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TNFRSF13C/BAFFR P21R and H159Y polymorphisms in multiple sclerosis

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ABSTRACT

Recent studies implicate B cells in multiple sclerosis (MS) pathogenesis, and consequently, several molecules participating in B cell survival and proliferation, including B-cell activating factor (BAFF), have recently been analyzed in MS patients. BAFF mediates its function through binding to three receptors; among them, its interaction with the BAFF receptor (BAFFR) is crucial in mediating its survival function. Interestingly, two common polymorphisms of the *TNFRSF13C* gene, encoding BAFFR, P21R (rs77874543) and H159Y (rs61756766), have been reported to affect BAFFR assembly and signaling. In order to evaluate the possible contribution of BAFFR in MS pathogenesis and/or phenotype, we analyzed both *TNFRSF13C/BAFFR* polymorphisms in 486 MS patients in relation to their disease severity, their disability status and the age of disease onset and duration. As control group, we used allele frequencies extracted from the Exome Aggregation Consortium (ExAC) Browser. Interestingly, we found a higher prevalence of the H159Y polymorphism in MS patients, suggesting that enhanced BAFFR-signaling might contribute to the disease pathogenesis.

1. Introduction

Multiple sclerosis (MS) is a chronic, debilitating neurological disease that begins in early adulthood, with a strong autoimmune background, but no clear etiology. Inflammation is considered a hallmark of acute MS lesions, characterized by infiltration of T and B cells, activated macrophages and microglia (Claes et al., 2015; Dendrou and Fugger, 2017; Hadjigeorgiou et al., 2019; Hemmer et al., 2002), resulting in tissue damage with axonal de- and re-myelination (Stadelmann, 2011). Recent studies implicate B cells in disease pathogenesis, a notion that is also supported by the evidence of the therapeutic role of B cell depletion in the management of the disease (Disanto et al., 2012; Franciotta et al., 2008).

Consequently, several molecules participating in B cell proliferation and homeostasis have recently been analyzed in MS patients (Srivastava et al., 2012; Steri et al., 2017). Amongst them is B-cell activating factor (BAFF), a TNF-family member which supports the survival of B cells (Mackay and Schneider, 2009). BAFF is predominantly expressed by cells of myeloid origin, such as macrophages, monocytes and dendritic cells (Woodland et al., 2006); however, non-hematopoietic cells, as astrocytes, can overexpress BAFF, either experimentally in mice following viral brain infection, or in pathological conditions, as primary central nervous system (CNS) lymphomas (Krumbholz et al., 2005; Lokensgard et al., 2016; Mackay and Schneider, 2009). Interestingly, BAFF has been detected in MS lesions (Krumbholzet al., 2005; Mackay and Schneider, 2009) and changes in BAFF concentrations in the cerebrospinal fluid have been associated with MS severity in humans (Ragheb et al., 2011; Thangarajh et al., 2004; Wang et al., 2012). Recent genetic studies revealed that defined genetic changes stabilizing *BAFF* mRNA and increasing its expression closely correlate with the incidence of MS (Steri et al., 2017).

BAFF binds to three receptors, namely BAFFR (BAFF receptor), BCMA (B cell maturation antigen), and TACI (transmembrane activator and calcium modulating ligand interactor) (Kim et al., 2003; Mackay and Schneider, 2009). While TACI is expressed by mature and terminally differentiated B cells, such as switched memory B cells, marginal zone B cells and plasma cells, and BCMA only by plasma cells, BAFFR expression starts already at the stage of immature/transitional B cells, acting as essential pro-survival receptor of mature follicular and marginal zone B cells (MacKay and Schneider, 2009; Smulski and

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Eibel, 2018). Interestingly, two functional common polymorphisms of the *TNFRSF13C* gene, encoding BAFFR, namely P21R (rs77874543) and H159Y (rs61756766), disturb either ligand-independent assembly of BAFFR into oligomers (P21R, Pieper et al., 2014), or increase ligand-independent BAFFR signaling (H159Y, Hildebrand et al., 2010). *TNFRSF13C*-P21R was found in a high frequency in patients with common variable immunodeficiency (CVID), but was low to undetectable in patients with chronic lymphocytic leukemia (Jasek et al., 2016; Losi et al., 2005; Pieper et al., 2014). On the other hand, the *TNFFRSF13C*-H159Y polymorphism results in a sustained overactivated BAFF-induced signaling and it was found in a high frequency in patients with Non-Hodgkin lymphomas and a Sjogren's syndrome-related lymphoproliferation (Hildebrand et al., 2010; Papageorgiou et al., 2015).

Since increased BAFF levels were found to associate with susceptibility for MS, we started to study the possible contribution of BAFFR signaling by analyzing the correlation of both *TNFRSF13C/BAFFR* polymorphisms with MS pathogenesis and/or phenotype.

2. Materials & methods

2.1. Study population

The study consisted of 486 consecutive patients with MS (male/female: 156/330, mean age: 44 years, range: 72 years), treated and followed-up in the University Hospital of Larissa, Greece. All patients fulfilled the 2010 revised McDonald diagnostic criteria for MS (Polman et al., 2011) and the recent diagnostic MRI criteria (Filippi et al., 2016). Details relating to age of disease onset and disease duration were collected for all patients; disability status was assessed using the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983), while disease severity was evaluated by means of the Multiple Sclerosis Severity Score (MSSS) (Roxburgh et al., 2005). MSSS scores were sorted into 3 categories, namely benign: 0–1.999, moderate: 2–6.999, and severe: 7–10 (Dardiotis et al., 2017). As a control, we used the publicly available data from the Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org) (Lek et al., 2016), since they include much larger numbers of individuals analyzed yielding higher significance. These data do not differ from our cohort of 316 healthy individuals previously analyzed for the prevalence of *TNFRSF13C/BAFFR* polymorphisms in a Greek population (Kompoti et al., 2015).

The ExAC database lists 32 *TNFRSF13C* variants composed from 30 missense mutations and 2 copy number variants. As control group for the Greek MS cohort, we used then (Non-Finnish) European population, in which 455 of 65,566 total alleles carry the H159Y missense mutation encoded by the *TNFSRF13C* variant 22:42,321,451 G/A (dbSNP rs1756766). This corresponds to an allele frequency of 1/147 (0.006787). The allele frequencies of the other populations listed in the ExAC database are: Finnish 1/400, Latino 1/133, African 1/462, East Asian 0/8608, South Asian 1/179 alleles.

Written informed consent was obtained from all the participants. The study was approved by the local institutional review board (University Hospital of Larissa, Greece) and was carried out in accordance with the principles of the Helsinki Declaration.

2.2. Molecular studies

Genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen, Crawley, UK), according to manufacturer's instructions. The detection of P21R and H159Y polymorphisms was performed by PCR amplification of exon 1 and 3 of TNFRSF13C, respectively, followed by restriction fragment length polymorphism (RFLP) analysis, as previously described in detail (Kompoti et al., 2015).

2.3. Statistical analysis

Allele frequencies were analyzed using Fischer's exact test. Pearson's chi-square test was used for comparison of categorical variables. For non-canonical variables, Mann-Whitney U non-parametric tests were performed. EDSS scores were analyzed as ordinal values. The dominant model of expression (Pieper et al., 2014) was chosen to examine any association between disease manifestation and the presence of the polymorphisms. Multinomial logistic regression was performed to conduct the comparison between stages of disease severity; univariate and multivariate analysis was performed as appropriate. The statistical significance threshold was set at two-sided $p < 0.05$. The statistical package SPSS v22 was employed for the analysis.

3. Results

Fifty-nine MS patients (12.1%) carried the P21R polymorphism, with one being a homozygote and 22 of the these P21R carrying patients (4.5% out of the total, or 37.3% out of P21R-positive patients) also had in heterozygosity the H159Y mutation. The analysis revealed that both polymorphisms were in Hardy-Weinberg Equilibrium (P21R $p = 0.262$, and H159Y $p = 0.260$). While, the frequency of the P21R polymorphism did not differ significantly between MS patients and controls, the H159Y polymorphism was found more frequently in the MS patient group (2.2% vs. 0.68%), correlating with a significantly ($p < 0.0001$) higher relative risk (3.3, 95%CI: 2.168–4.913) and odds ratio (3.4, 95%CI: 2.170–5.170) for MS (Table 1).

To study whether the presence of P21R and H159Y polymorphisms could influence MS disease severity, we examined the impact of both polymorphisms on “age of disease onset” and “MSSS” (the later combines EDSS and disease duration). Finally, we conducted a subgroup analysis to examine any difference of the clinical characteristics in the P21R-positive population regarding the presence of the H159Y alteration.

The mean age of MS patients at initial symptoms appearance was 32.3 years (range 56 years) and the mean duration of the disease was 11.7 years. The mean severity of MS as expressed by the MSSS was 4.5, while the median disability score (EDSS) was 3. No substantial differences regarding the clinical characteristics were noted between the genders (Supplementary Table 1).

Table 1
Allele frequencies of *TNFRSF13C/BAFFR*-P21R and *TNFRSF13C/BAFFR*-H159Y polymorphisms in the patients of the study.

Frequencies	MS patients n 486 *n* (%)	Controls* n 933 *n* (%)	*p*	OR (95%CI)	RR (95%CI)
wt/wt	912 (93.8%)	1740 (93.2%)			
P21R/wt	58 (6.0%)	120 (6.4%)	0.187	0.801 (0.583–1.092)	0.864 (0.690–1.061)
P21R/P21R	2 (0.2%)	6 (0.32%)			
wt/wt	464 (97.7%)	64,676 (99.3%)			
H159Y/wt	22 (2.3%)	445 (0.7%)	< 0.001	3.389 (2.170–5.170)	3.270 (2.168–4.913)
H159Y/H159Y	0 (0%)	0 (0%)			

Abbreviations: CI = Confidence Interval, MS = Multiple Sclerosis, OR = Odds Ratio, RR = Relative Risk, wt: wild-type. *data for P21R: http://exac.broadinstitute.org/variant/22-42322716-G-C; data for H159Y: http://exac.broadinstitute.org/variant/22-42321451-G-A.

Table 2
Association of *BAFFR*-P21R and *BAFFR*-H159Y polymorphisms with clinical characteristics of the patients of the study.

	P21R(+)	P21R(-)	p	H159Y(+)	H159Y(-)	p
Sex	*n 59*	*n 427*	*0.78**	*n 22*	*n 464*	*0.62**
Male n(%)	18 *(30.5%)*	138 *(32.3%)*		6 *(27.3%)*	150 *(32.3%)*	
Female n(%)	41 *(69.5%)*	289 *(67.7%)*		16 *(72.7%)*	314 *(67.7%)*	
Age of Onset	*n 59*	*n 427*	*0.78***	*n 22*	*n 464*	*0.24***
Mean	32.8	32.3		29.8	32.5	
Median	31.5	31		28	31	
Range	45	56		34	56	
MSSS	*n 59*	*n 427*	*0.89***	*n 22*	*n 464*	*0.32***
Mean	4.44	4.55		4	4.6	
Median	3.89	3.94		3.89	3.94	
Range	9.47	9.73		7.56	9.73	
Benign MS n(%)	7 *(11.9%)*	72 *(16.9%)*	*0.26**	3 *(13.6%)*	76 *(16.4%)*	*0.41**
Moderate MS n(%)	44 *(74.6%)*	272 *(63.7%)*		17 *(77.3%)*	299 *(64.4%)*	
Severe MS n(%)	8 *(13.6%)*	83 *(19.4%)*		2 *(9.1%)*	89 *(19.2%)*	

Abbreviations. SD: Standard Deviation, MS: Multiple Sclerosis, MSSS: Multiple Sclerosis Severity Score.
The statistical analysis was performed according to Pearson's chi-square test (*) and Mann-Whitney-U test (**).

Frequencies of the P21R-carriers and P21R-wt patients with benign, moderate and severe MS disease (according to the MSSS score) are depicted in Table 2. Disease severity did not differ between patients with the P21R polymorphism and wt patients ($p = 0.26$). Frequencies of the H159Y-carriers and H159Y-wt patients with benign, moderate and severe MS disease (according to the MSSS score) are also depicted in Table 2. Disease severity did not differ between patients with the H159Y polymorphism and H159Y-wt patients ($p = 0.41$). Differences between severe and benign disease status were further examined, among the mutational groups, by means of multinominal logistic regression. Since P21R & H159Y are in linkage disequilibrium, they were entered separately in the multivariate model, so that multicollinearity would not arise. However, statistically significant differences were not observed between patients with severe and benign disease status, for neither P21R nor H159Y polymorphism, either in the univariate, or the multivariate model adjusted for gender and age of onset ($p > 0.05$, in all cases). Furthermore, no significant differences were found concerning "the age of disease onset" neither for the P21R nor for the H159Y polymorphism (Table 2).

Finally, further analysis in the P21R-positive subgroup revealed that patients who also harbored the H159Y polymorphism had an earlier age of disease onset compared to H159Y-negative patients (median value 28.0 vs 32.5 years respectively); however, the difference was found marginally non-significant ($p = 0.096$). No statistically significant difference was noted for the rest of the clinical characteristics ($p > 0.05$, in all cases).

4. Discussion

Analyzing a large cohort of MS patients, we did not find any significant association of *TNFRSF13C/BAFFR*-P21R polymorphism with MS. In contrast, the *TNFRSF13C/BAFFR*-H159Y polymorphism was clearly overrepresented in the MS cohort. H159Y was first found in a high prevalence in patients with non-Hodgkin lymphoma and the polymorphism has been suggested to increase the risk of developing the disease (Hildebrand et al., 2010). Since the H159Y mutation was reported to increase the affinity of BAFFR for TRAF6 association resulting in a hyperactive receptor (Hildebrand et al., 2010), the correlation between an increased risk for MS and BAFFR hyperreactivity fits well to the observation made by Steri et al. that a polymorphism in the *BAFF* gene, which leads to higher BAFF levels, correlates positively with the risk of developing MS (Steri et al., 2017).

However, the relation between BAFF, BAFFR signaling and the susceptibility to MS may be more complex than just a direct correlation between higher BAFF levels, a stronger BAFFR signaling and the increased risk of developing the disease. In this context, it has been reported that blocking the biological activity of BAFF through the administration of BCMA-Ig, suppressed the onset and severity of experimental autoimmune encephalomyelitis (EAE) (Huntington et al., 2006). Likewise, neutralization of BAFF and APRIL in a marmoset (Callithrix jacchus) EAE model by treating the animals with belimumab (an anti-BAFF mAb) and anti-APRIL antibodies, delayed the development of this relevant preclinical MS model with a higher efficiency by belimumab than by anti-APRIL antibodies (Jagessar et al., 2012).

In contrast to these experimental models, the treatment of MS patients with soluble TACI-Ig (atacicept), which neutralizes both BAFF and APRIL, had to be terminated prematurely, since the disease had aggravated in the group of atacicept-treated patients in ATAMS (Kappos et al., 2014 and ATON (Sergott et al., 2015) trials. However, B cell depletion induced by treating MS patients with rituximab (an anti-CD20 mAb), suggested that the elimination of B cells in MS is safe and can ameliorate the disease (Rommer et al., 2016). Since the neutralization of BAFF and/or APRIL activity through the blocking of their receptors affects all circulating B cell subsets but spares a significant fraction of switched memory B cells, neutralization of BAFF and APRIL may deplete B cell subsets which are not involved into the pathology of MS leaving pathological switched memory B cells untouched (Baker et al., 2017). The presence of such pathological B cells may interpret the failure of some BAFF/APRIL-blocking agents in the management of MS.

Apart from BAFFR, Nogo is another BAFF-binding receptor that is expressed on neurons, inhibiting their outgrowth in response to BAFF binding (Vincent et al., 2013; Zhang et al., 2009) and may contribute to the correlation between increased BAFF levels and MS. Nogo-BAFF interaction may be relevant for CNS injuries and diseases, as they may be enhanced by increased local production of BAFF from astrocytes (Krumbholz et al., 2005; Vincent et al., 2013). Obviously, the precise role of Nogo-BAFF interaction in MS should be addressed in further studies.

Interestingly, there are no functional studies analyzing the contribution of BAFF/BAFFR polymorphisms in MS pathogenesis and/or phenotype, until now. As mentioned above, previous studies have demonstrated that the analyzed BAFFR polymorphisms P21R (rs77874543) and H159Y (rs61756766) have been associated with CVID, CLL and non-Hodgkin lymphoma pathogenesis (Hildebrand et al., 2010; Jasek et al., 2016; Losi et al., 2005; Pieper et al., 2014). Moreover, other variants of *BAFF* gene have been associated with other diseases with autoimmune pathophysiology. More precisely, the rs9514828, which is located in *BAFF* promoter region, has been associated with high levels of serum BAFF increasing the risk of NHL, CLL and hepatitis C-related mixed cryoglobulinemia (Ayad et al., 2015; Ferrer et al., 2009; Jasek et al., 2016; Lahiri et al., 2012), the rs1041569

has been associated with CLL risk (Jasek et al., 2016), while the AA genotype of the rs12583006 has been correlated with lupus and lupus related plaque formation (Theodorou et al., 2018). In addition, the rs2893321 seems to increase the risk for the development of Grave's disease (Lin et al., 2016) and the rs375946 has been associated with response to rituximab in patients with antineutrophil cytoplasmic antibody–associated vasculitis (Alberici et al., 2017).

A limitation of our study is the absence of data concerning the use of disease-modifying treatments, which have been reported to alter BAFF levels in blood of MS patients; particularly the use of INF-β is known to lead to a strong upregulation of BAFF in serum (Kannel et al., 2015). Obviously, further studies may clarify also this point.

Moreover, as presented in Table 1, we did not detect the H159Y/H159Y genotype in our cohort (both in MS patients and healthy controls). The latter could be attributed to the sample size that was genotyped, and it is a common phenomenon in candidate gene association studies examining low frequency variants (Rikos et al., 2019). Moreover, bearing in mind that there is no consensus method for measuring disease severity in MS (Roxburgh et al., 2005), we used the MSSS algorithm as a powerful method for comparing disease progression and severity. As presented, no significant associations were found between MSSS and BAFFR polymorphisms.

In conclusion, we report a significant association of the *TNFRSF13C/BAFFR*-H159Y polymorphism with MS in a cohort of Greek patients, suggesting that an imbalanced BAFF/BAFFR system increases the risk of developing MS. Since BAFF can also induce IL-10 by regulatory B cells (Yang et al., 2010) that were found to inhibit EAE development and progression (Matsushita et al., 2008), treatment of MS patients with the BAFF-neutralizing antibodies, as belimumab, may not only lead to the enrichment of potentially pathogenic switched memory B cells, but also to the depletion of disease-modifying regulatory B cells. Thus, treatment approaches interfering with the BAFF/BAFFR system are clearly less straight forward than originally anticipated and should integrate the complex role of BAFF and its receptors in B cell development and homeostasis.

Declaration of Competing Interest

All the authors declare no conflicts of interest associated with this manuscript.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.msard.2019.101422.

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