Antiviral Research 99 (2013) 136–138

Contents lists available at SciVerse ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Short Communication

# Association of single nucleotide polymorphism rs3775291 in the coding region of the *TLR3* gene with predisposition to tick-borne encephalitis in a Russian population

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ARTICLE INFO

*Article history:*
Received 18 March 2013
Revised 15 May 2013
Accepted 18 May 2013
Available online 27 May 2013

*Keywords:*
Tick-borne encephalitis (TBE)
Human genetic predisposition
Toll-like receptor 3 (*TLR3*) gene
Chemokine receptor 5 (*CCR5*) gene
Single nucleotide polymorphism

ABSTRACT

Tick-borne encephalitis (TBE) is a central nervous system (CNS) disease caused by the neurotropic, positive-sense RNA virus, tick-borne encephalitis virus (TBEV). A possible association between predisposition to TBE in a Russian population and two polymorphisms, a 32 bp deletion in the coding region of the chemokine receptor *CCR5* gene and the rs3775291 single nucleotide polymorphism (SNP) (G/A, Leu412Phe) in exon 4 of the toll-like receptor *TLR3* gene, was investigated. The genotypic and allelic frequencies of these polymorphisms were analyzed in 137 non-immunized TBE patients with different clinical manifestations, including fever (35), meningitis (62), and severe CNS disease (40), as well as in a control population (269 randomly selected Novosibirsk citizens). The frequencies of the *TLR3* G allele and G/G homozygotes were significantly higher among the patients with TBE compared with the control group ($P = 0.029$ and 0.037, respectively), especially among patients with severe disease ($P = 0.018$ and 0.017, respectively). These results indicate that the G allele (within the G/G homozygous genotype) of the *TLR3* rs3775291 SNP is associated with predisposition to TBE in the Russian population.



Tick-borne encephalitis (TBE) is caused by the tick-borne encephalitis virus (TBEV), a neurotropic, positive-sense RNA virus from the genus *Flavivirus* (family *Flaviviridae*). In Northern Eurasia, 6000–14,000 clinical cases of TBE are reported annually, including 3000–11,000 cases in Russia. In Siberia, a high number of TBE cases are observed as compared with all Russia; particularly, the TBE morbidity rate in the population of Novosibirsk varied from 5.5 to 26.8 per 100,000 between 1990 and 2006. Although the majority of individuals infected with TBEV will not develop any clinical symptoms (about 70–95%), a minority will develop a severe disease, since TBEV can cross the human blood–brain barrier and cause central nervous system (CNS) damage (Gritsun et al., 2003; Ruzek et al., 2010; Suss, 2008; Turtle et al., 2012; Tolokonskaya et al., 2007). Human genetic factors (as well as other factors, including prior immunization and TBEV subtype) can influence the severity and outcome of the disease.

Previously, variations in ABO blood groups and human leukocyte antigens were associated with TBE in the Russian population (Ierusalimsky, 2001). In the same population, six single nucleotide polymorphisms (SNPs) located in three human genes possibly involved in human antiviral defense (three SNPs in the *OAS2* gene, two SNPs in the *OAS3* gene, and one SNP in the *CD209* gene) were associated with severe forms of TBE (Barkhash et al., 2010, 2012). Other loci can also influence host genetic control of susceptibility to TBEV.

The current study investigated a possible association between predisposition to TBE and two polymorphisms, a 32 bp deletion (del32) in the coding region of the chemokine receptor *CCR5* gene and the rs3775291 SNP (G/A, Leu412Phe) in exon 4 of the toll-like receptor *TLR3* gene, in a Russian population. Previously, these polymorphisms were reported to be associated with an increased risk of TBEV infection in a Lithuanian population (Kindberg et al., 2008, 2011). Since Lithuanians and Russians belong to different ethnic groups, it is not correct to extrapolate genetic data obtained in the Lithuanian population to the Russian population. The aim of this study was to check these data in the Russian population residing in Novosibirsk (an area endemic for TBEV).

Blood samples were collected from unrelated TBE patients between 2002 and 2007. TBE was diagnosed based on clinical symptoms, seasonality, evidence of tick bite, and immunological tests. This research was approved by the Bioethics Committee of the Institute of Cytology and Genetics (Russian Academy of Sciences, Siberian Branch). All patients gave informed consent for participation in the research. Only non-immunized patients who self-reported that they had not previously received a TBEV vaccination or specific immunoglobulin after a tick bite were included in the study. All studied individuals were Caucasians (mainly Russians).

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http://dx.doi.org/10.1016/j.antiviral.2013.05.008

**Table 1**
Genotyping assays for studied polymorphisms.

| Gene, polymorphism | Primer sequences | Tm (°C) | Product length (bp) | Restriction enzyme | Genotype-dependent fragment lengths (bp) |
|---|---|---|---|---|---|
| *CCR5* +/del32 | 5′-cacctgcagctctcattttcc-3′[a]<br>5′-gtttttaggattcccgagtagca-3′[a] | 64 | 132 | – | +/+: 132<br>+/del32: 132, 100<br>del32/del32: 100 |
| *TLR3* rs3775291 | 5′-cttgctcattctcccttacacGta-3′[b]<br>5′-tacttctaggtggcccaaccaag-3′ | 64 | 96 | *Rsa*I | G/G: 73, 23<br>G/A: 96, 73, 23<br>A/A: 96 |

Tm, annealing temperature; bp, base pair; del32, 32 bp deletion.
[a] These primers were the same as those used by Kindberg et al. (2008).
[b] The G nucleotide designated by capitalization was changed in the primer sequence in order to generate a new restriction site, as described by Neff et al. (2002).

**Table 2**
Genotypic and allelic frequencies for the *CCR5* gene 32-bp deletion in tick-borne encephalitis (TBE) patients with different clinical manifestations and in the control group.

| Genotypes or alleles | Genotype (allele) frequency: % (number[b]) | | | | | | *P* values[c] |
|---|---|---|---|---|---|---|---|
| | Control group | TBE patients | | | | | |
| | | All | Fever | Meningitis | Fever plus meningitis | Severe forms | |
| +/+ | 73.5 (197) | 78.1 (107) | 71.4 (25) | 79.0 (49) | 76.3 (74) | 82.5 (33) | >0.05 |
| +/del | 25.0 (67) | 19.0 (26) | 22.9 (8) | 19.4 (12) | 20.6 (20) | 15.0 (6) | >0.05 |
| del/del | 1.5 (4) | 2.9 (4) | 5.7 (2) | 1.6 (1) | 3.1 (3) | 2.5 (1) | >0.05 |
| + | 86.0 | 87.6 | 82.9 | 88.7 | 86.6 | 90.0 | >0.05 |
| del | 14.0 | 12.4 | 17.1 | 11.3 | 13.4 | 10.0 | >0.05 |
| N[a] | 268 | 137 | 35 | 62 | 97 | 40 | |

+, allele without a deletion (normal allele); del, allele with a deletion.
[a] N – number of individuals.
[b] Number of subjects with a given genotype.
[c] *P* values were calculated: (1) for comparisons between TBE patients (all) and the control group, and (2) for comparisons between TBE patients with severe forms and each of TBE patient groups with other forms, as well as between TBE patients with severe forms and the control group.

**Table 3**
Genotypic and allelic frequencies for the *TLR3* gene rs3775291 single nucleotide polymorphism (SNP) in tick-borne encephalitis (TBE) patients with different clinical manifestations and in the control group.

| Genotypes or alleles | Genotype (allele) frequency: % (number[b]) | | | | | | *P* values[c] |
|---|---|---|---|---|---|---|---|
| | Control group | TBE patients | | | | | |
| | | All | Fever | Meningitis | Fever plus meningitis | Severe forms | |
| G/G | 42.4 (114) | 53.3 (73) | 51.4 (18) | 48.4 (30) | 49.5 (48) | 62.5 (25) | 0.037[d]<br>0.017[e] |
| A/G | 46.1 (124) | 39.4 (54) | 40.0 (14) | 43.5 (27) | 42.3 (41) | 32.5 (13) | >0.05 |
| A/A | 11.5 (31) | 7.3 (10) | 8.6 (3) | 8.1 (5) | 8.2 (8) | 5.0 (2) | >0.05 |
| G | 65.4 | 73.0 | 71.4 | 70.2 | 70.6 | 78.8 | 0.029[d]<br>0.018[e] |
| A | 34.6 | 27.0 | 28.6 | 29.8 | 29.4 | 21.2 | 0.029[d]<br>0.018[e] |
| N[a] | 269 | 137 | 35 | 62 | 97 | 40 | |

[a] N – number of individuals.
[b] Number of subjects with a given genotype.
[c] *P* values were calculated: (1) for comparisons between TBE patients (all) and the control group, and (2) for comparisons between TBE patients with severe forms and each of TBE patient groups with other forms, as well as between TBE patients with severe forms and the control group.
[d] *P* values for comparison between TBE patients and the control group.
[e] *P* values for comparison between TBE patients with severe forms and the control group.

A total of 137 samples from TBE patients were divided into three groups according to the clinical symptoms: fever ($n = 35$), meningitis ($n = 62$), and severe CNS disease ($n = 40$). In accordance with data reported by Gritsun et al., 2003, we assumed that the Siberian TBEV subtype prevails among bitten patients from Novosibirsk.

Control samples (Russian population) were obtained from 269 Novosibirsk citizens from The World Health Organization MONICA (Multinational MONItoring of trends and determinants in CArdiovascular disease) project. The control group was developed by random selection of individuals from the voting list of the population of one of the Novosibirsk district. No information about whether or not they had been previously infected with TBEV was available.

DNA was extracted from whole blood by phenol–chloroform deproteinization (Sambrook et al., 1989). Polymerase chain reaction (PCR) was used to genotype the *CCR5* +/del32 polymorphism, and PCR-restriction fragment length polymorphism (PCR-RFLP) analysis was used to genotype the *TLR3* rs3775291 SNP. The details of the genotyping assays are summarized in Table 1. The PCR and restriction products were visualized on a 5% polyacrylamide gel.

The genotype frequencies were assessed for consistency with Hardy–Weinberg equilibrium by both the $\chi$-square test and CHI-HW Software (Zaykin and Pudovkin, 1993). Genotypic and allelic frequencies were compared between all TBE patients (with both severe and mild illness) and the control group by $\chi$-square test

using SPSS Software (version 11.0). Comparisons were also made between patients with severe CNS disease vs. patients with mild illness (fever, meningitis, or fever plus meningitis group) and the control group. Differences were considered significant when the *P*-values were less than 0.05.

The genotype frequency distribution was consistent with Hardy–Weinberg equilibrium in all studied groups. No significant differences were observed between any of the groups for the *CCR5* +/del32 polymorphism (individuals with the del32/del32 genotype were found in each group) (Table 2). The frequency of the *TLR3* rs3775291 G/G homozygotes was greater in patients with TBE (53.3%), and this difference was more pronounced in patients with severe disease (62.5%), compared with the control group (42.4%) ($P = 0.037$ and 0.017, respectively). For the same SNP, a significant increase in the G allele frequency was observed in the patients with TBE (73.0%), especially in those patients with severe disease (78.8%), compared with the control group (65.4%) ($P = 0.029$ and 0.018, respectively) (Table 3).

The *CCR5* gene encodes a protein expressed on the surfaces of leukocytes that is involved in regulation of leukocyte migration and tissue-specific adhesion of effector T-helpers. The *CCR5* 32 bp deletion is a risk factor for the development of symptomatic West Nile virus infection (Glass et al., 2005, 2006; Lim et al., 2008, 2010) and TBE in the Lithuanian population (Kindberg et al., 2008). In the Russian population, we found no association between this polymorphism and predisposition to TBE.

The *TLR3* gene encodes a protein (a member of the Toll-like receptor family) that plays a crucial role in recognition of structural determinants typical for infectious agents and in activation of innate immunity. TLR3, mainly expressed on the endosomal membrane of dendritic cells, binds to viral dsRNA (intermediate products of viral RNA replication). This induces activation of NF-κB which leads to an increase in the production of interferon type I and activation of protective antiviral mechanisms. An important role of TLR3 in the response to viruses causing CNS diseases has been suggested by Kumar et al. (2009) and Matsumoto et al. (2011).

Our results coincide with results obtained in the Lithuanian population indicating that functional TLR3 is a risk factor (Kindberg et al., 2011). The rs3775291 SNP (G > A) results in a leucine to phenylalanine substitution that leads to impaired receptor functioning (Ranjith-Kumar et al., 2007). In our study, the frequencies of the G allele and G/G genotype, which corresponds to functional TLR3, were increased in TBE patients (particularly patients with severe disease), compared with the control group (Table 3). These data are also consistent with observations in mice; mice with non-functional TLR3 receptor were more protected from West Nile virus than mice with normal TLR3 (Wang et al., 2004). However, these results were contradicted by the subsequent observation of a protective effect of TLR3 against West Nile virus in mice (Daffis et al., 2008).

Thus, in addition to the *OAS2*, *OAS3*, and *CD209* gene SNPs that we previously reported (Barkhash et al., 2010, 2012), this study detected a new genetic factor (*TLR3* gene rs3775291 SNP) that appears to be involved in predisposition to TBE in the Russian population. Additional studies are required to validate this result. However, our data are similar to data in the Lithuanian population (Kindberg et al., 2011) and suggest that a functional TLR3 is a risk factor for TBEV infection. Future studies on different or larger TBE patient samples, as well as analysis of additional *TLR3* gene SNPs, will help to clarify the mechanisms of host-virus interaction during TBEV infection.

## Acknowledgment

This work was supported by the Russian Foundation for Basic Research (Grant No. 11-04-01206a). Authors are grateful to Pavel I. Pilipenko, Natalia G. Myasnikova, Yulia O. Bogdanova, and Olga V. Morozova for providing blood or DNA samples and clinical information from patients with TBE.

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