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Analysis of the relationship between single nucleotide polymorphism of the CD209, IL-10, IL-28 and CCR5 D32 genes with the human predisposition to developing tick-borne encephalitis

Analiza związku polimorfizmu pojedynczego nukleotydu w zakresie CD209, IL-10, IL-28 i CCR5 D32 z predyspozycją do rozwoju kleszczowe zapalenie mózgu

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
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Summary

Introduction: It is known that in the pathogenesis of tick-borne encephalitis (TBE) various molecules play a significant role. The most prominent factors include IL-10, IL-28B, CD-209 and CCR5. It is reasonable to search for genetic predispositions to the development of various clinical forms of TBE related to the genetic variation of IL-10, IL-28B, CD-209 and CCR5. In this study we aimed to search for the relationship between single nucleotide polymorphism in the promoter region of the CD209, IL-10, IL-28 and 32 base pair deletion in CCR5 coding region (Δ 32) with the human predisposition to development of various clinical presentations of TBE. We tried to assess the relation between the presence of particular alleles and genotypes with laboratory and clinical parameters.

Material/Methods: 59 patients with TBE and 57 people, bitten by a tick who never developed TBE (Polish cohort), were included in the study. To assess the distribution of single nucleotide polymorphisms, TaqMan SNP genotyping assays were used for IL10: rs1800872 and rs1800896, for CD 209 rs4804803 and rs2287886, rs12979860 for IL 28B SNPs according to the manufacturer's protocol using real-time PCR technology on the StepOne thermal cycler.

Results: Comparison between TBE patients and CG showed that in SNP rs2287886 CD 209 AG heterozygotes were more frequent in the TBE group, while homozygotes GG were more frequent in the CG group.

Conclusions: SNP rs2287886 CD 209 AG heterozygotes predispose humans to develop TBE. Single nucleotide polymorphism in the promoter region of the CD209, IL-10, IL-28 and CCR5 D32 genes does not correlate with the severity of TBE.

Keywords: **CD209 • IL-10 • IL-28 • CCR5 D32 • polymorphism • tick-borne encephalitis**

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Introduction

Tick-borne encephalitis (TBE) is a disease caused by the tick-borne encephalitis virus (TBEV) belonging to the Flaviviridae family. Since 1993 there has been an increase in the incidence of TBE in Poland, especially in the Eastern regions of the country (Warmia and Mazury and Podlasie region), which is an endemic area. In 2013, 225 cases have been reported, with 111 (49%) noticed in the Podlaskie province [22].

A wide variety of clinical TBE presentations have been observed, including meningitis, meningoencephalitis, meningoencephaloradiculitis, and meningoencephalomyelitis. This disease may also cause long-term sequeale, which may last for months to years, mainly pareses, ataxia, and other gait disturbances [3,9,29]. Despite the wide availability of vaccination, the disease poses a significant clinical and epidemiological threat.

It is still not known, why some patients develop clinically severe condition, while others present with mild or asymptomatic disease. It may be hypothesised that human genetic variability may play a significant role in the severity of the disease and it seems reasonable to search for genetic markers, predisposing to a specific response to infection with TBEV. Genetic association study could allow for prediction of the severity of the clinical course and development of serious neurological complications. Additionally, targeted treatment interventions could be implemented.

It is known that in the pathogenesis of TBE an array of molecules play a significant role. The most prominent factors include IL-10, IL-28B, CD-209 and CCR5 [7,8].

In literature numerous reports have been published on the role of genetic IL-10 and IL-28 polymorphisms in hepatitis C (also caused by virus belonging to Flaviviridae) susceptibility and treatment response; however, reports on the influence of genetics on the course of TBE are sparse. Differences in the clinical course of infection with West Nile virus (the virus also belongs to the Flaviviridae) in patients with a deletion of 32 base pairs in the coding region of CCR5 have been reported [10,18].

Considering that the hepatitis C virus and West Nile virus belong to the same family as the TBE virus and WNV causes similar symptoms as TBE virus, it is reasonable to search for genetic predispositions to the development of various clinical forms of TBE related to the genetic variation of IL-10, Il-28B, CD-209 and CCR5.

Aims

1. Describe the relationship between single nucleotide polymorphism in the promoter region of the CD209, IL-10, IL-28 as well as 32 base pair deletion in CCR5 coding region (Δ 32) with human predisposition to the development of various clinical presentations of tick-borne encephalitis.

2. Establish a relation between the presence of particular alleles and genotypes with laboratory and clinical parameters.

Materials and methods

The study included 59 Polish patients (22 women, 37 men) aged 18-80, mean age 47±18.9 years with a history of TBE (European virus subtype) treated in the Department of Infectious Diseases and Neuroinfections, Poland in the year 2012 and 2013. None of the patients had been vaccinated against TBE and all of them had a history of tick bites. Diagnosis was made on the basis of clinical manifestation, cerebrospinal fluid (CSF) examination and the presence of serum specific antibodies and CSF (Enzygnost Anti-TBE/FSME Virus [IgG, IgM] Siemens test). As far as the severity of the disease is concerned, patients were divided into two groups: TBE 1 - 26 patients with meningitis with no neurological symptoms; TBE 2-33 patients with meningoencephalitis or meningoencephalomyelitis.

29 patients (49.1%) reported prodromal phase of the disease. The mean pleocytosis in CSF was 135.5±192 cells/mm^3 and the mean protein concentration was 62.5±24.8 mg/dl. The mean time of hospitalization was 16.9±4.1 days.

Patients voluntarily agreed to participate in the study and gave their written informed consent. The study was approved by the Local Ethical Committee.

Control group

The control group (CG) consisted of 57 patients after tick bite, who had not developed TBE. Patients were admitted to the Department because of suspicion of TBE. Based on laboratory tests results and cerebrospinal fluid examination, TBE was excluded.

Genotyping

Blood samples for molecular genetic studies were collected in tubes containing EDTA as anticoagulant and stored at -80° C. QIAamp DNA Blood Mini Kit (QIAgen, Hilden, Germany) was used to extract genomic DNA from all samples. The extraction was performed following the manufacturer's protocol, DNA was resuspended in 200 µL of AE buffer (QIAgen, Hilden, Germany) and stored at 4°C for further analyses. To assess the distribution of single nucleotide polymorphisms, TaqMan SNP (Life Technologies, USA) genotyping assays were used for Il10: rs1800872 and rs1800896, for CD 209 rs4804803 and rs2287886, rs12979860 for IL 28B SNPs according to the manufacturer's protocol using real-time PCR technology on the StepOne thermal cycler (Applied Biosystems/Life Technologies, Foster City, CA).

Genotypes were identified using TaqMan Genotyper Software v1.0.1 (Applied Biosystems/Life Technologies, Foster City, CA), calculation of Hardy-Weinberg equilibrium for each analyzed set of genotypes was performed by this software. To analyze CCR5 Δ32 (rs 333) variation, PCR with sequence specific primers were used according to the previously described PCR technique [11]. Visualization under UV light was performed after electrophoresis on the 2.5% agarose gel (SIGMA, Saint Louis, USA) stained with DNA-star dye (Lonza Inc, Rockland, USA).

Laboratory tests

The inflammatory parameters in the peripheral blood (erythrocyte sedimentation rate – ESR, C-reactive protein concentration, leukocyte and platelet count) and in cerebrospinal fluid (CSF) (pleocytosis, protein and albumin concentration) were measured with standard laboratory techniques, on admission (examination 1) and after 12-16 days, in the early convalescent period (examination 2). In some patients CSF was examined also in the late convalescent period 6 weeks after admission (examination 3) (Table 1).

Table 1. Laboratory test results of TBE patients

	Examination 1 (at admission)		Examination 2 (2 weeks after the ex.1)		Examination 3 (4 weeks after the ex.2)	
	Mean	SD	Mean	SD	Mean	SD
Serum						
ESR (mm/h)	32.72	20.38	-	-	-	-
CRP (mg/dl)	12.3	14.6	1.7	2.1	-	-
WBC (10^3/µl)	10.7	4.6	9.4	6.3	-	-
Lymphocytes	1.96	3.17	3.76	6.01	-	-
PLT (tys/µl)	197.72	52.27	230.8	63.4	-	-
CSF						
Pleocytosis (cells/ml)	135.5	192	55.93	29.21	22.21	17.85
Mononuclear cells (cells/ml)	91.66	120.57	52.29	29.53	18.7	16.22
Neutrophil cells (cells/ml)	44.5	152.9	3.8	13.93	3.5	10.95
Lymphocytes (cells/ml)	81.5	120.95	49.66	29.8	28.1	14.8
Monocytes count (cells/ml)	15.17	16.27	4.43	3.61	4	7.24
Protein concentration (mg/dl)	62.5	24.82	65.37	30.74	47.89	24.46
Albumin concentration (mg/dl)	43.88	19.63	42.89	20.6	33.91	18.7

Statistical analysis

Statistical analysis was performed using Statistica 10 Statasoft, Poland.

SNP allelic and genotypic frequencies (the portion of chromosomes with a particular allele and the portion of individuals with a particular genotype, respectively) were compared between groups by χ^2 and for continuous variables by Mann-Whitney test.

The link between the presence of particular alleles with laboratory and clinical parameters was assessed with U Mann-Whitney test. The comparison between different IL28B, CCR5, IL10 and CD209 genotypes was performed with Kruskall-Wallis ANOVA and significant differences verified with two-side comparisons.

P value <0.05 was considered statistically significant.

Results

Genotypic frequencies for these SNPs were calculated for each of the different TBE patients groups (severe CNS disease, meningitis) and for control group (patients after tick bite, who did not develop TBE) (Table 2).

No statistically significant differences were found in genotype frequencies between the patients with severe TBE and patients with meningitis for any of the SNPs.

Comparison between TBE patients and CG showed that in SNP rs2287886 CD 209 AG heterozygotes were more frequent in TBE group (p<0.05) while homozygotes GG were more frequent in CG group (p<0.05) (Table 2).

Also patients with a combination of AG genotypes in either rs2287886 or rs4804803 were more frequent in TBE group (result on the edge of statistical significance p=0.06). As far as other examined SNPs are concerned, the only difference between TBE and CG groups was observed in only for ILB28 CT heterozygotes (p=0.09) (Table 2).

Analysis of other relations

CCR5

Specific IgG antibody titers on admission tended to be higher in wild type homozygotes than in *CCR5D32* heterozygotes, which was statistically significant in CSF (p<0.05). The mean total CSF pleocytosis, lymphocyte and monocyte count in the examination 2 was about two-fold higher in *CCR5D32* heterozygotes, suggesting delayed resolution of the inflammatory infiltrate, but the difference was not statistically significant.

IL28B

The presence of at least one T allele correlated with lower CSF IgG anti-TBEV antibody titer, higher total and mononuclear pleocytosis and higher CSF protein and albumin in examination1, as well as higher CSF neutrophils in examination 2.

CT heterozygotes had a higher maximum body temperature than TT homozygotes.

CT heterozygotes had a higher CSF total and mononuclear pleocytosis and higher CSF protein and albumin concentration than CC homozygotes.

The presence of T allele and CT genotype were linked to more pronounced intrathecal inflammatory response, but delayed intrathecal IgG response and possibly less intense peripheral inflammation (a non-significant tendency for lower ESR, CRP and leukocytosis).

IL10

IL10 rs1800872

There was a higher blood lymphocyte count in patients with at least one T allele and lower in patients with at least one G allele (in examination 1). There were only two TT homozygotes in the study group, both with untypically high lymphocytosis on admission (2200-2300/ml), and the difference between them and GG homozygotes was statically significant (p<0.05).

IL 10 rs1800896

There was lower leukocytosis and lymphocytosis in patients with at least one C allele, significantly in examination 2 (in examination 1 for leukocytosis p = 0,0504). CC homozygotes had significantly higher median lymphocytosis in examination 2 than TT homozygotes and TC heterozygotes (p < 0,05).

CD209

CD209 rs2287886

There was a tendency for higher total blood leukocytosis and lymphocytosis in patients without G allele (AA homozygotes) in comparison with the rest of the group, but it was not statistically significant with the non-parametric test.

Patients with G allele tended to present with higher CSF pleocytosis in examination 1, significant for CSF neutrophil count (50 vs 10/mm^3).

AA homozygotes had lower median neutrophil CSF count than GG homozygotes (p< 0.05) and GA heterozygotes (p< 0.01), which did not differ between themselves.

There was a tendency for higher specific IgG serum titers in heterozygotes than in both AA and GG homozygotes, but it was not significant (all p between 0.06 and 0.09).

Table 2. Genotype frequencies for the CD209 rs2287886, CD 209 rs4804803, IL 10 rs1800872, IL 10 rs1800896, CCR5Δ32 rs 333, IL B28 rs12979860 single nucleotide polymorphisms (SNPs) in tick-borne encephalitis (TBE) patients with different clinical manifestations and control group (CG)

	CG	TBE	p	TBE1	TBE2	p
CD 209 rs2287886						
AA	9 (15.8%)	9 (15.3%)	0.9	3	6	0.49
AG	21 (36.8%)	34 (57.6%)	0.02	14	20	0.61
GG	27 (47.4%)	16 (25.4%)	0.02	9	7	0.25
CD 209 rs4804803						
AA	38 (66.7%)	38 (64.4%)	0.8	15	23	0.34
AG	14 (24.6%)	19 (32.2%)	0.36	10	9	0.37
GG	5 (8.8%)	2 (3.4%)	0.22	1	1	0.88
IL 10 rs1800872						
TT	1 (1.8%)	2 (3.4%)	0.59	1	1	0.88
TG	22 (38.6%)	18 (30.5%)	0.36	10	8	0.25
GG	34 (59.6%)	39 (66.1%)	0.48	15	24	0.23
Il 10 rs1800896						
TT	13 (22.8%)	12 (20.3%)	0.82	5	7	0.89
TC	32 (56.1%)	28 (47.5%)	0.42	14	14	0.46
CC	12 (21.1%)	19 (32.2%)	0.3	7	12	0.53
			IL10 (rs1800872+ rs1800896)			
TT/TT	1 (1.8%)	2 (3.4%)	0.58	1	1	0.86
TT/TC	0	0		0	0	
TT/CC	0	0		0	0	
TG/TT	5 (8.8%)	6 (10.2%)	0.8	3	3	0.76
TG/TC	17 (29.8%)	12 (20.3%)	0.24	7	5	0.26
TG/CC	0	0		0	0	
GG/TT	7 (12.3%)	4 (6.8%)	0.31	1	3	0.43
GG/TC	15 (26.3%)	16 (27.1%)	0.97	7	9	0.98
GG/CC	12 (21.1%)	19 (32.2%)	0.4	7	12	0.44
			CD209 (rs2287886 + rs4804803)			
AA/AA	9 (15.8%)	9 (15.3%)	0.94	3	6	0.48
AA/AG	0	0		0	0	
AA/GG	0	0		0	0	
AG/AA	15 (26.3%)	20 (33.9%)	0.37	7	13	0.32
AG/AG	6 (10.5%)	14 (23.7%)	0.06	7	7	0.61
AG/GG	0	0		0	0	
GG/AA	14 (24.6%)	9 (15.3%)	0.21	5	4	0.45
GG/AG	8 (14%)	5 (8.5%)	0.34	3	2	0.45
GG/GG	5 (8.8%)	2 (3.4%)	0.22	1	1	0.86
			CCR5Δ32 rs 333			
wt/wt	42 (73.6%)	49 (82.1%)	0.2	23	26	0.33
wt/Δ32	15 (26.4%)	10 (17.9%)	0.2	3	7	0.33
			IL B28 rs12979860			
CC	27 (47.4)	20 (33.9%)	0.14	10	10	0.51
CT	20 (35.1%)	30 (50.8%)	0.09	13	17	0.91
TT	10 (17.5%)	9 (15.3%)	0.74	3	6	0.48

TBE1 - meningitis
TBE2 – meningoencephalitis or meningoencephalomyelitis

CD209 rs4804803

A lack of G allele correlated with higher lymphocytosis in examination 1 ($p<0.01$).

AA homozygotes had higher median examination 1 lymphocytosis than AG heterozygotes. Only two patients had GG genotype, which coincided in both cases with GG in rs2287886 position.

CD209 rs2287886/CD209 rs4804803

Patients with AA/AA and AG/AA genotypes had higher median lymphocytosis than AG/AG group, and other differences between CD209 genotypes were not significant.

Total pleocytosis tended to be lower in GG/AG and GG/GG genotypes than in AA/AG, AG/AG and GG/AA, with a two-fold median difference and AA/AA genotype having intermediate median value. The difference between GG/AG and AA/AG, AG/AG and GG/AA combinations was significant ($p<0.05$).

Patient with AG/AG and AA/GG genotypes had higher mononuclear pleocytosis than patients with GG/AG genotypes. Patients with AA/AA tended to have lower neutrophil pleocytosis than was the case with other allele combinations, but the only significant difference was in the AA/AG group ($p<0.05$). Patients with AG/AA had higher monocyte pleocytosis than patients with GG/AG. AG/AG and GG/AA genotypes exhibited a tendency for a still higher number of monocytes in comparison with AG/AA, AA/AA and GG/AG, but below a level of statistical significance (p between 0.06-0.07).

Discussion

So far no study investigating correlations of SNPs and TBE was performed in the Polish population. There are differences between the results of studies performed in the European and Siberian patient populations, which may stem from a different genetic background or different properties of the Western (European) and Siberian subtype of TBEV [2,10].

CD209, a c-type lectin expressed by dendritic cells (DCs), acts as a pathogen recognition receptor. Dendritic cells in the skin and intestine probably play an important role in the early stages of TBEV infection, acting as a source of pro-inflammatory and anti-viral mediators (including type I IFNs, TNFa, IL-1b and IL-6) and as antigen-presenting cells initiating specific immune response. At the same time they are susceptible to TBEV themselves and may be responsible for the initial spread of the virus from the primary infection focus [12].

A SNP in the promoter region of CD209 (-336 A/G; rs4804803) affects transcription and is associated with the severity of dengue fever and the complication of disease. SNP with AG genotype affects the cell surface CD209 expression related to immune augmentation of immune response and less viral replication [28]. It has been noted that rs4804803, an SNP in the CD209 promoter, contributes to the severity of liver disease in chronic HCV infection [23].

There are also studies indicating a possible association between two SNPs in the promoter region of the CD209 gene (rs4804803 and rs2287886) and predisposition to severe forms of TBEV. Barkhash et al. observed an increase in the frequency of the rs2287886 SNP AA homozygotes and the A allele among patients with severe TBE (encephalitis and encephalomyelitis) compared with the group of patients with meningitis, or a combined group of patients with mild forms (fever and meningitis), or the control group [2]. The authors suggested that the CD209 gene promoter region rs2287886 SNP is associated with predisposition to severe forms of TBE in the Russian population. They hypothesized that, unlike other Flaviviruses studied, high dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin expression facilitates spread of TBEV and is detrimental, although the difference with previous studied may be also caused by a different genetic background in different study populations [2].

No studies of CD209 SNPs have been performed in patients infected with Western European TBEV so far. In our study, conducted in the Polish population, we observed that in SNP rs2287886 CD209 gene heterozygotes AG were more frequent in TBE group, while homozygotes GG were more frequent in CG group. This tendency is similar to the one observed in studies conducted by Barkhash et al. [10]. Also patients with a combination of AG genotypes in either rs2287886 or rs 4804803 were more frequent in TBE group. This information may have an impact on vaccination policy by revealing patients with a predisposition to TBE development, as they would then have to be vaccinated.

We compared our results with available data on SNPedia and observed that the distribution of alleles for both CD 209 SNPs in CG are comparable to the European population. This allows us to conclude that the results of our study, even on such a small group, are reliable [24].

Interleukin-10 (IL-10) is a cytokine synthesized by activated macrophages, dendritic cells, B and T CD4+ lymphocytes that perform multiple, mainly anti-inflammatory and immunoregulatory functions [20].

The data on IL-10 role in *Flavivirus* infections are limited, but its increased synthesis seems to be linked to the severe clinical course of Dengue fever [14,27]. TBEV elicits only minimal IL-10 synthesis in dendritic cells, suggesting that it plays no or little role in the first stage of the infection, in the skin. According to Günther et al. the concentration of IL-10 in the CSF of patients with TBE was increased, but significantly lower than in the group of patients with aseptic encephalomeningitis

caused by other viruses, including enteroviruses, herpes simplex type 2 and cytomegalovirus, in parallel with on average more severe and complicated clinical course of TBE [7]. In a study by Atrasheuskaya at al. an increase of serum IL-10 tended to correlate with the favorable clinical course in TBE patients, in line with strong humoral response [1].

Polymorphism of genes for IL-10 was analysed in HCV patients. Sun et al. concluded that the IL-10-1082GG genotype may increase the risk of chronic HCV infection in the Caucasian population, and people carrying the IL-10-592A allele are more likely to clear HCV spontaneously [25]. Li et al. in a group of 1,140 subjects found significantly higher IL-10 production in HCV patients, especially patients with the GG genotype [20]. There are many studies on IL-10 polymorphism in various infections, caused by, e.g. HIV, enteroviruses, Ebstain–Barr virus, etc., but no single study on TBE infection. In our study no association was found between this SNP and TBE severity.

Another interesting molecule, which influences the course of viral infections is IL-28B. Polymorphisms in the genes for type III IFNs present another level of genetic variability which could influence the susceptibility to TBEV. This phenomenon has been thoroughly studied in the infection caused by a member of *Flaviviridae*, HCV [13]. Recent genome-wide association studies have shown that a genetic polymorphism at rs12979860 near IL28 gene on chromosome 19, encoding interferon-lambda-3, is associated with variable responses to the drugs. CC genotype of IL-28B is associated with two to three fold higher probability of sustained virologic response compared to T allele carriers [4]. These seminal studies have opened a new chapter in the field of HCV host-viral interactions. Genetic variations in IL28B region may also predict the probability of a sufficiently strong innate immune response to spontaneously clear virus after acute hepatitis C. Early therapeutic intervention could be recommended for individuals with unfavourable IL-28B genotypes [6].

In our study conducted in patients infected with other *Flaviviridae* TBEV we observed a difference between TBE and CG groups only for ILB28 CT heterozygotes. The study of these relations in TBE patients reveals a predisposition to TBE.

Simultaneously with results of the present study we observed a dependence of IFNl3 expression in csf on rs12979860 in a small group of TBE patients, which however requires further confirmation (unpublished data). Surprisingly, IFNl3 concentration was higher in bearers of unfavorable CT than CC genotype and the difference was significant only in the convalescent period, so this particular SNP seem to influence rather kinetics than the initial level of IFNl3 synthesis in TBE and may not be of clinical importance. In HCV infection, other SNPs associated with *Il28B* have been found to influence treatment response more significantly than rs12979860: rs8099917 and rs12980275 [23]. The minor allele of rs8099917 confers both a two-fold increased risk of progression to chronic hepatitis and five-fold increased risk of a treatment failure and is related to significantly lower IFNl3 expression in PBMC [26]. Further study of these loci in TBE patients might reveal a stronger correlation with clinical picture than we have detected for rs12979860.

CCR5 is a receptor for chemokines CCL3 (MIP-1a), CCL4 (MIP-1b) and CCL5 (RANTES), which is expressed by Th1 CD4+ and T CD8+ lymphocytes, as well as by monocytes [21]. Signaling through CCR5 not only drives migration of the immune cells, but also contributes to T lymphocyte activation, proliferation and differentiation towards Th1 phenotype [28].

The *CCR5Δ32* deletion results in the synthesis of a truncated CCR5 protein, which is not expressed on the cell surface [21]. In Northern Europe, the *CCR5Δ32* homozygocity, resulting in the lack of a functional CCR5 protein, is found in less than 1% of the population [10,17]. The allele frequency decreases southward and is very rare in non-Caucasian populations [15,17].

In the context of our study, it has been linked to the increased susceptibility to a symptomatic infection with WNV, a neurotropic Flavivirus closely related to TBEV [24,28]. Glass et al. have detected homozygocity (but not heterozygocity) for *CCR5Δ32* to be a highly significant risk factor of a symptomatic WNV infection in the Caucasian US population, with a relative risk increased at least four-fold [5]. The correlation with the clinical outcome was not obvious, possibly because of the small number of cases, but the data strongly suggested the increased mortality of *CCR5Δ32* homozygotes [5]. Lim et al. confirmed *CCR5Δ32* homozygocity to be significantly more frequent in four cohorts of patients with symptomatic WNV infection than in the general population, with an odds ratio of 4.2 for all the groups of 619 patients. No influence of *CCR5Δ32* homozygocity on either clinical form of the symptomatic disease (uncomplicated febrile disease versus central nervous system involvement) or any effect of heterozygocity for *CCR5Δ32* was observed. The latter observation may suggest that even a limited ability to express CCR5 is still sufficient for protection against WNV [16].

This finding prompted the epidemiological study on the *CCR5Δ32* distribution in TBE patients from northern Europe, which suggests the allele is responsible for some fraction of, but certainly not all, TBE morbidity [10]. Besides *CCR5Δ32* homo- and heterozygocity, it is conceivable that differently mediated and more subtle differences in CCR5 activity and expression may influence the response and susceptibility to the infection in the analogous manner, which has not been studied so far. In our study we found no differences between TBE patients and healthy controls.

Our study is the first study of human genetic predisposition to tick-borne encephalitis in the Polish population. However, because of the small number of patients, additional studies are needed.

Conclusions

1. SNP rs2287886 CD 209 AG heterozygotes predispose humans to develop TBE.

2. No predisposition to severe forms of TBE and single nucleotide polymorphism in the promoter region of the CD209, IL-10, IL-28 and CCR5 D32 genes was stated.

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