus, the direct relationship of NCOA5 with IL-6 expression has been demonstrated [20].

In this study, we aimed to investigate whether the rs2903908 polymorphism in the NCOA5 gene has an effect on MS disease.

Methods

Study subjects

This study was performed in line with the principles of the Declaration of Helsinki. The study was started after the approval of Tokat Gaziosmanpaşa University Faculty of Medicine Ethics Committee (15-KAEK-016). Informed consent was obtained from all individual participants included in the study. Only 5 ml blood samples were taken into EDTA tubes for DNA isolation from a total of 316 (n:160 control group and n:156 MS patients) voluntaries, who had all given informed consents. Patients were included from the Neurology Department of Tokat Gaziosmanpasa University Research Hospital, Tokat, Turkey, according to 2005 revised McDonald criteria. Physical disability and disease severity were measured using The Expanded Disability Status Scale (EDSS) and Multiple Sclerosis Severity Scale (MSSS). The patient group was constructed with 45 males and 111 females (mean age (± SD) = 37.06 ± 9.36 years). The gender data and age of the all participants are given in Table 1.

Table 1
Age and gender information of MS and Control Groups

		MS	Control
Sample Size		156	160
Gender	**Male**	111 (71.15%)	99 (61.88%)
	Female	45 (28.85%)	61 (38.12)
Age		37.06 (9.36)	37.29 (± 12.13)

Genomic Analysis

We performed DNA purification with PureLink™ Genomic DNA Mini Kit K1820-02 (Invitrogen Life Technologies, Carlsbad, CA, USA) from peripheral venous blood samples (preserved in EDTA tubes) of the all participants. Genetic analyses were performed by using a TaqMan SNP genotyping assay in a StepOnePlus Real-Time Polymerase Chain Reaction (RT-PCR) system (Applied Biosystems, Foster City, CA, USA) by following the conditions recommended by the manufacturer.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS 16.0, SPSS Inc., Chicago, IL, USA) software program was used to calculate the frequency of Single Nucleotide Polymorphism (SNP) allele/genotype for each patient and control group. Deviation from Hardy Weinberg balance for the detected genotype frequencies was investigated by Fischer's exact chi-square test. SPSS 16.0 software program was used to compare data and calculate OR (Odds Ratio) values of patient and control groups.

Results

The demographical and clinical findings of studied subjects are shown in Tables 1 and 2. The mean age of the patients and healthy controls were 37.06 and 37.29, respectively. The MS group was composed of 111 (71.15%) males and 45 (28.85%) females, while the control group was composed of 99 (61.88%) males and 61 (38.12%) females (Table 1). The mean age at onset of the disease was 29.71 ± 8.88 and the mean duration of MS was 7.33 ± 6.31 years (Table 2). 3.85% (n:6) of the patients had seizures more than once in a week, 16.02% (n: 25) 1–4 in a month, 51.28% (n:80) 1–11 a year 28.85% (n: 45) less than once in a year. No statistically significant difference was found between the MS and Control groups in terms of age and gender ($p > 0.05$) (Table 1).

Table 2
Clinical features of MS Group

Features	MS (n = 156)	
MS Duration	Mean (SD)	7.33 (± 6.31)
Age at onset of MS (years)	Mean (SD)	29.71 (± 8.88)
Frequency of episodes	> Once a week	6
	1–4 in a month	25
	1–11 in a year	80
	< 1 in a year	45

The percentages of CC, TT, and TT genotypes among overall participants were 12. 66% (n:40), 40.82% (n:129), and 46.52% (n:147), respectively. The frequency of CC, CT, and TT genotypes for the MS group was 19.87% (n:31), 37.82% (n:59) and 42.32% (n:66), whereas the frequencies of the genotypes for the and the control group was 5.63% (n:9), 43.75% (n:70), and 50.62% (n:81) respectively. The frequency of the CC genotype was found significantly higher in the MS patient group (p = 0.0002; OR,95%CI = 4.16, 1.91–9.05) than healthy controls. On the other hand, although the frequency of TT genotype was found to be lower in MS patients, the difference was not statistically significant (p > 0.05) (Table 3).

Table 3
NCOA5 rs2903908 polymorphism comparison of MS and Control Groups

Genotype	MS (n = 156)		Control (n = 160)		P	*OR, 95% - CI%
	N	%	N	%		
CC	**31**	**19.87**	**9**	**5.63**	**0.0002**	**4.16, 1.91–9.05**
CT	59	37.82	70	43.75	0.304	0.78, 0.50–1.22
TT	66	42.31	81	50.62	0.144	0.72, 0.46–1.11
Allele						
C	**121**	**38.78**	**88**	**27.50**	**0.003**	**1.67, 1.20–2.33**
T	**191**	**61.22**	**232**	**72.50**		
OR: Odds Ratio CI: confidence interval; *- Statistics for each genotype						

In terms of allele frequencies, the C allele was found significantly higher in the patient group than controls (p = 0.003; OR.95%CI = 1.67, 1.20–2.33). These results suggest that the C allele may recessively predispose to MS disease (Table 3).

Discussion

MS is a relapsing, chronic, autoimmune disease that affects CNS and may cause motor, sensory and cognitive disorders and imbalance. MS usually occurs between the ages of 18–50 [21]. MS patients have limited physical activity due to the risk of relapse [21–23]. Although the pathogenesis of MS has not been elucidated entirely, genetic and environmental factors are collectively effective in the pathogenesis. Results of familial studies demonstrated a 0.2% prevelance in general population. The incidence is 10 to 20fold higher in siblings (2–4%). Monozygotic twins have the highest risk rate of 20% [4, 24]. The higher rate in siblings and monozygotic twins indicates the genetic factors may be more predominant [4]. Also, distinctive worldwide prevalence supports the heritability of MS. Some subpopulations or ethnic communities like Amerindians, native Siberians, New Zealand Maori, African blacks, Japanese Uzbeks, Chinese Samis, Turkmen, Kyrgyzis, Kazakhs have low incidence of the disease whereas northern Europeans and Americans have higher risk [25]. Having MS-resistant or low-incidence inheritance points out the record and genetic makeup of a population that influences its prevalence of developing MS. Notably, International Multiple Sclerosis Genetics Consortium (IMSGC) explained up to 48% of MS's heritability with large-scale GWAS data [12, 26].

There are studies on many genes that are related to MS in the literature. HLA-DRB1, CD40 and retinoid acid-related orphan receptor A RORA (Retinoic acid receptor-associated orphan receptor alpha), are potential risk factors for MS and other diseases, especially heterozygosity of these genes seems to increase the risk for several autoimmune diseases [13–18, 27–29]. NCOA5 is a coactivator for alpha and beta estrogen receptors among with orphan nuclear receptor NR1D2. NCOA5 is localized in the 20q12-q13.12 chromosome region. Interaction with the nuclear receptor is independent of the AF2 domain at the receptor, known as regulating interaction with other co-receptors such as RORA. Several alternatively spliced transcript variants are present for NCOA5 gene (NCBI Gene ID: 57727). It is thought that NCOA5 may have a bidirectional effect in terms of the genes it regulates, and have both coactivator and corepressor functions. As a transcriptional regulator, the NCOA5 gene suppresses the expression of IL-6 [30]. Simultaneously, the insufficiency of NCOA5 increases IL-6 levels [20, 31]. IL-6 is an essential mediator of many inflammatory processes and has a significant relationship between demyelinating and inflammatory diseases like MS [15, 32]. IL-6 is more frequently detectable in the CSF of MS patients [32]. Likewise, an excessive number of IL-6 expressing cells in demyelinated MS lesions demonstrated by Schönrock et al., indicating a valid role for IL-6 in MS [15].

Although IMSGC has illustrated evidence of MS susceptibility and linkage disequilibrium of NOCA5, no studies in the literature focused on the relationship between the NOCA5 gene and MS.[11,29] Studies examining the relationship between the NCOA5 gene and polymorphisms and autoimmune diseases are also too few. Raychaudhuri et al. demonstrated a potential association between the NCOA5 gene and rheumatoid arthritis (RA) [33]. Zervou et al. established a significant relationship between psoriasis and polymorphism of the NCOA5 gene rs2903908 [17]. Langefeld et al. provided a possible linkage with systemic lupus erythematosus and NCOA5 [29]. In various autoimmune diseases, like Type I Diabetes (T1D), lupus, RA and coeliac disease having multiple risk alleles generates greater risk than having two

copies of the same risk allele (compound risk allele heterozygosity) [27–30]. Heterozygous deletion of the NCOA5 gene was linked with hepatic inflammation, steatois, and Hepatic Cell Carcinoma (HCC), infertility and glucose intolerance in mice [30, 34]. In a previous study, we have expressed a possible relationship between NCOA5 gene rs2903908 polymorphism and Behçet's Disease.[19] In the present study, we detected significant differences between MS patients and healthy controls for NCOA5 gene rs2903908 polymorphism, both in terms of general genotype distribution (p = 0.004, data not shown) and CC genotype (p = 0.0002; OR, 95%CI = 4.16, 1.91–9.05). We also found that there was a significant difference between MS patients and controls in terms of allele distribution and that the C allele was high in the patient group (p = 0.003; OR.95%CI = 1.67, 1.20–2.33). These results suggest a possible relationship between the NCOA5 gene rs2903908 polymorphism and therefore between the NCOA5 gene and MS. The results show that the C allele at the rs2903908 polymorphic locus may predispose to MS dominantly (Table 3).

Limited studies with NCOA5 have shown that NCOA5 has effects on the expression of IL-6. Gao et al. suggested in their studies that NCOA5 is a transcriptional repressor of the IL-6 gene and the haploiusuffiency of NCOA5 causes an increase in IL-6 expression [20, 31]. On the other hand, preclinical and clinical data highlights the pivotal role of IL-6 in MS, RA, HCC, Type 2 Diabetes (T2D), psoriasis, Experimental Autoimmune Encephalomyelitis (EAE) and infertility [20, 30, 34–36]. Although IL-6 has an anti-inflammatory effect, it also augments inflammatory tissue damage by promoting TH17 differentiation and preventing Treg cell development. Treatment with anti-IL-6 mAb proven to be promising in MS and protective in EAE, RA and Neuromyelitis Optica (NO), an autoimmune disease of the optic nerve and spinal cord similar to MS [36, 37]. NCOA5 seems to be providing a genetic link between autoimmune diseases through its effects on IL-6 expression [30]. On the other hand, Gao et al. (2019) showed that IL-6 expression was significantly increased in haploinsufficient $Ncoa5^{+/-}$ mice. Thus, Ncoa5 deficiency has been shown to clearly increase IL-6 expression [20]. In another study conducted in recent years, Koptan et al. (2022), while investigating the rs2903908 polymorphism of the NCOA5 gene in Behçet's patients, also investigated the effects of this polymorphism on NCOA5 gene expression. Their results showed that NCOA5 gene expression in Behçet's patients differed in genotypes due to the rs2903908 polymorphism, with the lowest expression in CC genotypes and the highest in TT genotypes [38]. All these data suggest that NCOA5 may also be effective in the pathogenesis of MS. This is the first study in the literature to determine this connection. Although our study has some limitations (the relatively small number of samples and we do not ourselves tested our hypothesis), the results we obtained gave us hope that we could be right in this thought.

However, the presence of studies with different results in terms of rs2903908 polymorphism in other autoimmune diseases in the literature suggests that NCOA5 may be effective not only through IL-6 but also through other pathways [17, 19, 38]. Another candidate gene known to play a role in the pathogenesis of MS is RORA. Studies in the literature have shown that RORA plays a role in the pathogenesis of MS by promoting the differentiation of Th17 cells and that the decrease in RORA expression has a crucial role in various neuroimmunological pathways such as inflammatory responses

[18, 35, 39, 40]. Eftekharian et al. showed that some polymorphisms in the RORA gene are associated with MS [18]. Sayad et al. revealed a significant downregulation in the expression of the RORA in MS patients versus healthy controls (Expression ratio = 0.62, P-value = 0.006) [35]. The fact that NCOA5 also actively role in E2- mediated upregulation of RORA expression make us to the thought that NCOA5 may also play a role in the pathogenesis of MS via this pathway. Insufficiency of NCOA5 may be resulting in inefficient upregulation of RORA, which explains the overall down regulation of the RORA gene in MS patients.

Conclusion

With this study, the relationship between NCOA5 gene rs2903908 polymorphism and MS was investigated for the first time. NCOA5 gene may be a novel candidate for future studies investigating genetic susceptibility for chronic, inflammatory and autoimmune diseases that share similar pathogenesis. The results obtained suggest that the NCOA5 gene may play a role in MS pathogenesis. The results obtained in this preliminary study show that research groups working on MS pathogenesis should also be focused on the NCOA5 gene too.

Abbreviation List

CIA- Coactivator Independent of AF2

CNS- Central Nervous System

CSF- Cerebrospinal Fluid

EAE- Experimental Autoimmune Encephalomyelitis

EDSS- Expanded Disability Status Scale

GWAS- Genome-Wide Association Studies

HCC- Hepatic Cell Carcinoma

HLA- Human Leukocyte Antigen

IL-6- Interleukin-6

IMSGC- International Multiple Sclerosis Genetics Consortium

MHC- Major Histocompatibility Complex

MS- Multiple Sclerosis

MSSS- Multiple Sclerosis Severity Scale

NCOA5- The Nuclear Receptor Coactivator 5

NO- Neuromyelitis Optica

NR1D2.- Nuclear Receptor Subfamily 1 Group D Member 2

RA- Rheumatoid Arthritis

RORA- Retinoic Acid Receptor-Associated Orphan Receptor Alpha

RT-PCR- Real-Time Polymerase Chain Reaction

SNP- Single Nucleotide Polymorphism

SPSS- Statistical Package for the Social Sciences

T1D- Type I Diabetes

T2D- Type 2 Diabetes

TAGAP- T Cell Activation RhoGTPase Activating Protein

TNF- Tumor Necrosis Facto

TYK2- Tyrosine Kinase 2

Declarations

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Compliance with Ethical Standards

Conflict of interest

The authors declare no competing interest.

Ethical approval

This study was performed in line with the principles of the Declaration of Helsinki. The study was started after the approval of Tokat Gaziosmanpaşa University Faculty of Medicine Ethics Committee (15-KAEK-016).

Informed consent

Informed consent was obtained from all individual participants included in the study.

Consent for publication

The authors confirm that the MS patients and control subjects included in the study gave voluntary consent for the publication of study data and clinical data.

Data availability statements

Detailed data on the findings of this study are obviously not available due to sensitivity and are available from the corresponding author upon reasonable request.

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Aydin Rustemoglu, Sema Atasever, Betul Cevik and Husniye Rustemoglu; data analysis, evaluation and article writing were performed by Husniye Rustemoglu, Erdem Arslan, Ahmet Bulent Turhan, Filiz Taspinar and Aydin Rustemoglu. The first draft of the manuscript was written by Husniye Rustemoglu and Erdem Arslan. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Statements and Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence.

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