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Mannose-Binding Lectin 2 Gene Polymorphism in PANDAS Patients

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ABSTRACT

Introduction: Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS), a subgroup of obsessive-compulsive disorder (OCD), has received much attention even though the specific underlying mechanisms remain unknown. Mannose-binding lectin (MBL) is a key factor in the innate immune response. The aim of this study was to investigate the role of MBL2 gene polymorphisms in pediatric OCD patients diagnosed as PANDAS, PANDAS-Variant and non-PANDAS.

Methods: The study included 102 pediatric OCD patients (59 [57.8%] PANDAS, 20 [19.6%] non-PANDAS, and 23 [22.5%] PANDAS-Variant) and 60 healthy controls. Polymorphisms at codon 52, 54 and 57 of the MBL2 gene were investigated.

Results: Codon 54 polymorphism and any variant of MBL2 gene were significantly more frequent in the OCD group than in the control group (OR=2.97, 95% CI: 1.26–6.97; and OR=2.66, 95% CI: 1.32–5.38, respectively). According to regression analysis, the presence of any variant of MBL2 gene was found in 14.50-fold increased frequency in the PANDAS subgroup compared with the non-PANDAS subgroup (95% CI: 2.49–84.19).

Conclusions: Our findings support an association between MBL2 genotypes and pediatric OCD, particularly PANDAS-OCD.

Keywords: Mannose-binding-lectin2 gene, obsessive-compulsive disorder, pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections.

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INTRODUCTION

Obsessive-compulsive disorder (OCD) is a neurobiological disorder that affects 1–3% of all children (1). Although the pathophysiology of childhood OCD has yet to be fully clarified (2), the possible link between infection-triggered autoimmune processes and neuropsychiatric symptoms has been emphasized in the literature. This clinical condition is also known as pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS). The diagnostic criteria for PANDAS were first described by Swedo et al. (3). They suggested that the immune system plays an important role based on the causal relationship between psychopathology and immune dysfunction in the development of neuropsychiatric disorders. The five diagnostic criteria for PANDAS are as follows (all criteria must be met): 1. Presence of OCD or a tic disorder (TD); 2. Prepubertal symptom onset; 3. Acute symptom onset and episodic (relapsing-remitting) course; 4. Temporal association between group A beta-hemolytic streptococcal (GABHS) infection and symptom onset/exacerbation; 5. Association with neurological abnormalities (particularly motoric hyperactivity and choreiform movements) (3, 4).

Nonetheless, the environmental, neurological, immunological and genetic factors affecting the clinical features of PANDAS have not yet been clarified (5). More specifically, it is emphasized that a familial link between autoimmune disease and OCD/TD (6). Recently, Chang et al. suggest that screening familial autoimmunity and OCD history for PANDAS/PANS patients (7).

The complement system can be activated via three pathways: the classic, the alternative and the mannose binding lectin (MBL) pathway (8). Previous studies have shown that MBL plays a significant role in the innate immune response by binding to carbohydrates on the surface of pathogens where it can activate the complement system, or directly act as an opsonin. The three most common polymorphisms of the MBL2 gene at codon 52 (arginine to cysteine; D), codon 54 (glycine to aspartic acid; B), and codon 57 (glycine to glutamic acid C) are located in exon 1 that cause significant reduction of serum MBL level, and production of dysfunctional proteins (9). The frequency of MBL deficiency due to MBL2 gene polymorphisms in Caucasians was estimated to be 25%-30% (10).

Individuals with MBL deficiency exhibit an increased vulnerability to both infectious diseases (11), and autoimmune diseases, such as rheumatoid arthritis (12), systemic lupus erythematosus (13), and Kawasaki disease (14). Previous studies have also suggested that there is an association between MBL2 gene polymorphisms, and increased susceptibility to

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rheumatic heart diseases (RHD) (15), and acute rheumatic fever (ARF) (16). MBL has the capacity to bind to a broad range of microorganisms (8, 17). Moreover, it was reported that GABHS strongly bind to MBL (18).

Based on these data, the present study aimed to investigate the possible association between MBL2 gene polymorphisms and childhood OCD. The second aim of this study was to investigate whether there was a significant difference in the distribution of polymorphisms in the MBL2 gene between PANDAS, non-PANDAS and PANDAS-Variant subgroups of the OCD patients.

METHODS

Participants

The study included 102 pediatric OCD patients aged 6–17 years and 60 age-matched healthy controls. All patients were followed-up around 3 years to establish diagnostic accuracy for subgroups of patients with OCD. The patients were chosen from among those treated at Çukurova University, School of Medicine, Child and Adolescent Psychiatry Outpatient Department. The controls were recruited from a primary health care clinic in collaboration with the family physician that works in the clinic. The project was approved by the Ethics Committee of the X University (B.10 OTHG. 0.79.00.07). Informed consent was obtained from all of the patients and their parents; therefore, only volunteers were included. All participants enrolled in this study were Turkish population. Antistreptolysin O (ASO) titers were measured ≥2 times to confirm an antecedent streptococcal infection.

The OCD patients were subdivided into three groups as follows: 1. The PANDAS subgroup included patients who met the diagnostic criteria for PANDAS (3); 2. The non-PANDAS subgroup included patients who did not meet the criteria for PANDAS; 3. The PANDAS-Variant subgroup included patients who met at least 3 of the first 4 criteria (19).

Exclusion criteria for the OCD patients were as follows: mental retardation, pervasive developmental disorders, or dysmorphic features. In addition to these, the exclusion criteria for the control group included a current or past history of psychiatric disorders.

Demographic and Clinical Parameters

Demographic data, including age, gender, and ages at onset of symptoms were recorded. Anamnesis was obtained from the participants and their families. Additionally, detailed information about previous infectious diseases, including recurrent upper respiratory tract infections (RURTIs), tonsillectomy, penicillin prophylaxis and the occurrence of emotional and/or behavioral symptoms following infections was recorded. Furthermore, family history of ARF and/or RHD and OCD and/or TDs was noted.

DNA Sequence Analysis

Molecular genetic analysis was performed using genomic DNA extracted from EDTA-anticoagulated venous blood using the RTA Genomic DNA Isolation Kit from BLOOD, according to the manufacturer's instructions (GEPOSB RTA, Kocaeli, Turkey) and DNA was stored at-20°C until using. Genotyping of the MBL2 gene was performed using a polymerase chain reaction (PCR) sequence-based typing technique. The DNA fragment on the 10th chromosome -including the GRCh37.p5 region, starting with the number 54527897 nucleotide and ending with 54528286– was sequenced. The DNA was amplified via PCR using the sense primer 5′-GGC CAG GGA TGG GTC ATC TAT T-3′ and the antisense primer 5′ GAC ATC AGT CTC CTC ATA TCC CCA G-3′. PCR was performed at 95°C for 10 min, followed by 40 cycles of 30 s at 95°C, 1 min at 60°C, 40 S at 72°C, 7 min at 72°C and a final 4 min forever PCR amplification was performed using a Corbett Palm-Cycler gradient thermal cycler (Corbett Life Science, Australia). The resulting PCR product was treated with ExoSAP-IT (GML A. G., Wallerau, Switzerland) and subjected to direct cyclic sequencing via a BigDye Terminator v.3.1 cycle sequencing kit (Applied Biosystems, Warrington, United Kingdom), according to the manufacturer's instructions. The sequencing reactions were analyzed using an automated capillary DNA sequencer (ABI-3500 Gene Analyzer, Applied Biosystems). SeqScape v.2.7 sequencing analysis software was used for sequence evaluation.

Psychometric Measurement

Psychiatric diagnoses were made according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM IV-TR) criteria, and the Turkish version of the Schedule for Affective Disorders and Schizophrenia for School-Aged Children, Present and Lifetime Version (K-SADS-PL) (20, 21). The Children's Yale-Brown Obsessive-Compulsive Scale (CY-BOCS) was used to evaluate the severity of OCD symptoms. The CY-BOCS is a modified version of the Y-BOCS; it is a 10-item, clinician-rated semi-structured instrument designed to assess OCD symptom severity during the previous week. The CY-BOCS consists of 5 primary sections, including Instructions, Obsessions Checklist, Severity Items for Obsessions, Compulsions Checklist, and Severity Items for Compulsions (22, 23).

Statistical Analysis

SPSS v. 16.0 (SPSS Inc.; Chicago, IL, USA) for Windows was used for statistical analysis. The distribution of continuous variables was tested for normality using the Shapiro-Wilks test. Because continuous variables were not normally distributed, non-parametric tests (Kruskal-Wallis and Mann-Whitney U Tests) were used. Categorical variables were compared between groups using the chi-square test.

The genotypic distribution for the MBL2 gene polymorphisms in the study population was in Hardy-Weinberg equilibrium. The wild-type allele was called as allele A; and the variant alleles in exon 1 at codon 52, 54, and 57 were called allele D, allele B, and allele C, respectively (AB [heterozygous] and BB [homozygous] variants for codon 54; AC [heterozygous] and CC [homozygous] variants for codon 57; AD [heterozygous] and DD [homozygous] variants for codon 52) (8, 9). Because of the small number of the carriers of other genotypes, the statistical analysis included only codon 54 polymorphism. The presence of any variant in exon 1 (AB, BB, AC, CC, AD or DD genotypes for heterozygosus and/or homozygosus) was also analyzed.

Binary logistic regression and a multinomial regression model was applied to identify independent variables associated with each OCD subgroups. Two different multinomial regression analyses with PANDAS and PANDAS variant groups as dependent variables were performed. We used non-PANDAS group as reference category in the each of the regression models while. In the first model; any variant in exon 1, gender, comorbidity, a history of tonsillectomy, a family history of OCD/TDs or ARF/RHD were considered as independent variables. In the second model the same independent variables and codon 54 polymorphism were included, with the exception of any variant in exon 1. The results are presented as mean ± SD and median (range). The degree of association between these variables was determined via the odds ratio (OR) and its 95% confidence interval (CI).

RESULT

Clinical and Sociodemographic Characteristics

Of the 102 OCD patients, 54 (52.9%) were female and 48 (47.1%) were male. Of the 60 controls, 31 (51.7%) were female and 29 (48.3%) were

male. Mean age in the OCD group was 10.3±2.9 years (range 4–17 years), and 12.9±3.3 years (range 4–17 years) in the control group. Among the OCD patients, 59 (57.8%) were in the PANDAS subgroup, 20 (19.6%) were in the non-PANDAS subgroup, and 23 (22.5%) were in the PANDAS-Variant subgroup. The clinical and demographic characteristics of the groups are shown in Table 1.

The percentage of OCD patients who had comorbid attention-deficit hyperactivity disorder (ADHD), a history of symptom exacerbation following infections, OCD symptoms onset before the age of 11 years, RURTIS, tonsillectomy and elevated ASO titers (>200 IU mL-1), and a family history of OCD and/or TDs or ARF and/or RHD were significantly higher in the PANDAS subgroup compared with the non-PANDAS subgroup.

There was no significant difference in the mean CY-BOCS score between the PANDAS subgroup (23.6±7.4) and both the non-PANDAS (21.3±5.6; p=0.247) and PANDAS-Variant subgroups (21.9±4.6; p=0.376), as well as between the non-PANDAS and PANDAS-Variant subgroups (p=0.713). In addition, there was no significant difference in the mean CY-BOCS score between the patients with and without at least one polymorphism in exon 1 (2.0±0.9 and 2.0±0.8, respectively; p=0.983).

Genotypes and Allele Frequencies for the MBL2 Gene

The frequencies of the carriers of any variant allele in exon 1 (D, B, or C alleles) were significantly higher in the total OCD group (57/204, 27.9%; p<0.05) and PANDAS subgroup (45/118, 38.1%; p<0.05) than in the control group (16/120, 13.3%). The frequency of the BB genotype was 5.9% (N=6) in the OCD group and 8.5% (N=5) in the PANDAS subgroup, whereas this genotype was not observed in the control group (for both; p<0.05). The genotypic distribution for the MBL2 gene polymorphisms in the study population was in Hardy-Weinberg equilibrium for both patients and controls and also in total study groups (p>0.05 for all).

The distribution of MBL2 genotypes and allele frequencies in the study groups are shown in Table 2.

MBL2 Gene Polymorphisms According to Groups

As compared to the control group, codon 54 polymorphism and any variant in exon 1 were significantly more frequent in the OCD group by chi-square test (OR=2.97, 95% CI: 1.26–6.97; p=0.010, and OR=2.66, 95% CI: 1.32–5.38; p=0.005, respectively). The frequencies of polymorphism at codon 54 and at any variant in exon 1 were significantly higher in the PANDAS subgroups than in the control group with an odds of 4.15 (95% CI: 1.67–10.31; p=0.001), and 5.05 (95% CI: 2.29–11.08; p=0.0001), respectively. The DNA sequencing image of heterozygozis C>T nucleotide change in MBL2 gene codon 54 is shown in Figure 1, DNA agarose gel image is shown in Figure 2.

The frequencies of polymorphism at codon 54 (OR=3.62, 95% CI: 0.95–13.74; p=0.049) and any variant in exon 1 (OR=5.05, 95% CI: 1.61–15.79; p=0.003) were significantly higher in the PANDAS subgroup (39.0%) than in the non-PANDAS subgroup (15.0%) by chi-square test. The distribution of MBL2 exon 1 polymorphisms according to groups is shown in Table 3.

Results of the Regression Analyses

According to multinomial regression analyses, the presence of any variant was found in 14.50-fold increased frequency in the PANDAS subgroup when compared with the non-PANDAS subgroup as reference category (95% CI: 2.49–84.19; p=0.003). In addition, according to both regression models, a history of tonsillectomy (for the first model; OR=28.03, 95% CI: 2.11–370.92; p=0.011, and for the second model; OR=17.25, 95% CI: 1.63–182.74; p=0.018) and a family history of ARF and/or RHD (for the first model; OR=49.62, 95% CI: 6.12–401.71; p=0.0001, and for the second model; OR=26.82, 95% CI: 4.06–177.06; p=0.011) were significant risk factors for PANDAS. As seen in the Table 4, when we compared to

Table 1. Demographic and clinical variables, according to group

		OCD Subgroups n (%)			OR (95% CI) Comparison Between	
	OCD Group (N=102)	PANDAS (Gr1; N=59)	PANDAS-V (Gr2; N=23)	N-PANDAS (Gr3; N=20)	Gr1and Gr2	Gr1 and Gr3
Gender						
Male	48 (47.1)	35 (59.3)	7 (30.4)	6 (30.0)		
Female	54 (52.9)	24 (40.7)	16 (69.6)	14 (70.0)	0.30 (0.10–0.83)	3.40 (1.14–1.10)
An onset <11 ys	94 (92.2)	57 (96.6)	21 (91.3)	16 (80.0)	2.71 (0.35–20.51)	7.12 (1.19–42.49)*
ASO >200	36 (35.3)	31 (52.5)	3 (13.0)	2 (10.0)	**7.38 (1.97–27.53)** $^{\alpha}$	**9.96 (2.12–46.83)** $^{\alpha}$
Comorbidity	96 (94.1)	55 (93.2)	23 (100)	18 (90.0)	0.70 (0.61–0.81)	1.52 (0.25–9.04)
ADHD	71 (69.6)	44 (74.6)	18 (78.3)	9 (45.0)	0.81 (0.25–2.57)	**3.58 (1.24–10.32)** $^{\beta}$
TDs	27 (26.5)	18 (30.5)	7 (30.4)	2 (10.0)	1.00 (0.35–2.85)	3.95 (0.82–18.84)
Exacerbation	74 (72.5)	55 (93.2)	17 (73.9)	2 (10.0)	**4.85 (1.22–19.23)** $^{\beta}$	123.75 (20.89–733.98)*
RURTIs	78 (76.5)	54 (91.5)	20 (87.0)	4 (20.0)	1.62 (0.35–7.41)	43.20 (10.35–180.20)*
Tonsillectomy	36 (35.3)	31 (52.5)	5 (21.7)	-	**3.98 (1.30–12.15)** $^{\beta}$	1.71 (1.35–2.17)*
F-ARF/RHD	82 (80.4)	56 (94.9)	18 (78.3)	8 (40.0)	**5.18 (1.12–23.86)** $^{\beta}$	28.00 (6.46–121.30)*
F-OCD/TDs	88 (86.3)	54 (91.5)	20 (87.0)	14 (70.0)	1.62 (0.35–7.41)	**4.62 (1.23–17.40)** $^{\beta}$

Notes: Chi-square test was used for comparisons between the groups; $^{\beta}$, *p* value ≤0.05; $^{\alpha}$, *p* value ≤0.005; *, *p* value=0.0001; OR=odds ratio; CI, confidence interval; Gr1 and Gr2, comparison between the PANDAS and PANDAS-Variant subgroups; Gr1 and Gr3, comparison between the PANDAS and non-PANDAS subgroups
Abbreviations: ADHD, attention-deficit hyperactivity disorder; An onset <11 ys, OCD symptoms onset before the age of 11 years; ASO >200, an Antistreptolysin O titer of more than 200 IU mL^{-1}; Exacerbation, a history of symptom exacerbation following infections; F-ARF/RHD, a family history of acute rheumatic fever/rheumatic heart disease; F-OCD/TDs, a family history of obsessive compulsive disorder/tic disorders; OCD, obsessive compulsive disorder; N-, non-PANDAS; RURTIs; recurrent upper respiratory tract infections; PANDAS, pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections;-TDs, tic disorders;-V, PANDAS-Variant

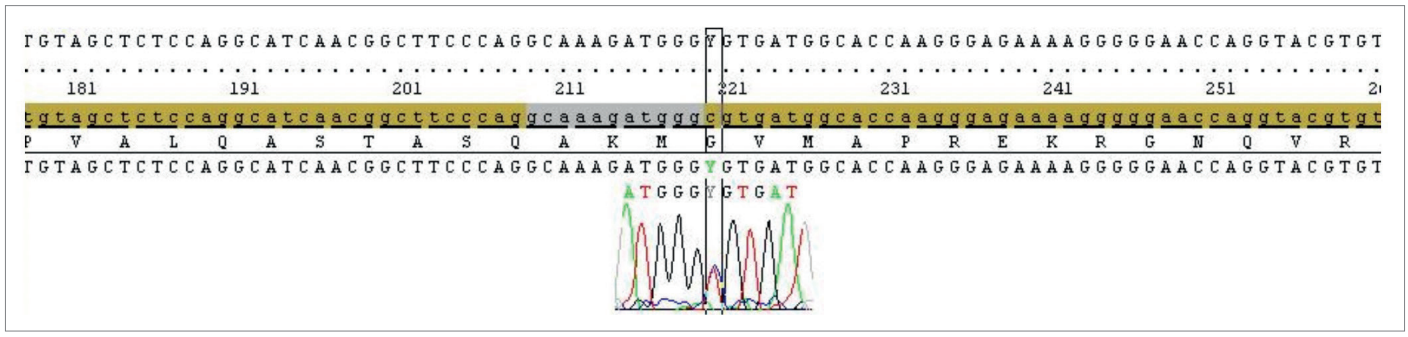

Figure 1. The DNA sequencing image of heterozygozis C>T nucleotide change in MBL2 gene codon 54.

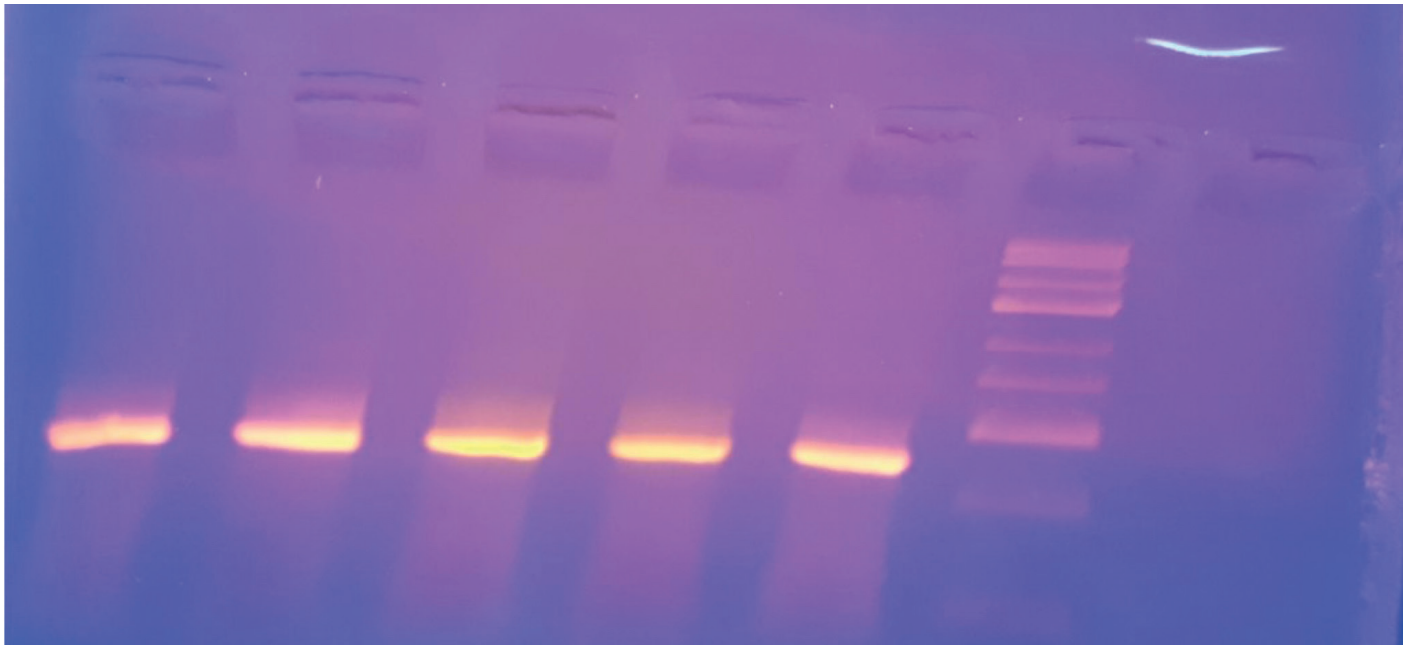

Figure 2. DNA agarose gel

Non-PANDAS group as reference category, a history of tonsillectomy was found to be only significant risk factor for PANDAS-Variant group, and only in the first regression model (OR=5.49, 95% CI: 1.06–28.38; p=0.042).

DISCUSSION

This is the first study, to our knowledge, reporting an association between PANDAS and MBL2 gene polymorphisms in pediatric OCD patients. Carrying any variant in exon 1 of the MBL2 gene was significantly more common in both the OCD group and the PANDAS subgroup compared to the control group. According to regression analysis, the presence of any variant in exon 1 increased the risk of PANDAS by a factor of 14.5 compared to the non-PANDAS group that served as the reference category, whereas it did not have any significant effect on the risk of PANDAS-Variant. Moreover, the frequency of variant alleles (D, B, or C alleles) was significantly higher in the OCD group and PANDAS subgroup than in the control group. Among 102 OCD patients, 6 were homozygous for the codon 54 BB allele, and 1 was homozygous for variant codon 57 DD allele. Interestingly, all of these patients were in the PANDAS subgroup. Similar with our findings, a small sized study researching an association between MBL-2 gene polymorphism and cardiomyopathy in Turkish samples, the distribution of AA, AB, and BB genotypes for MBL codon 54 were 65%, 25% and 10% in children with CMP. However, there was no study demonstrating MBL-2 Polymorphism prevalence in healthy population in Turkey (24).

The rates of TDs and OCD in first-degree relatives of pediatric probands with PANDAS were reported higher than in the general population and similar to typical TDs and OCD (25). Consistent with earlier findings, the present findings show that a family history of OCD and/or TDs was more common in the PANDAS subgroup than in the non-PANDAS subgroup, which, as previously reported, may be due to the heritability of genetic variants of the MBL2 gene (26). Additional research on the possible association between familial aggregation of OCD and/or TDs and variations of the MBL2 gene is warranted.

Codon 54 polymorphism of the MBL2 gene significantly affects serum levels of MBL and can lead to an increase in susceptibility to recurrent infections (27). It is reported that codon 54 polymorphism may be related with SLE-vasculitis (28). In addition, there is evidence suggesting that MBL2 genotypes play a role in the development of ARF, and RHD as a result of susceptibility to GABHS infections (15). It has been suggested that PANDAS shares the same mechanisms with ARF and SC, and the same genetic factors may be involved in the predisposition to both disorders (3, 4). Consistently, we found that family history of ARF/RHD was strongly associated with PANDAS subgroup in both regression models. Our findings may provide new insight to investigate the MBL2 gene in a wide spectrum of post-streptococcal autoimmune conditions. Additionally, the cumulative effects of genetic vulnerabilities, environmental factors, and both the lectin pathway and other immune mechanisms may contribute to the pathogenesis of PANDAS.

Table 2. Distribution MBL2 genotypes and allele frequency, according to group

		Study Groups n (%)		OCD Subgroups n (%)		
		Controls (n=60)	OCD (n=102)	PANDAS (n=59)	PANDAS-V (n=23)	N-PANDAS (n=20)
Allele	A	104 (86.7)	147 (72.1)	73 (61.9)	39 (84.8)	35 (87.5)
	B, C or D	16 (13.3)	57 (27.9) $^{\beta}$	45 (38.1) $^{\beta}$	7 (15.2)	5 (12.5)
Genotypes						
Codon 54	AA	52 (86.7)	70 (68.6)	36 (61.0)	17 (73.9)	17 (85.0)
	AB	8 (13.3)	26 (25.5)	18 (30.5)	5 (21.7)	3 (15.0)
	BB	-	6 (5.9) $^{\beta}$	5 (8.5) $^{\beta}$	1 (4.3)	-
Codon 57	AA	59 (98.3)	100 (98.0)	57 (96.6)	23 (100.0)	20 (100.0)
	AC	1 (1.7)	2 (2.0)	2 (3.4)	-	-
	CC	-	-	-	-	-
Codon 52	AA	54 (90.0)	86 (83.3)	45 (76.3)	22 (95.7)	19 (95.0)
	AD	5 (8.3)	15 (15.7)	13 (22.0)	1 (4.3)	1 (5.0)
	DD	1 (1.7)	1 (1.0)	1 (1.7)	-	-
Any variant	AA	45 (75.0)	54 (52.9)	22 (37.3)	17 (73.9)	15 (75.0)
	AB, AC or AD	14 (23.3)	41 (40.2)	31 (52.5)	5 (21.7)	5 (25.0)
	BB or DD	1 (1.7)	7 (6.9) $^{\beta}$	6 (10.2) $^{\beta}$	1 (4.3)	-

Notes: The genotypic distribution for the MBL2 gene polymorphisms in the study population was in Hardy-Weinberg equilibrium for both patients and controls and also in total study groups (p>0.05 for all). A, wild type allele; AB, heterozygous variant for codon 54; AC, heterozygous variant for codon 57; AD, heterozygous variant for codon 52; BB, homozygous variant for codon 54; CC, homozygous variant for codon 57; DD, homozygous variant for codon 52; Any variant, presence of AB, BB, AC, CC, AD or DD genotypes; MBL2, mannose binding lectin-2 gene; N-, non-PANDAS; OCD, obsessive compulsive disorder; PANDAS, pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections;-V, PANDAS-Variant

Table 3. Distribution of *MBL2* gene polymorphisms according to groups

	MBL2 Gene Polymorphisms n (%)		OR (95% CI) Comparison with		
	Yes (n=63)	No (n=99)	Controls	Non-PANDAS	PANDAS
Any variant					
Control Group	15 (25.0)	45 (75.0)			
OCD Group	48 (47.1)	54 (52.9)	2.66 (1.32–5.38) $^{\alpha}$		
OCD Subgroups					
PANDAS	37 (62.7)	22 (37.3)	5.05 (2.29–11.08)*	5.04 (1.61–15.79) $^{\alpha}$	
PANDAS-Variant	6 (26.1)	17 (73.9)	1.05 (0.35–3.17)	1.05 (0.26–4.18)	0.21 (0.07–0.61) $^{\beta}$
Non-PANDAS	5 (25.0)	15 (75.0)	1.00 (0.31–3.21)		
***MBL2* codon 54**					
Control Group	8 (13.3)	52 (86.7)			
OCD Group	32 (31.4)	70 (68.6)	2.97 (1.26–6.97) $^{\beta}$		
OCD Subgroups					
PANDAS	23 (39.0)	36 (61.0)	4.15 (1.67–10.31) $^{\alpha}$	3.62 (0.95–13.74) $^{\beta}$	
PANDAS-Variant	6 (26.1)	17 (73.9)	2.29 (0.69–7.55)	2.00 (0.42–9.33)	0.55 (0.19–1.60)
Non-PANDAS	3 (15.0)	17 (85.0)	1.14 (0.27–4.81)		

Notes: Chi-square test was used for comparisons between the groups; $^{\beta}$, *p* value ≤0.05; $^{\alpha}$, *p* value ≤0.005; *, *p* value=0.0001; OR, odds ratio; CI, confidence interval
Any variant, presence of AB, BB, AC, CC, AD or DD genotypes; MBL2 gene, mannose binding lectin-2 gene; N-, non-PANDAS; OCD, obsessive compulsive disorder; PANDAS, pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections;-V, variant;

Table 4. Results of two models of multinomial logistic regression analyses

	PANDAS			PANDAS-Variant		
	B	**OR (95% CI)**	***p* value**	**B**	**OR (95% CI)**	***p* value**
First Model						
Gender	0.857	2.35 (0.48–11.51)	0.290	-0.029	0.97 (0.20–4.51)	0.970
Comorbidity	-2.120	0.12 (0.00–3.99)	0.236	15.666	636.8 (636.8–636.8) x^3	-
OCD/TD in family	0.565	1.76 (0.25–12.34)	0.570	0.605	1.83 (0.28–11.65)	0.522
Tonsillectomy	3.334	28.03 (2.11–370.92)	0.011	1.697	5.45 (0.41–71.22)	0.196
ARF/RHD in family	3.904	49.62 (6.12–401.71)	0.000	1.703	5.49 (1.06–28.38)	0.042
Any variant	2.675	14.50 (2.49–84.19)	0.003	0.794	2.21 (0.40–12.19)	0.362
Second Model						
Gender	0.836	2.30 (0.51–10.42)	0.277	-0.083	0.92 (0.19–4.25)	0.915
Comorbidity	-1.574	0.20 (0.00–4.6)	0.322	16.626	940.0 (940.0–940.0) x^3	-
OCD/TD in family	0.734	2.08 (0.33–13.03)	0.432	0.506	1.65 (0.26–10.29)	0.587
Tonsillectomy	2.848	17.25 (1.63–182.74)	0.018	1.391	4.01 (0.34–46.59)	0.266
ARF/RHD in family	3.289	26.82 (4.06–177.06)	0.001	1.443	4.23 (0.95–18.75)	0.050
Codon 54 polymorphism	1.457	4.29 (0.75–24.40)	0.101	1.009	2.74 (0.47–15.89)	0.260

The Reference category was non-PANDAS group for each model; OR, odds ratio; CI, confidence interval; x^3, value presented as 10^3
Abbreviations: ARF/RHD, a family history of acute rheumatic fever/rheumatic heart disease; any variant, presence of AB, BB, AC, CC, AD or DD genotypes; OCD/TDs, a family history of obsessive compulsive disorder/tic disorders; PANDAS, pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections

Recently, significant revisions have been proposed for the diagnosis of PANDAS to facilitate more accurate diagnosis and treatment. Two new clinical entities –pediatric acute-onset neuropsychiatric syndrome (PANS) and childhood acute-onset neuropsychiatric syndrome (CANS)– have been proposed in order to identify a homogenous group of patients characterized by dramatic onset of symptoms as a hallmark clinical feature for this group (3). PANS removes the requirement of temporal association with GABHS infections, and includes acute onset OCD both preceding other infections and without an apparent environmental precipitant. It is essential to rule out probable medical causes since acute neuropsychiatric symptoms (eating refuse to separation anxiety) can present in a wide variety in patients with PANS. (7).

In the present study, we thought that, the PANDAS-Variant subgroup exhibited genotypic and phenotypic features on a continuum somewhere between those observed in the PANDAS and non-PANDAS subgroups. There is a need for future investigation to determine unique characteristics of this subgroup. In other word, changing nosological classification from PANDAS to PANS, supports defining new clinical subgroup associated with other infectious triggers besides streptococcus. MBL and its clinical importance on PANS related phenotype might be future research interest area.

The primary limitation of the present study is that the MBL serum level could not be assessed and so could not be correlated with other associated variables. Secondly, there are other important genetic variants that are involved in variable MBL2 levels such as-550 H/L and the-221 X/Y variants although we could not examine these regions (29). Haplotype based variant analysis and/or genome wide studies may provide better understanding of ethiopathogenesis of autoimmune neuropsychiatric disorders in the future. Additionally, fewness of the control patients may have affected the main results of the study.

CONCLUSION

Based on the present findings, we conclude that MBL2 genotypes might be associated with PANDAS and may serve as candidate marker for susceptibility in immune-mediated subgroups of patients with childhood-onset OCD. Future research should focus on assessing the association between various genotypes and comorbid conditions, other immune pathways (such as immunoglobulins), and the potential role of dopaminergic system in these cases. Better understanding role of the MBL2 gene may lead to novel therapeutic targets.

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