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Analysis of chosen SNVs in *GPC5*, *CD58* and *IRF8* genes in multiple sclerosis patients

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ABSTRACT

Purpose: Multiple sclerosis (MS) is an autoimmune disease of the central nervous system with a neurodegenerative compound. Heterogenetic background of autoimmunity pathway components has been suggested in the MS pathogenesis. The main aim of our study was to evaluate the association between selected polymorphisms of the*CD58*, *IRF8* and *GPC5* genes and treatment effectiveness in a group of relapsing-remitting MS patients. This is the first study of MS patients from Podlaskie Region in the Polish population.
Materials and methods: The study group comprised 174 relapsing-remitting MS patients diagnosed under 40 years of age. Genotyping was performed using ready to use TaqMan assays.
Results: We demonstrate a strong association of the polymorphisms with sex, age of onset and response to the treatment applied. A significant correlation was observed in the presence of allele T of rs10492503 polymorphism in*GPC5* gene with sex and age of MS onset. Logistic regression analysis revealed an increased risk of the interaction of rs17445836 in *IRF8* gene with male sex and the type of treatment (OR = 3.80, $p < 0.05$), and a decreased risk in the interaction of female sex with disease progress according to the EDSS scale (OR = -2.33, $p < 0.05$).
Conclusions: The analysis of the correlation between different alleles, genotypes and clinical status confirmed the interaction between the genetic factors of age of onset and response to therapy. The study suggests that genetic variants in*GPC5*, *CD58* and *IRF8* genes may be of clinical interest in MS as predictors of age of onset and response to therapy.

1. Introduction

Multiple sclerosis (MS) is a chronic demyelinating and inflammatory disease leading to neurodegenerative destruction of the central nervous system. The autoimmune background of this disease has been commonly postulated [1,2]. The prevalence of MS varies between 2 and 160 per 100,000 individuals in different countries and ethnic groups [3]. MS is the most common neurological disease among young people, with onset between 20 and 40 years of age [4]. In most patients, the disease begins as an episodic disorder and evolves over time into a progressive one. The reported models of the pathogenesis support the occurrence of two overlapping and connected effector arms — inflammatory and neurodegenerative [5]. Bursts of focal inflammation are thought to underlie the episodic, relapsing–remitting phase of MS, whereas axonal loss and neurodegeneration are responsible for progressive symptoms, which are the predominant cause of disability [6,7].

The etiology of MS remains elusive, but has been suggested to be affected by both environmental and genetic factors and their complex interaction. A large number of studies suggest a multifactorial etiology on the basis of genetic susceptibility [8]. The human leukocyte antigen (HLA) locus on chromosome 6p21 is an important genetic factor of MS risk [9–11]. In the past decade, several reports, including some from independent genome wide association studies (GWAS), have identified the association between MS risk and the single nucleotide variants (SNVs) of several non-HLA genetic loci, including *CD58*, *IL2RA*, *JAK2*, *IRF8*, *GPC5*, etc. [12–14]. In this paper, we focus on *IRF8, CD58* and *GPC5* genes and their correlation with the MS development and

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response to MS therapy.

CD58 gene encodes a ligand for the T-cell specific CD2 membrane molecule, an adhesion molecule that forwards important signals for T-cell proliferation and differentiation [15]. In MS patients, the control of activated T-cells by $CD4^+CD25^+$ regulatory T-cells is damaged; therefore, the *CD58* gene polymorphisms have been an appealing target when considering the function of genetic mutation in the immune system dysfunction related to MS [16,17].

IRF8 is a transcription factor of the interferon (IFN) regulatory factor (IRF) proteins family. These proteins are composed of a conserved DNA-binding domain in the N-terminal region and a divergent C-terminal region that serves as the regulatory domain. IRF family proteins bind to the IFN-stimulated response element (ISRE) and regulate the expression of genes stimulated by type I IFNs, namely IFN-alpha and IFN-beta. The IRF control the expression of IFN-alpha- and IFN-beta-regulated genes that are induced by viral infection [18] and are specifically expressed in immune cells. Upregulation of interferon responses has been noted in peripheral blood of a subset of untreated MS patients [19,20]. However, the role of interferons in the onset of MS is still unclear. Results of a recently performed expression study show that the susceptibility allele near *IRF8* is associated with a higher mRNA expression of interferon-response pathway genes in subjects with MS [21].

The *GPC5* gene codes a glypican 5, a type of heparan sulfate proteoglycan that fulfills signaling functions in the extracellular matrix. Northern blot analysis revealed that the gene is expressed as a 3-kb message in the brain and several other human tissues [22]. Although its exact mechanisms are still unknown, glypicans have been shown to contribute to neuronal development and function [14].

A number of genetic polymorphisms have been identified and analyzed in the three genes: rs2300747 in *CD58* gene, rs17445836 and rs13333054 in *IRF8* gene and rs10492503 in *GPC5* gene [21,23]. All those variants were previously described as major factors in pharmacogenomics studies and their role in the response to interferon β and glatiramer acetate therapy in MS patients in different populations was discussed [21,23,24]. Pharmacogenomics investigates the impact of the genome on the response to pharmacotherapy [25].

The main aim of our study was to confirm and understand the potential role and association of the selected polymorphisms of *CD58*, *IRF8* and *GPC5* genes with not only the therapeutic approach but also different clinical MS data of our relapsing-remitting MS (RRMS) groups of patients. This is the first study of MS patient population living in Eastern Poland.

2. Materials and methods

The study population consisted of unrelated 174 patients (124 women and 50 men) with clinically defined RRMS according to McDonald criteria [26]. All of them were diagnosed under 40 years of age and treated with interferon β (a/b)(32%/31%), glatiramer acetate (22.4%), natalizumab (6.3%) or fingolimod (5.7%). In the present study participants the age at disease diagnosis is the same as the age of onset. Chosen clinical features of all MS patients, men and women separately, are presented in Table 1.

In Poland, only patients meeting the National Health Fund (NFZ) inclusion criteria can apply for the NFZ funded treatment as a part of the Multiple Sclerosis Drug Prescription Treatment Programs. There are two MS treatment programs - first and second line. Participants of the study took part in the first line MS treatment program and were treated with interferon beta preparations. Inclusion criteria were: 18 years of age or older, diagnosis of RRMS based on the McDonald criteria [26], incidence of at least one relapse or at least one new GD + focus (MRI) within 12 months before the qualification to the study. Exclusion criteria were as described in the Summary of Product Characteristics (SmPC).

All participants of the study were examined by a neurologist every 3 months and had an MRI with contrast every 12 months. After each 12 months of therapy the efficacy of treatment was assessed. If there were relapses and new active demyelinating lesions then the treatment was changed to second-line drugs (natalizumab, fingolimod). If there were only relapses or only new active lesions in the MRI then the treatment was changed to glatiramer acetate.

All the patients included in the study were assessed according to the Expanded Disability Status Scale (EDSS) at the beginning of the therapy and then every 3 months. The change in the EDSS scale was defined as an increase or decrease in the EDSS score by 1 point sustained over 3 months not associated with a relapse. An increase in the EDSS score represented disability progress and worsening of symptoms in the MS patients. A decrease in the EDSS score represented partial improvement of neurological symptoms.

2.1. Ethics issues

The study was approved by the local bioethics committee in Medical University of Bialystok (approval number: R-I-002/71/2016). All the study participants signed informed consent.

2.2. DNA extraction and SNV analysis

DNA was extracted from the peripheral whole blood leukocytes using two extraction methods, the salting-out and the exchange membrane method column separation (QIAamp DNA Blood Mini Kit, Qiagen, Germany).

The single nucleotide variants (SNV) analysis with the allelic discrimination technique was performed using the 7900 H T Fast Real-Time PCR System (Applied Biosystems, USA). All SNVs (rs2300747, rs17445836, rs13333054 and rs10492503) were genotyped by fluorogenic TaqMan technology from ready to use human assays library (Applied Biosystems, Foster City, CA, USA). No significant deviation from Hardy–Weinberg equilibrium was observed for the SNVs studied.

2.3. Statistical analysis

To assess whether there was a relationship between the genotype or allele occurrence and selected feature median unbiased estimator (mid-p) of odds ratio, the exact confidence interval and associated p-value, both obtained with the use of the mid-p method were used. Generalized Linear Models with the logit or Gaussian link functions were constructed in order to determine the dependency between the selected features and chosen outcome variables (e.g. change in EDSS) with potential confounders taken into consideration (e.g. patient's age).

To determine whether there were statistically significant differences

Table 1
Chosen clinical features of MS patients.

Characteristics	MS patients, n = 174	Women, n = 124	Men, n = 50
Age at onset (years), mean ± SD	41.14 ± 0.79	42.78 ± 0.98	37.14 ± 1.11
Disease duration (years), mean ± SD	8.12 ± 0.42	8.18 ± 0.51	7.96 ± 0.75
EDSS[a] (mean ± SD)	1.85 ± 0.10	1.84 ± 0.12	1.87 ± 0.19

[a] Expanded Disability Status Scale.

Table 2
Generalized Linear Models with the disease onset set as the dependent variable with 95% confidence intervals for models' coefficients.

Predictors	Women	Men
rs2300747	P = 9.45e-01	P = 2.27e-01
	95% CI: (-5.71; 5.32)	95% CI: (-3.53; 15.28)
rs10492503	P = 6.11e-01	**P = 1.93e-02**
	95% CI: (-0.85; 4.17)	**95% CI: (0.91; 8.61)**
rs17445836	P = 6.71e-01	P = 7.73e-01
	95% CI: (-2.58; 4.01)	95% CI: (-3.08; 4.15)
rs13333054	P = 9.93e-01	P = 3.79e-01
	95% CI: (-3.44; 3.41)	95% CI: (-4.97; 1.87)
rs2300747	P = 9.05e-01	P = 9.42e-01
rs10492503	95% CI: (-5.93; 5.25)	95% CI: (-1.15; 16.95)
rs17445836	P = 6.17e-01	**P = 7.83e-03**
rs13333054	95% CI: (-2.31; 3.90)	**95% CI: (1.62; 9.31)**
	P = 6.66e-01	P = 3.66e-01
	95% CI: (-2.73; 4.27)	95% CI: (-1.93; 5.31)
	P = 8.27e-01	P = 2.42e-01
	95% CI: (-4.09; 3.27)	95% CI: (-5.53; 1.36)

between the groups of interest, either one-way analysis of variance model [27] was fitted or non-parametric approach was applied (Wilcoxon rank-sum test [28] or Kruskal-Wallis test [29]). The choice of an appropriate method was made upon fulfilling the normality and the homogeneity of variance assumptions and in case of violation of at least one of the conditions, the non-parametric approach was employed. The normality of features' distribution was checked with the Shapiro-Wilk test [30] and the homogeneity of variances with the Levene's test [31]. The R software environment [32] was used for all calculations and the significance level alpha equal to 0.05 was accepted.

3. Results

Analysis of the selected polymorphisms in *CD58*, *IRF8* and *GPC5* genes in MS patients and the correlation between their presence and different variables (sex, age, length of treatment, type of medication taken, drug change, changes in the EDSS score) clearly showed that sex is the only determinant of any difference in MS patients. The female gender was the main factor for the differences noted within the EDSS scale and the age of onset. However, in the group of men with MS all the polymorphisms studied correlated with some variables, such as age, and length and type of treatment.

3.1. Polymorphisms vs clinical MS data

The mean age of disease onset is a differentiating factor only in women with MS and affects the drug change depending on the presence of specific alleles within the examined SNP. This trend was not demonstrated in MS men.

For the *IRF8* gene polymorphism rs17445836 we found an association between the presence of risk allele and the age of onset in the female MS patients. Patients, who became ill at the age of 37 with the allele risk A were statistically significantly more likely to switch from interferon beta 1b to glatiramer acetate than patients with normal G alleles who were diagnosed at younger age (approx. 22 years) and whose interferon beta 1b was changed to natalizumab ($p < 0.006$). We found no evidence for alleles of this SNV distribution among the male MS patients. We observed in the second SNV of *IRF8* gene (rs13333054) that in women with a later disease onset (37 years) and normal C allele polymorphism, rs13333054 was statistically significantly more likely to have changed interferon beta 1b to glatiramer acetate as compared to patients with earlier disease onset (approx. 22 years) and showed both normal C alleles and risk alleles T, but who had interferon beta 1b changed to natalizumab ($p < 0.002$, $p < 0.01$). We found no evidence for allele distribution in the male MS group. The results of this analysis are presented in Supplementary Tables S1 and S2.

We observed a strict correlation between the occurrence of allele T of rs10492503 polymorphism of *GPC5* gene, sex and age of onset in MS patients. Male patients with allele T were diagnosed earlier than female patients with the same allele (28 ± 0.94 vs. 34.4 ± 0.84, $p < 0.01$) and both female and male patients with ancestral allele A ($p < 0.01$, $p < 0.05$). No statistically significant association was found between the clinical characteristics, such as the scheme of treatment and the EDSS score changes. This may suggest that the allele T of rs10492503 polymorphism of *GPC5* gene is a strong factor of early-onset MS in male patients.

Our study additionally revealed a relationship between the presence of risk allele G polymorphism rs2300747 in *CD58* gene, sex and the length of treatment. Women who had the risk allele G were diagnosed later and treated shorter than men with normal allele A in whom the disease was diagnosed earlier (34 ± 2.5 vs 29 ± 0.8, $p < 0.04$) but who were treated for longer (39 ± 7.8 vs. 62 ± 3.8 months, $p < 0.01$). This may suggest that risk allele G predisposes females to develop MS over the age of 30 and to have shorter treatment to achieve a therapeutic effect as compared to men with normal allele A and the age of onset under 30 but with longer MS treatment.

Additionally, we observed a correlation between wild-type allele G in rs17445836 in *IRF8* gene, sex and the age of onset. Men with allele G were diagnosed with MS earlier than those with the same allele (29 ± 0.98 vs 35 ± 0.78, $p < 0.0004$) and risk allele A (29 ± 0.98 vs 34 ± 0.5, $p < 0.03$). The analysis of the second SNV in this gene (rs13333054) shows an association between the presence of risk allele T and the age of MS onset. In the group of men with allele T the age of onset was statistically significantly lower than in women with wild-type allele C (27 ± 1.98 vs 33 ± 0.8, $p < 0.003$) and risk allele T (27 ± 1.89 vs 34 ± 1.44, $p < 0.01$) (Table 2).

3.2. Logistic regression

In additive model of inheritance we analyzed all data of patients (age, sex, EDSS) with MS and SNP. We showed a statistically significant association between rs17445836 of *IRF8* gene in men with MS and the MS treatment, but not in MS women. The presence of a risk allele A is associated with an increased chance for men to change the treatment from interferon 1a/b to glatiramer acetate (OR = 3.80, $p < 0.05$, CI 95%) (Table 3). Additionally, regardless of the current SNP polymorphism, age of disease onset and other variables analyzed, a correlation with the change in the EDSS score was noted only in the group of MS women, compared to MS men (OR = -2.33, $p < 0.05$, CI 95%). In addition, we found an association between the change in the EDSS score and the treatment applied in the group of MS women alone. In the

Table 3
Generalized Linear Models with changing treatment (interferon 1a/b, glatiramer acetate, natalizumab) set as the dependent variable with odds ratios and corresponding 95% confidence intervals.

Predictors	Women	Men
rs2300747	P = 5.63e-01	P = 4.13e-01
	OR = 0.71 (0.23; 2.47)	OR = 0.30 (0.01; 8.12)
rs10492503	P = 1.41e-01	P = 8.82e-02
	OR = 0.58 (0.27; 1.16)	OR = 3.21 (0.91; 14.83)
rs17445836	P = 4.59e-01	P = 7.68e-02
	OR = 1.34 (0.63; 3.08)	OR = 3.19 (0.97; 13.25)
rs13333054	P = 6.10e-01	P = 9.36e-01
	OR = 0.82 (0.36; 1.74)	OR = 0.96 (0.30; 2.50)
rs2300747	P = 6.82e-01	P = 6.20e-01
rs10492503	OR = 0.78 (0.24; 2.80)	OR = 0.45 (0.01; 14.98)
rs17445836	P = 1.55e-01	P = 8.35e-02
rs13333054	OR = 0.58 (0.27; 1.19)	OR = 4.15 (0.91; 26.69)
	P = 3.10e-01	**P = 4.51e-02**
	OR = 1.54 (0.69; 3.74)	**OR = 3.80 (1.11; 15.99)**
	P = 5.89e-01	P = 3.80e-01
	OR = 0.79 (0.33; 1.81)	OR = 0.61 (0.18; 1.75)

female MS group treated with glatiramer acetate, statistically significantly more often the EDSS score was altered as compared to women treated with interferon 1a/b (OR = -2.37, $p < 0.003$, CI 95%).

4. Discussion

Genome-wide association studies identified hundreds of potential genetic risk loci associated with numerous autoimmune diseases such as MS. Genes discovered by GWAS are now the focus of numerous ongoing studies. According to our knowledge, this is the first study to report the evidence of association of polymorphisms analyzed in *GPC5*, *CD58* and *IRF8* genes with gender and age of MS onset.

Previous studies on the rs2300747, rs10492503, rs17445836 and rs13333054 polymorphisms in the MS patients revealed their positive correlation with the response to interferon or glatiramer acetate treatment. It was Sellebjerg et al. [33] who conducted a prospective study in a group of Danish patients and found no relationship between rs13333054 and rs17445836 in *IRF8* and rs10492503 in *GPC5* gene and with clinical disease activity in MS patients beginning *de novo* treatment. These authors evaluated mainly patients' response to first-line treatment with interferon β and found no correlation between the two SNVs in the *IRF8* gene and clinical response to interferon β. These polymorphisms did not increase the risk of MS, either [33]. Our study, however, showed that the presence of the risk allele A at rs17445836 in a group of men and in a group of women with MS with the disease onset after the age of 35 has a negative impact on interferon β treatment. These patients responded more poorly to interferon β and had to switch to glatiramer acetate more frequently. In the study by Sellebjerg et al. [33], the analysis of the rs10492503 polymorphism in *GPC5* gene did not confirm its relationship as a response predictor in the interferon β therapy, which had been reported by other authors [14,23]. Our research also failed to find this association, which may be due to the fact that we did not take into consideration the duration of the interferon β therapy alone and the subsequent switch for another drug. There are undoubtedly some differences in the genotype distribution – their penetration in the European populations – in the group from Spain in both studies [14,23], in the Danish group [33] and in our present Polish group. Nevertheless, the assessment of rs10492503 polymorphism in *GPC5* gene in our study revealed a correlation between the presence of the risk allele T, sex and the age of disease onset. The presence of the risk allele T in male MS patients was associated with the disease onset under the age of 30 as compared to women with the same allele, who were diagnosed over the age of 30. This may suggest that allele T is a potent factor of younger age of MS onset in male patients. Until now, no such correlation has been described in the literature. This is an interesting and unanticipated observation taking into consideration that women get affected by autoimmunity-based diseases 7–10 times more often than men, probably due to the immunomodulatory role of oestrogens [34].

A study by Bashinskaya et al. [35], analysing rs13333054 and rs17445836 polymorphisms in *IRF8* gene and rs2300747 in *CD58* gene, showed a strong correlation of these polymorphisms with gender in a population of Russian MS patients. A specific effect of the presence of genotype rs17445836*G/G of *IRF8* gene was found only in a group of women with MS (but only in combination with another polymorphism in *TNFRSF1A* gene), whereas rs2300747*A/A of the *CD58* gene was present only in a group of men with MS (but only in combination with another polymorphism in the *IL7RA*) [35]. Similar results concerning the genotype-sex relationship were reported by Baranzini et al. [36] and Sawcer et al. [37] in GWAS studies. Their findings are in contradiction to our observations. We revealed not only the relationship between the presence of the risk allele G polymorphism rs2300747 and gender, in the group of MS women, but also an association with the treatment duration. The risk allele G was a factor affecting the morbidity among women over the age of 30 and contributing to a shorter treatment allowing for positive therapeutic effect as compared to male patients with normal allele A, who fell ill under the age of 30 but were treated longer. Differences in the current analyses may result from the number of patients in the study group and a smaller number of polymorphisms in our study. It seems likely that a larger study group, recruiting healthy volunteers or adding polymorphisms in genes *TNFRSF1A* and *IL7RA* would allow for similar results.

Our last observation results from the fact that the analysis of polymorphisms in the group of patients with MS and an attempt to search for mutual relationships between their presence against many variables, including: sex, age of onset, treatment duration, type of administered drugs, a switch from one drug to another and disability progress according to the EDSS scale, showed evidently that female gender was the only factor determining any differences in the group of patients with MS. The EDSS scale determines the possibility of patient's inclusion to therapy using first- or second-line drugs and to clinical investigations, and assesses patient's neurological impairment and disability. Only in the female MS patients, the disease progressed in the form of changes in the EDSS score much more frequently. Additionally, in our study the female MS patients were less likely to respond to the first-line drug, i.e. interferon, and often due to disease progress, assessed with the EDSS scale, required the administration of glatiramer acetate at earlier stages of therapy. We found no available literature data that would describe such correlations with female gender.

4.1. Study limitations

Pronounced discrepancies observed in numerous studies in differentiated populations can be explained by a lower statistical power of the results due to insufficient number of experimental samples and population stratification. Difficulty in the repetition of GWAS results can be due to non-homogeneity in the genetic structure of the disease studied [38,39]. Moreover, it is likely that the relationship between certain SNVs is stronger in some populations and weaker in others. This may depend on the allele frequency, genome location on chromosomes, various linkage disequilibrium patterns in the populations, various evolutionary histories of genes affecting complex diseases [38]. We are aware of the fact that the presented findings can be contradictory to earlier published investigations. However, we attempt to launch a cooperation with other centres in Poland to increase the study group. It seems that the benefits of this type of study are huge, since identification of certain molecular variants in a group of MS patients could contribute to more precise selection of treatment depending on the age of onset and sex.

5. Conclusions

The analysis of the correlation between different alleles, genotypes and clinical status confirmed the interaction between the genetic factors of age of onset and response to therapy. The study suggests that genetic variants in *GPC5*, *CD58* and *IRF8* genes may be of clinical interest in MS as predictors of age of onset and response to therapy.

Conflict of interests

The authors declare no conflict of interests.

The author contribution

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.advms.2018.12.004.

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