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Research Article

PROMOTER POLYMORPHISM IN TGFBR2 IS ASSOCIATED WITH THE ONSET OF THE RELAPSING-REMITING MULTIPLE SCLEROSIS

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Summary: Multiple sclerosis (MS) is an organ-specific autoimmune disorder that affects the central nervous system. Transforming growth factor beta (TGF-β) and its receptor II (TGFBR2) are two key components of the TGF-β signaling pathway, which play a pivotal role in immune tolerance. Our aim was to investigate the promoter polymorphism -875G/A in TGFBR2 and MS susceptibility as well as the age of disease onset in Bulgarian patients. The case-control study included 159 patients with relapsing-remitting multiple sclerosis (RRMS) subjected to disease-modifying therapy and 307 age-gender-matched healthy controls. We observed the following genotype frequencies for TGFBR2 in cases: GG-68%, AG-26%, AA-6% and controls: GG-63%, AG-33%, AA-4%. No significant differences in genotype and allele frequencies between cases and controls were observed. However, we estimated that the AG genotype and the combination of AA+AG genotypes were significantly more often represented in patients with an early onset compared to those with a late onset of the disease (OR=2.637, 95%CI=1.162÷6.050, p=0.011; OR=2.377, 95%CI=1.122÷5,069, p=0.014). The results indicate that carrying a variant allele-A in promoter polymorphism -875G/A TGFBR2 (rs3087465) might be a risk factor for the early onset of RRMS.

Keywords: -875G/A polymorphism; rs3087465; RRMS; transforming growth factor receptor II.

Abbreviatios: CI – confidence intervals; EDSS – Expanded Disability Status Scale; MS – Multiple sclerosis; OR – odds ratios; PIRA-PCR – primer-introduced restriction analyses -polymerase chain reaction; RRMS – relapsing-remitting multiple sclerosis; SNP – single nucleotide polymorphism; TGF-β – transforming growth factor; TGFBR2 – transforming growth factor receptor II.

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INTRODUCTION

Multiple sclerosis (MS) is a chronic systemic neuron inflammatory disease affecting thousands of people worldwide every year that is a common reason for heavy physical disability in young adults (Dendrou et al., 2015). Relapsing-remitting MS is described by unpredictable relapses followed by a period of months of remission with no new symptoms of disease activity (Compston and Coles, 2008). Multiple sclerosis is accepted as an immune-mediated disorder resulting in imbalance of the pro- and anti-inflammatory cytokines due to an inflammatory process triggered by T cells (Navikas and Link, 1996; Compston and Coles, 2008). It is well known that both genetic and environmental factors are involved in the pathogenesis of MS (Gourraud et al., 2012; Nakahara et al., 2012).

The transforming growth factor β (TGF- β) signaling pathway takes a central position in controlling immune regulation, cell differentiation, apoptosis, cell growth and adult tissue homeostasis (Lee et al., 2017; Derynck et al., 2001; de Caestecker et al., 2000). TGF-β is a regulatory cytokine produced by many cell types and especially common in the immune cells, CNS glial and gut epithelial cells (Jin et al., 2007). The TGF beta family includes TGFβ1, TGFβ2 and TGFβ3. The expression of each isoform is restricted by differentiated cell type. TGF-β1 is the most abundant member of the TGF-β family and a major immunosuppressive cytokine with an important role in maintaining immune homeostasis; its deficiency resulting in lethal autoimmunity in mice (Mo et al., 2006).

Therefore, TGF-β1 is regarded as one of the most extensively studied cytokines, in respect of the immunopathogenesis of RRMS, especially as a major factor of autoimmune regulation (Lee et al., 2017). TGF- β1 binds to the TGF-b receptor II (TGFBR2), which recruits a type I receptor dimer to create a hetero-tetrameric complex with the ligand. These receptors are serine/threonine kinase receptors that induce the phosphorylation of SMAD proteins. SMAD proteins serve as transcription factors, which could perform positive or negative regulation of target genes (Massagué et al., 2000; Jin et al., 2008). Collectively, TGF-β1 is involved in several intracellular signaling pathways that differ in their consequences depending on the cell type and grade of differentiation (epithelial cells, endothelial cells, smooth muscle, myofibroblast, immune cells, etc.). Some studies indicate that overexpression of TGF-β1 is associated with reduced expression or inactivation of TGF-β1 receptors, especially TGFBR2, in correlation with cancer progression (Fukai et al., 2003; Kim et al., 2003). The study of Seijo et al. pointed out that a promoter single nucleotide polymorphism (SNP) G-875A in TGFBR2 was implicated in the transcription activity in the normal epithelial cells of the gene (Seijo et al., 2000). They suggested an increased expression of the receptor by -875A allele variant of TGFBR2 (Seijo et al., 2000).

Based on the important role of TGF-β1 signaling pathway in the autoimmune diseases, we aimed to investigate the promoter polymorphism -875G/A in TGFBR2 and susceptibility to MS in Bulgarian patients.

MATERIALS AND METHODS

Subjects

In this case-control study 159 patients (114 women and 45 men, average age 40.1±8.4) with RRMS were included. Blood samples of the patients were collected at the Department of Neurology, Medical University, Plovdiv, Bulgaria. The type of the disease - relapsing-remitting multiple sclerosis was diagnosed according to the McDonald criteria (Polmanet al., 2011). All included patients were in a remission phase of the disease (defined as a period of improvement or stable clinical condition for at least 3 months); age between 18 and 60 years; undergoing disease-modifying therapy with interferon-beta or glatiramer acetate for at least 6 months. The disease severity was determined by means of the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983). All required cases were with EDSS below 4.5. The patient group was divided into two subgroups according to the onset of the disease: early onset - under 30 years (n=82 patients) and late onset - above 30 years (n=77 patients). The control group included 307 age-gender matched unrelated healthy volunteers (130 women and 50 men, average age - 40.19±10.64 years). From all participants in the study a written informed consent was obtained according to the ethical guidelines of the Helsinki Declaration.

DNA extraction and genotype analyses

Genomic DNA was extracted from blood leukocytes by using Chelex® 100 (Bio-Rad, USA) following the manufacturer's instructions. SNP analysis of the TGFBR2 at position -875 was detected by using a primer-introduced restriction analysis (PIRA-PCR) assay (Keet al., 2001; Guangfuet al., 2006). For the TGFBR2 G-875A, mismatched G was introduced into the sense primer to replace A at -2bp from the polymorphic site to create a RsaI restriction site. The primer sequences were: sense-5'-GCAA GAAAGGAAATTGAAAGTTTGT-3' and antisense-5'-TCACCTGAATGCTT GTGCTTTT-3'. PCR reactions were performed under the following conditions: denaturation at 94°C for 5min, followed by 94°C (45sec), 57°C (45sec) and 72°C (45sec) for 30 cycles, followed by final extension at 72°C for 7 min. The genotyping analyses were performed after amplification by a GeneAmp PCR System 9700 (Applied Biosystems).The PCR product was digested overnight at 37°C by restriction enzyme RsaI (Thermo Fisher Scientific, MA, USA) and then separated on 3.5% agarose gel by electrophoresis (Fig. 1).

Statistical analyses

Genotype distribution and allele frequencies between the cases and controls were evaluated using thechi-square test. To compare the difference in age between patients with RRMS and the controls independent samples t-test was used. The association between genotypes of promoter polymorphism -875G/Ain TGFBR2 and risk of multiple sclerosis was estimated by computing the odds ratios (ORs), their 95% confidence intervals (CIs) and the P-value by using logistic regression analyses. The Hardy-Weinberg equilibrium was assessed

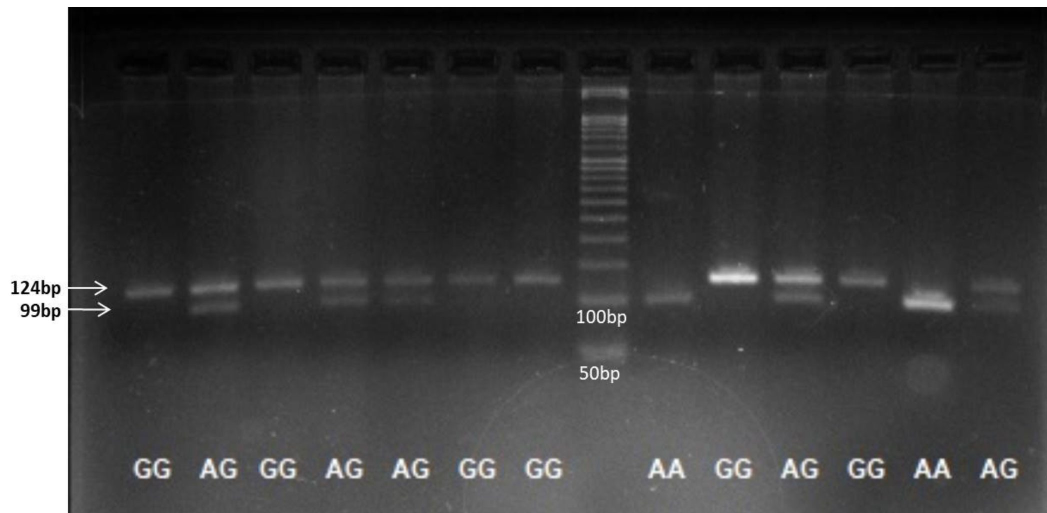

Figure 1. Agarose gel electrophoresis of PIRA-PCR for TGFBR2 -875G/A genotype analysis. After PCR amplification, the 124bp product was digested with a RsaI restriction enzyme. In AA-genotype, two bands (99bp and 25bp) were visualized after staining with ethidium bromide, in GG-genotype - one band (124bp) and in heterozygous GA genotype – three bands (124bp, 99bp and 25bp), respectively (25bp fragment was too short to be detected). 50bp DNA ladder was used as a marker.

using the chi-square test. The results are regarded as significant at $P<0.05$. All statistical analyses were performed using StatSoft software.

RESULTS

Genotype and allele frequency of promoter polymorphism -875G/A TGFBR2 (rs3087465)

All 159 RRMS patients and 307 healthy volunteers were successfully genotyped for a promoter polymorphism -875G/A in TGFBR2 gene. There were no significant differences in gender and age between cases and controls (chi-square test). In our study, the genotype distribution among the cases and controls did not deviate from Hardy-Weinberg equilibrium (P=0.540, P=0.980, respectively, chi-square test). We observed the following genotype frequencies for TGFBR2 G-875A in RRMS patients: GG-68%, AG-26%, AA-6% and controls: GG-63%, AG-33%, AA-4% (Table 1). However, no significant differences of genotypes and allele frequencies between the cases and controls were observed ($\chi 2=3.125$, df=2, P=0.209).

A significant difference in the distribution of the genotypes in the studied polymorphism (rs3087465) was observed when the patients were divided into two subgroups according to the onset of the disease. The allele and genotype distribution of -875G/A polymorphism in TGFBR2 according to the onset of RRMS in cases and controls is presented in Table 2. Our results demonstrated that AG genotype was significantly more often represented in patients with an early onset than those with a late onset of the disease (34% vs

Table 1. Allele and genotype distribution of the promoter polymorphism -875G/A in TGFBR2 among RRMS patients and healthy controls.

Genotype	Cases (n=159)	Controls (n=307)	OR (95%CI)	p-value
GG	109 (68%)	193 (63%)	1	
AG	41 (26%)	102 (33%)	0.712 (0.451÷1.120)	0.122
AA	9 (6%)	12 (4%)	1.338 (0.497÷3.512)	0.534
AG+AA	50 (31.5%)	114 (37.1%)	0.777 (0.506÷1.190)	0.223
G allele	259 (81%)	488 (80%)	1	
A allele	59 (19%)	126 (20%)	0.882 (0.616÷1.262)	0.475

17%; OR=2.637, 95%CI=1.162÷6.050, p=0.011). A similar association was revealed when the combination of the genotypes AA+AG from the patients with an early onset was compared to those with a late onset of the disease (40% vs 22%;OR=2.377, 95%CI=1.122÷5,069, p=0.014).

DISCUSSION

TGF-β is a multifunctional cytokine playing a diverse set of roles involving cell differentiation, immune regulation and tumor suppression (Huang et al., 2014). TGF-β1 is an essential cytokine for maintaining the balance between

Table 2. Genotype and allele distribution of -875G/A polymorphism in TGFBR2 according the onset of RRMS.

Genotype	Early onset (n=82)	Late onset (n=77)	OR (95%CI)	p-value
GG	49 (60%)	60 (78%)	1	
AG	28 (34%)	13 (17%)	2.637 (1.162÷6.050)	0.011
AA	5 (6%)	4 (5%)	1.531 (0.332÷7.264)	0.540
AG+AA	33 (40%)	17 (22%)	2.377 (1.122÷5.069)	0.014
GG vs. AG+AA	49 (60%) 33 (40%)	60 (78%) 17 (22%)	0.421 (0.197÷0.891)	0.014
AG vs. AA+GG	28 (34%) 54 (66%)	13 (17%) 64 (83%)	2.553 (1.113÷5.803)	0.013
G allele	126 (77%)	133 (86%)	1	
A allele	38 (23%)	21 (14%)	1.910 (1.024÷3.580)	0.029

inducible T regulatory cells (iTregs) and Th17 cells, which secrete pro-inflammatory cytokines (Eisenstein et al., 2009). The TGF-β signaling pathway initiated from TGF-β1 binding to TGFBR2, initiate an intracellular signaling. The polymorphic variant in the promoter region of TGFBR2 (rs3087465) causing a G to A transition was reported by Seijo et al. (Seijo et al., 2000). Moreover, these authors established that the presence of A polymorphic variant at the position -875 increased the activity of TGFBR2 transcription in normal epithelial cells and affected a specific binding oligonucleotide probe (Seijo et al., 2000). Collectively, all these data determine that promoter polymorphism as a candidate SNP for genetic predisposition to multiple sclerosis as an autoimmune disease. In our study, we investigated the association of -875G/A TFGBR2 and RRMS among 159 patients and 307 healthy volunteers.The results showed no significant differences in the genotypic distribution in cases and controls by the $\chi 2$ test. On the other hand, TGFBR2 heterozygous genotype was associated with a non significantly decreased risk of MS compared with the wild-type (TGBR2-875GG) genotype. We found several studies on the same SNP in association with cancerogenesis which showed the significance of that SNP in the development of breast and lung cancers. Jin et al. (2007; 2008) studied 636 patients with breast cancer, and 223 patients with an esophageal squamous cell carcinoma in a Chinese population, and demonstrated a significantly decreased risk to the diseases related to -875GA genotype in TGFBR2 compared with -875GG genotype. Moreover, Huang et al. (2014) performed a meta-analysis involving 9 case-control studies analyzing the relation of TGFBR2G-875A and the risk of cancer. These authors suggested that A allele was associated with a trend of decreased cancer risk. To our knowledge, the role of -875G/A polymorphism in TGFBR2 for RRMS has not been previously explored.

In the present study, we showed that -875G/A polymorphism influenced the onset of RRMS among a Bulgarian population. When the patient group was divided into two subgroups according to the onset of the disease, we found that AG and AA+AG genotypes were more often represented compared to GG genotype in patients with an early onset of the disease. These results show that the promoter variant allele of TGFBR2 -875A might be one of the factors that determine the early onset of the disease, which has a great clinical significance for the debilitating progress of the disease. Taking into account the effect of this polymorphism on the activity of TGFBR2, we may assume that higher transcriptional activity determined by variant A-allele may increase the TGFBR2 density and utility of active TGFb1 by target cells, including Th17 and iTreg cells. The -875G/A polymorphism in TGFBR2 has the potential to modulate the TGFB-signaling pathway resulting in an early onset of RRMS.

Some limitations of our study need to be noted. The sample size was relatively small including only patients with EDSS less than 4.5 due to the disease-modifying therapy. Thus, the impact of -875G/A polymorphism in TGFBR2 in cases with severe disability (EDSS>5.0) should be considered in the future.

CONCLUSION

To our knowledge, this is the first study investigating the role of -875G/A TFGBR2 SNP in multiple sclerosis genetic predispositions. The results of our study performed among a Bulgarian population indicated that carrying a variant allele-A in promoter polymorphism -875G/A in TGFBR2 (rs3087465) might be a risk factor for an early onset of RRMS.

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REFERENCES

Compston A, Coles A, 2008. Multiple sclerosis, Lancet, 372 (9648): 1502–17.

deCaestecker MP, Piek E, Roberts AB, 2000. Role of transforming growth factor-beta signaling in cancer. J Natl Cancer Inst, 92: 1388–1402.

Dendrou, C. A., Fugger, L., &Friese, M. A., 2015. Immunopathology of multiple sclerosis. Nature Reviews Immunology, 15(9): 545.

Derynck R, Akhurst RJ, Balmain A, 2001. TGF-beta signaling in tumor suppression and cancer progression. Nat Genet, 29: 117–129.

Eisenstein, E. M., & Williams, C. B., 2009. The T (reg)/Th17 cell balance: a new paradigm for autoimmunity. Pediatr Res, 65(5 Pt 2), 26R–31R.

Fukai Y, Fukuchi M, Masuda N, Osawa H, Kato H, Nakajima T, Kuwano H, 2003. Reduced expression of transforming growth factor-beta receptors is an unfavorable prognostic factor in human esophageal squamous cell carcinoma. Int J Cancer, 104: 161–166.

Gourraud P.A., H.F. Harbo, S.L. Hauser, S.E. Baranzini, 2012. The genetics of multiple sclerosis: An up-to-date review, Immunol. Rev, 248 (1): 87–103.

Huang, Y. S., Zhong, Y., Yu, L., & Wang, L., 2014. Association between the TGFBR2 G-875A polymorphism and cancer risk: evidence from a meta-analysis. Asian Pac J Cancer Prev, 15: 8705–8708.

Jin, G., Deng, Y., Miao, R., Hu, Z., Zhou, Y., Tan, Y., ...& Chen, W., 2008. TGFB1 and TGFBR2 functional polymorphisms and risk of esophageal squamous cell carcinoma: a case–control analysis in a Chinese population. Journal of Cancer Research and Clinical Oncology, 134(3): 345–351.

Jin, G., Wang, L., Chen, W., Hu, Z., Zhou, Y., Tan, Y., ...& Zhang, Z., 2007. Variant alleles of TGFB1 and TGFBR2 are associated with a decreased risk of gastric cancer in a Chinese population. International Journal of Cancer, 120(6): 1330–1335.

Ke X, Collins A, Ye S., 2001. PIRA PCR designer for restriction analysis of single nucleotide polymorphisms. Bioinformatics, 17: 838–9.

Kim SJ, Im YH, Markowitz SD, Bang YJ., 2000. Molecular mechanisms of inactivation of TGF-b receptors during carcinogenesis. Cytokine Growth Factor Rev, 11: 159–68.

Kurtzke, J. F., 1983. Rating neurologic impairment in multiple sclerosis an expanded disability status scale (EDSS), Neurology, 33(11): 1444–1452.

Lee, P. W., Severin, M. E., &Lovett-Racke, A. E., 2017. TGF-β regulation of encephalitogenic and regulatory T cells in multiple sclerosis. European Journal of Immunology, 47(3): 446–453.

Li Mo, Wan Y.Y, Sanjabi S., Robertson A.K., Flavell R.A., 2006. Transforming growth factor-beta regulation of immune responses. Annu Rev Immunol, 24: 99–146.

Massagué, J. and Wotton, D., 2000. Transcriptional control by the TGF-β/Smad signaling system. The EMBO Journal, 19(8), pp.1745–1754.

Nakahara J, Maeda M, Aiso S, Suzuki N, 2012. Current concepts in multiple sclerosis: autoimmunity versus oligodendrogliopathy. Clinical Reviews in Allergy & Immunology, 42 (1): 26–34.

Navikas V., H. Link, 1996. Cytokines and the pathogenesis of multiple sclerosis, J. Neurosci. Res, 45 (4-6): 322–333.

Polman, C. H., S. C. Reingold, B. Banwell, , M. Clanet, , J. A. Cohen, et al., 2011. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria, Annals of Neurology, 69(2): 292–302.

Seijo ER, Song H, Lynch MA, Jennings R, Qong X, Lazaridis E, Muro-Cacho C, Weghorst CM and Munoz-Antonia T, 2000. Identification of genetic alterations in the TGFbeta type II receptor gene promoter. Mutat Res, 483: 19–26.