Antiviral Research 93 (2012) 64–68

Contents lists available at SciVerse ScienceDirect

Antiviral Research

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

Single nucleotide polymorphism in the promoter region of the *CD209* gene is associated with human predisposition to severe forms of tick-borne encephalitis

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ARTICLE INFO

Article history:
Received 25 June 2011
Revised 5 September 2011
Accepted 19 October 2011
Available online 28 October 2011

Keywords:
Tick-borne encephalitis (TBE)
Human genetic predisposition
Dendritic cell-specific intercellular adhesion molecule-3 (ICAM3)-grabbing non-integrin (DC-SIGN)
CD209 gene
Single nucleotide polymorphism (SNP)

ABSTRACT

Tick-borne encephalitis virus (TBEV) is a neurotropic, positive-sense RNA virus of the genus *Flavivirus* (family *Flaviviridae*) which can cause a variety of clinical manifestations in humans. Previously the severity and outcome of dengue fever and hepatitis C (diseases caused by viruses from the family *Flaviviridae*) were associated with the rs4804803 single nucleotide polymorphism (SNP) located in the promoter region of the human *CD209* gene. This gene encodes dendritic cell-specific ICAM3-grabbing nonintegrin (DC-SIGN), a C-type lectin pathogen-recognition receptor expressed on the surface of dendritic cells and some types of macrophages. In the current study, a possible association between two SNPs in the promoter region of the *CD209* gene (rs4804803 and rs2287886) and predisposition to severe forms of TBEV-induced disease was investigated. The genotypic, allelic and haplotypic frequencies of these SNPs were analyzed in 136 non-immunized Russian patients with different clinical manifestations of tick-borne encephalitis (TBE) and in a control group. An increase in the frequency of the rs2287886 SNP AA homozygotes and the A allele was detected among patients with severe central nervous system disease compared with the group of patients with meningitis ($P = 0.003$ and 0.019), or a combined group of patients with mild forms (fever and meningitis) ($P = 0.003$ and 0.026), or the control group ($P = 0.007$ and 0.035). Thus, our results suggest that the *CD209* gene promoter region rs2287886 SNP is associated with predisposition to severe forms of TBE in the Russian population.

1. Introduction

Tick-borne encephalitis virus (TBEV) is a neurotropic, positive-sense RNA virus that can cross the human blood–brain barrier and cause severe central nervous system (CNS) disease. TBEV belongs to the genus *Flavivirus*, family *Flaviviridae*; this genus also includes yellow fever, West Nile, Japanese encephalitis and dengue viruses. In Northern Eurasia, 6000–14,000 clinical cases of tick-borne encephalitis (TBE) are reported annually, including 3000–11,000 cases in Russia. Although TBEV infections in humans can cause a variety of clinical manifestations, about 70–95% of these infections are asymptomatic (Gritsun et al., 2003; Suss, 2008). It is known that human genetic factors, as well as other factors, including prior immunization and virus type, can influence the severity and outcome of viral diseases. Variations in AB0 blood groups and human leukocyte antigens, as well as polymorphisms in human genes encoding the chemokine receptor CCR5, 2′–5′-oligoadenylate synthetases 2 and 3 (OAS2 and OAS3) and toll-like receptor 3 (TLR3) were previously reported to be associated with susceptibility to TBEV-induced disease in different human populations (Barkhash et al., 2010a,b; Ierusalimsky, 2001; Kindberg et al., 2008, 2011). However, since host genetic control of susceptibility to infectious diseases in humans is likely to be complex, variations in additional genetic loci may also be involved in predisposition to TBE.

The severity and outcome of a TBEV infection in a particular individual depends on the efficiency of both the innate and adaptive immune responses in suppressing virus replication. At the initial stage of the infectious process, TBEV is thought to infect dendritic cells and macrophages, cells which mediate the interaction between the innate and adaptive immune responses; the efficiency of virus propagation in these cells may influence the subsequent distribution of infection through the host organism (Dorrbecker et al., 2010). Dendritic cell-specific intercellular adhesion molecule 3 (ICAM3)-grabbing non-integrin (DC-SIGN) is

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doi:10.1016/j.antiviral.2011.10.017

a C-type lectin expressed on the surface of dendritic cells and certain macrophages that has several functions. As a pathogen recognition receptor (PRR), DC-SIGN allows the cell to detect specific pathogen-associated molecular patterns and then to capture viruses and other pathogens, process pathogen antigens and present them to effectors of the adaptive immune response (such as T-lymphocytes). As a cell adhesion receptor, DC-SIGN is involved in dendritic cell migration and adhesion as well as in T-lymphocyte activation. This receptor contains an extracellular domain (composed of a carbohydrate recognition domain and a neck domain), a transmembrane region and a cytoplasmic domain (van Vliet et al., 2008; Zhou et al., 2006). It was previously reported that both dengue and hepatitis C viruses (which also belong to the *Flaviviridae* family) use DC-SIGN as a receptor to infect dendritic cells and macrophages (Tassaneetrithep et al., 2003; van Vliet et al., 2008). The role of DC-SIGN in TBEV infections has not been studied.

Human DC-SIGN is encoded by the *CD209* gene located in the p13.3 region of chromosome 19. This gene comprises seven exons and produces multiple transcripts by alternative splicing (**Ensembl ID: ENSG00000090659**). Previously the severity and outcome of dengue fever and hepatitis C were associated with the rs4804803 single nucleotide polymorphism (SNP) located in the promoter region of this gene. In the current study, a possible association between two SNPs located in the promoter region of the human *CD209* gene (rs4804803 and rs2287886) and predisposition to severe forms of TBE in the Russian population was investigated.

2. Materials and methods

2.1. Subjects

Blood samples were collected from unrelated symptomatic patients with TBE from hospitals in Novosibirsk (Russia) between 2002 and 2007. TBE was diagnosed based on clinical symptoms, seasonality, evidence of a tick bite and positive immunological diagnosis. 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 those 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 white (mainly Russians). A total of 136 samples were obtained from TBE patients (76 males and 60 females) and divided into three groups according to the following clinical symptoms: fever ($n = 35$), meningitis ($n = 61$), and severe CNS disease such as encephalitis or poliomyelitis-like syndrome ($n = 40$). The mean age of patients was approximately 48 years (56 years for patients with fever, 42 – with meningitis, 50 – with severe disease). The TBEV sub-type was not determined; however, since the studied patients were Novosibirsk citizens, we assumed that most patients were infected with the Siberian TBEV sub-type (Gritsun et al., 2003).

Control samples (Russian population) were obtained from 263 Novosibirsk citizens (130 males, 133 females, mean age approximately 44 years) from The World Health Organization MONICA project (Russia). 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.

2.2. Genotyping

DNA was extracted from whole blood by phenol and chloroform deproteinization as described previously (Sambrook et al., 1989). Allelic Discrimination TaqMan SNP genotyping assays and a RealTime PCR 7500 machine (Applied Biosystems) were used to genotype the *CD209* rs2287886 SNP in the TBE patient samples collected from 2002 to 2005. A polymerase chain reaction and restriction fragment length polymorphism (PCR–RFLP) analysis was used to genotype the *CD209* rs2287886 SNP in the 2006 to 2007 TBE patient samples and in the control group as well as the *CD209* rs4804803 SNP in all TBE samples and in the control group. To facilitate these SNP assays, new restriction sites were generated in the PCR products by substituting nucleotides in one of the primers for each assay (Neff et al., 2002). The details of the genotyping assays developed are summarized in Table 1. The restriction products were visualized on 5% polyacrylamide gels stained with ethidium bromide. The PCR–RFLP assay conditions were optimized using DNA samples with known genotypes. Initially, the genotypes of randomly selected DNA samples were determined by sequencing PCR fragments containing the studied SNPs using an automated ABI Prism 310 Genetic Analyzer in the Inter-institutional DNA Sequencing Center of the Siberian Branch of Russian Academy of Sciences. The sequencing primers are listed in Table 1.

2.3. Statistical analysis

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-square test using SPSS Software (version 11.0). The difference between two groups was considered significant if the *P*-value was less than 0.05. The correspondence of the genotype frequencies to the Hardy–Weinberg equilibrium was assessed using both the χ-square test and CHIHW Software (Zaykin and Pudovkin, 1993). Prediction of potential haplotypes was performed using a maximum-likelihood method and Arlequin Software (version 3.0) (Excoffier et al., 2005). Permutation tests using the EM algorithm were applied to analyze pairwise linkage disequilibrium (LD) between SNPs (Slatkin and Excoffier, 1996).

3. Results

The promoter region of the *CD209* gene was selected for the analysis because the rs4804803 SNP (located 336 nts upstream of the translation start codon) was previously reported to be associated with the disease severity and outcome of infections with dengue and hepatitis C viruses, which, as well as TBEV, belong to the family *Flaviviridae* (Ryan et al., 2010; Sakuntabhai et al., 2005; Wang et al., 2011). Two SNPs from the *CD209* gene promoter region, rs4804803 and rs2287886 (located 139 nts upstream of the translation start codon) were selected as genetic markers to study a possible association between genetic variation in this promoter and predisposition to TBE in humans.

Genotypic and allelic frequencies for these SNPs were calculated for each of the different TBE patient groups (severe CNS disease, fever, meningitis, and a combined group of patients with fever and meningitis) and for a control Russian Novosibirsk population group (Table 2). No statistically significant differences were found in genotype and/or allele frequencies between the overall group of patients with TBE and the control group for either of the *CD209* gene SNPs studied. For the majority of individual groups the genotype frequency distribution corresponded to the Hardy–Weinberg equilibrium. Only in the TBE severe CNS disease group was a statistically significant deviation from the Hardy–Weinberg equilibrium observed for the SNP rs2287886 ($\chi^2 = 5.46$; d.f. = 1) because of the abundance of AA homozygotes and a low number of heterozygotes. An increase in the frequency of the rs2287886 SNP AA homozygotes was detected among patients with severe disease (28.2%) compared with meningitis patients (6.7%) ($P = 0.003$) or fever plus meningitis patients (8.4%) ($P = 0.003$), or

Table 1
Single nucleotide polymorphism (SNP) genotyping assays.

SNP	Primer sequences	T_m (°C)	Product length (bp)	Restriction enzyme	Restriction fragment lengths, bp
rs4804803	5′-aactgggggtgctacctgGc-3′[a] 5′-ggatggtctgggggttgacag-3′[a]	62	153	*Hae*III	AA: 153 AG: 153, 134, and 19 GG: 134 and 19
	5′-aatgaggacagcagcagctc-3′[b] 5′-ggagaaggactcatccactc-3′[b] 5′-actcatccactcatggatg-3′[c]	62	196	–	–
rs2287886	5′-atgctctgatgctttccacGag-3′[a] 5′-cactcatgtcaccccactctcc-3′[a]	64	168	*Hinf*I	GG: 168 GA: 168, 146, and 22 AA: 146 and 22
	5′-tgctcagccatccatgagtg-3′[b,c] 5′-cctcctctgaatggatagacgtg-3′[b]	64	410	–	–

T_m, annealing temperature; bp, base pair.
[a] Primers for SNP genotyping by RFLP; nucleotides designated by capitalization were changed in sequences of primers, as described by Neff et al. (2002).
[b] Primers for SNP genotyping by DNA sequencing (for PCR of DNA fragment containing SNP).
[c] Primers for SNP genotyping by DNA sequencing (sequencing primers).

Table 2
Genotypic and allelic frequencies for the *CD209* gene single nucleotide polymorphisms (SNPs) in tick-borne encephalitis (TBE) patients with different clinical manifestations and in the control group.

SNPs (genotypes or alleles)	Genotype (allele) frequency, %					
	Control group	TBE patients				
		All	Fever	Meningitis	Fever plus meningitis	Severe forms
rs4804803						
AA	66.2	68.4	62.9	63.9	63.5	80.0
AG	30.8	27.9	31.4	32.8	32.3	17.5
GG	3.0	3.7	5.7	3.3	4.2	2.5
A	81.6	82.4	78.6	80.3	79.7	88.8
G	18.4	17.6	21.4	19.7	20.3	11.2
N[a]	263	136	35	61	96	40
rs2287886						
GG	49.0	47.0	45.7	51.6	49.5	41.0
GA	39.0	38.8	42.9	41.7	42.1	30.8
AA	12.0[b]	14.2	11.4	6.7[b]	8.4[b]	28.2
G	68.5	66.4	67.1	72.5	70.5	56.4
A	31.5[c]	33.6	32.9	27.5[c]	29.5[c]	43.6
N[a]	249	134	35	60	95	39

[a] *N* – number of individuals.
[b] $P < 0.01$ for comparison between group of TBE patients with severe forms of the disease and other groups.
[c] $P < 0.05$ for comparison between group of TBE patients with severe forms of the disease and other groups.

the control group (12.0%) ($P = 0.007$). In addition, for this SNP, an increase in the A allele frequency was found among patients with severe disease (43.6%), compared to meningitis patients (27.5%) ($P = 0.019$) or to fever plus meningitis patients (29.5%) ($P = 0.026$), or to the control group (31.5%) ($P = 0.035$).

Although no significant differences in genotypic or allelic frequencies were found between TBE patients with severe disease and the other groups studied for the *CD209* gene rs4804803 SNP, an increase in the AA homozygote frequency was observed among patients with severe disease (80.0%) compared with all other groups studied (from 62.9% in patients with fever to 66.2% in the control group). There was also a corresponding decrease in the frequency of AG heterozygotes among patients with severe disease (17.5%) compared with each of the other groups studied (from 30.8% in the control group to 32.8% in patients with meningitis).

The frequencies of different genotype combinations for these two SNPs were calculated using only samples with known genotypes for each of the SNPs in both the TBE (133 patients including 35 with fever, 59 with meningitis, and 39 with severe disease) and control (157 individuals) groups (Table 3). Six out of nine possible combined genotypes were found in each of the groups studied. One genotype combination, AG/AA (the first genotype corresponds to the SNP rs4804803 and the second corresponds to the SNP rs2287886) was found for only one individual from the control group. Two of the genotype combinations (GG/AG and GG/AA) were not found in the tested samples. Statistically significant differences were detected in the frequency of the AA/AA combined genotype between patients with severe disease (28.2%) and those with meningitis (6.8%) ($P = 0.004$) or fever plus meningitis (8.4%) ($P = 0.003$) or the control group (12.7%) ($P = 0.018$).

Since the two analyzed SNPs are located only 197 bp apart, a linkage analysis for these SNPs was performed. In each of the TBE groups as well as in the control group these two SNPs were found to be in a significant linkage disequilibrium (LD) (data not shown), and the haplotype frequencies were calculated for these SNPs in TBE patients with severe disease compared to those for patients with fever or meningitis, the combined fever and meningitis group and the control group (Table 4). The order of the nucleotides shown in the haplotype structure corresponds to the order of SNPs on the chromosome (rs4804803, rs2287886). Three out of four possible haplotypes (AG, AA, and GG) were found in each of the groups studied. The GA haplotype composed of the minor alleles of both of the studied SNPs was found only in one individual in the control group (0.3%). A significant increase in the AA haplotype frequency was observed among patients with severe disease (43.6%) compared to the meningitis group (28.0%) ($P = 0.024$), combined fever plus meningitis group (29.8%) ($P = 0.03$), or the control group (30.9%) ($P = 0.033$).

Table 3
Frequencies of genotype combinations for the *CD209* gene SNPs rs4804803 and rs2287886 in TBE patients with different clinical manifestations and in the control group.

Genotype combination (rs4804803/rs2287886)	Genotype combination frequency, %					
	Control group	TBE				
		All	Fever	Meningitis	Fever plus meningitis	Severe forms
AA/GG	28.0	27.8	25.7	28.8	27.7	28.2
AA/AG	25.5	26.3	25.7	28.8	27.7	23.0
AA/AA	12.7[b]	14.3	11.4	6.8[a]	8.4[a]	28.2
AG/GG	20.4	15.0	14.3	18.6	17.0	10.3
AG/AG	10.2	12.8	17.2	13.6	14.9	7.7
AG/AA	0.6	0	0	0	0	0
GG/GG	2.6	3.8	5.7	3.4	4.3	2.6
GG/AG	0	0	0	0	0	0
GG/AA	0	0	0	0	0	0
N[c]	157	133	35	59	94	39

[a] $P < 0.01$ for comparison between group of TBE patients with severe forms of the disease and other groups.
[b] $P < 0.05$ for comparison between group of TBE patients with severe forms of the disease and other groups.
[c] *N* – number of individuals.

Table 4
Haplotype frequencies for the *CD209* gene SNPs rs4804803 and rs2287886 in TBE patients with different clinical manifestations and in the control group.

Haplotype[a]	Haplotype frequency, %					
	Control group	TBE				
		All	Fever	Meningitis	Fever plus meningitis	Severe forms
AG	51.0	48.5	45.7	52.5	50.0	44.9
AA	30.9[b]	33.8	32.9	28.0[b]	29.8[b]	43.6
GG	17.8	17.7	21.4	19.5	20.2	11.5
GA	0.3	0.0	0.0	0.0	0.0	0.0
N[c]	157	133	35	59	94	39

[a] The order of nucleotides in the haplotype structure corresponds to the order of SNP alleles on the chromosome (rs4804803, rs2287886).
[b] $P < 0.05$ for comparison between group of TBE patients with severe forms of the disease and other groups.
[c] *N* – number of individuals.

4. Discussion

Because the symptoms of non-immunized patients were considered to more accurately indicate individual susceptibility to TBEV-induced disease, only patients who had not received vaccination or specific immunoglobulin after a tick bite were included in this study. For both SNPs studied, no statistical differences were found between the overall group of patients with TBE and the control group. This result was expected because TBE is a complex disease with various clinical manifestations, and it was not reasonable to consider all TBE patients as a single group. Non-immunized patients with severe CNS disease were considered to be more susceptible to TBE virus-induced disease than individuals with mild fever and/or meningitis. Since neither blood donors nor other individuals in the Russian population are routinely screened for a TBEV infection, it was not possible to collect samples from non-immunized, asymptomatic TBEV infected individuals.

Both the AA genotype and A allele of the *CD209* rs2287886 (−139) SNP were associated with predisposition to severe forms of TBE in the Russian population. Although no association between the rs4804803 (−336) SNP genotypes or alleles and predisposition to severe forms of TBE was detected, a significant increase in the frequency of the combined rs4804803 and rs2287886 SNPs AA/AA genotype, as well as in the frequency of the AA haplotype, was found suggesting that both the rs2287886 and rs4804803 SNPs may contribute to predisposition to severe forms of TBE in the Russian population. However, further studies are required to investigate the role of the rs4804803 SNP in TBE pathogenesis.

Previously the G allele of the *CD209* gene rs4804803 SNP (both GG and GA genotypes) was associated with a significant risk of dengue hemorrhagic fever compared to dengue fever in a Thai population (Sakuntabhai et al., 2005) and with dengue pathogenesis in a Taiwanese population (Wang et al., 2011). However, in a Brazilian population, no association of the rs4804803 or some other *CD209* gene SNPs with dengue pathogenesis was found (Silva et al., 2010). The G allele of the rs4804803 SNP was also associated with more advanced liver disease, higher liver fibrosis scores and levels of alanine transaminase in Irish patients with a chronic hepatitis C virus infection (Ryan et al., 2010). It was also demonstrated that the G allele of the rs4804803 SNP is associated with lower *CD209* expression levels *in vitro* (Chan et al., 2010; Sakuntabhai et al., 2005). In contrast, our results demonstrated that the alternative A allele of this SNP (within the combined rs4804803/2287886 genotype or haplotype) may contribute to increased susceptibility to TBE. This finding may reflect genetic differences between Asian, Irish and Russian populations and/or different roles of DC-SIGN in the pathogenesis of the different flaviviruses studied. Since the A allele of the SNP rs4804803 has been associated with higher *CD209* expression levels *in vitro* (Sakuntabhai et al., 2005), individuals with this allele would be expected to have higher concentrations of DC-SIGN molecules on the surfaces of their dendritic cells and macrophages compared to individuals with the G allele. This may lead to enhanced spread of TBEV in an infected person. While the A allele of the rs4804803 SNP was shown to be associated with increased susceptibility to tuberculosis in a sub-Saharan African population (Vannberg et al., 2008), the same allele was reported to be associated with the protection from this disease in a South African population (Barreiro et al., 2006). No association between the rs2287886 SNP and susceptibility to dengue fever in a Thai population was previously reported (Sakuntabhai et al., 2005). In contrast, the G allele of this SNP was associated with rapid progression of AIDS in HIV type 1-infected Japanese hemophiliacs (Koizumi et al., 2007).

Although the rs2287886 SNP in the *CD209* promoter region was not included in some of the previous virus-induced disease

association studies in which the rs4804803 SNP was analyzed, different rs4804803/rs2287886 LDs have been observed in various ethnic populations. For example, a significant LD was observed in an Indian population (Selvaraj et al., 2009) but was not detected in a Thai population (Sakuntabhai et al., 2005). In the present study, significant LD between these two SNPs in a Russian population was detected. However, only the rs2287886 SNP (AA genotype and A allele) was found to be associated with predisposition to severe forms of TBE. In the *CD209* promoter region, the rs2287886 SNP is located 7 bps from one of the predicted binding sites for the transcription factor AP-1 (Liu et al., 2003), and variation in this site may affect the expression level of the *CD209* (Koizumi et al., 2007).

Our results suggest that the rs2287886 SNP in the *CD209* gene promoter region is associated with predisposition to severe forms of TBE in the Russian population. To our knowledge, this is the first study of a possible association between variation in the *CD209* gene and the outcome of a TBEV infection. Additional studies are required to further validate these results. Analysis of additional *CD209* gene SNPs (including those in the coding region) may be useful in determining the involvement of this gene in modulating the severity and outcome of TBE. Biochemical analyses performed either on samples obtained from patients during the course of their illness (cytokine levels etc.) or on cells obtained from convalescent patients with different clinical symptoms should also be done to supplement the results obtained in future studies. Identification of genetic variants associated with predisposition to severe TBEV-induced disease will be important for better understanding viral pathogenesis mechanisms and may be useful for the development of specific therapy for treatment of TBEV infections.

Acknowledgements

We are grateful to Aida G. Romaschenko for reading of this manuscript and helpful comments, to Pavel I. Pilipenko, Natalia G. Myasnikova, Yulia O. Bogdanova, and Olga V. Morozova for providing blood or DNA samples as well as clinical information from patients with TBE, and to Viktor F. Kobzev for providing oligonucleotide primers used in this research. This work was supported by the Russian Foundation for Basic Research (Grant No. 11-04-01206a to A.V.B. and V.N.B), National Center for Infectious Diseases, Centers for Disease Control and Prevention (Grant No. CI000216 to M.A.B. and A.A.P.) and the Global Partnership Grant from the University System of Georgia to A.A.P.

References

Barkhash, A.V., Perelygin, A.A., Babenko, V.N., Myasnikova, N.G., Pilipenko, P.I., Romaschenko, A.G., Voevoda, M.I., Brinton, M.A., 2010a. Variability in the 2′–5′-oligoadenylate synthetase gene cluster is associated with human predisposition to tick-borne encephalitis virus-induced disease. J. Infect. Dis. 202, 1813–1818.

Barkhash, A.V., Babenko, V.N., Kobzev, V.F., Romaschenko, A.G., Voevoda, M.I., 2010b. Polymorphism of 2′–5′-oligoadenylate synthetase (*OAS*) genes, associated with predisposition to severe forms of tick-borne encephalitis, in human populations of North Eurasia. Mol. Biol. 44, 985–993.

Barreiro, L.B., Neyrolles, O., Babb, C.L., Tailleux, L., Quach, H., McElreavey, K., Helden, P.D., Hoal, E.G., Gicquel, B., Quintana-Murci, L., 2006. Promoter variation in the DC-SIGN-encoding gene CD209 is associated with tuberculosis. PLoS Med. 3, e20.

Chan, K.Y., Xu, M.S., Ching, J.C., So, T.M., Lai, S.T., Chu, C.M., Yam, L.Y., Wong, A.T., Chung, P.H., Chan, V.S., Lin, C.L., Sham, P.C., Leung, G.M., Peiris, J.S., Khoo, U.S., 2010. CD209 (DC-SIGN) -336A>G promoter polymorphism and severe acute respiratory syndrome in Hong Kong Chinese. Hum. Immunol. 71, 702–707.

Dorrbecker, B., Dobler, G., Spiegel, M., Hufert, F.T., 2010. Tick-borne encephalitis virus and the immune response of the mammalian host. Travel Med. Infect. Dis. 8, 213–222.

Excoffier, L., Laval, G., Schneider, S., 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evol. Bioinform. Online 1, 47–50.

Gritsun, T.S., Lashkevich, V.A., Gould, E.A., 2003. Tick-borne encephalitis. Antiviral Res. 57, 129–146.

Ierusalimsky, A.P., 2001. Tick-borne Encephalitis. Manual for Physicians. State medical academy publishers, Novosibirsk [in Russian].

Kindberg, E., Mickiene, A., Ax, C., Akerlind, B., Vene, S., Lindquist, L., Lundkvist, A., Svensson, L., 2008. A deletion in the chemokine receptor 5 (CCR5) gene is associated with tickborne encephalitis. J. Infect. Dis. 197, 266–269.

Kindberg, E., Vene, S., Mickiene, A., Lundkvist, A., Lindquist, L., Svensson, L., 2011. A functional toll-like receptor 3 gene (TLR3) may be a risk factor for tick-borne encephalitis virus (TBEV) infection. J. Infect. Dis. 203, 523–528.

Koizumi, Y., Kageyama, S., Fujiyama, Y., Miyashita, M., Lwembe, R., Ogino, K., Shioda, T., Ichimura, H., 2007. RANTES-28G delays and DC-SIGN-139C enhances AIDS progression in HIV type 1-infected Japanese hemophiliacs. AIDS Res. Hum. Retroviruses 23, 713–719.

Liu, H., Yu, W., Liou, L.Y., Rice, A.P., 2003. Isolation and characterization of the human DC-SIGN and DC-SIGNR promoters. Gene 313, 149–159.

Neff, M.M., Turk, E., Kalishman, M., 2002. Web-based primer design for single nucleotide polymorphism analysis. Trends Genet. 18, 613–615.

Ryan, E.J., Dring, M., Ryan, C.M., McNulty, C., Stevenson, N.J., Lawless, M.W., Crowe, J., Nolan, N., Hegarty, J.E., O'Farrelly, C., 2010. Variant in CD209 promoter is associated with severity of liver disease in chronic hepatitis C virus infection. Hum. Immunol. 71, 829–832.

Sakuntabhai, A., Turbpaiboon, C., Casadémont, I., Chuansumrit, A., Lowhnoo, T., Kajaste-Rudnitski, A., Kalayanarooj, S.M., Tangnararatchakit, K., Tangthawornchaikul, N., Vasanawathana, S., Chaiyaratana, W., Yenchitsomanus, P.T., Suriyaphol, P., Avirutnan, P., Chokephaibulkit, K., Matsuda, F., Yoksan, S., Jacob, Y., Lathrop, G.M., Malasit, P., Desprès, P., Julier, C., 2005. A variant in the CD209 promoter is associated with severity of dengue disease. Nat. Genet. 37, 507–513.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual (second edition). Cold Spring Harbor Laboratory Press, New York.

Selvaraj, P., Alagarasu, K., Swaminathan, S., Harishankar, M., Narendran, G., 2009. CD209 gene polymorphisms in South Indian HIV and HIV-TB patients. Infect. Genet. Evol. 9, 256–262.

Silva, L.K., Blanton, R.E., Parrado, A.R., Melo, P.S., Morato, V.G., Reis, E.A., Dias, J.P., Castro, J.M., Vasconcelos, P.F., Goddard, K.A., Barreto, M.L., Reis, M.G., Teixeira, M.G., 2010. Dengue hemorrhagic fever is associated with polymorphisms in JAK1. Eur. J. Hum. Genet. 18, 1221–1227.

Slatkin, M., Excoffier, L., 1996. Testing for linkage disequilibrium in genotypic data using the Expectation–Maximization algorithm. Heredity 76, 377–383.

Suss, J., 2008. Tick-borne encephalitis in Europe and beyond – the epidemiological situation as of 2007. Eurosurveillance 13, 1–8.

Tassaneetrithep, B., Burgess, T.H., Granelli-Piperno, A., Trumpfheller, C., Finke, J., Sun, W., Eller, M.A., Pattanapanyasat, K., Sarasombath, S., Birx, D.L., Steinman, R.M., Schlesinger, S., Marovich, M.A., 2003. DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. J. Exp. Med. 197, 823–829.

Vannberg, F.O., Chapman, S.J., Khor, C.C., Tosh, K., Floyd, S., Jackson-Sillah, D., Crampin, A., Sichali, L., Bah, B., Gustafson, P., Aaby, P., McAdam, K.P., Bah-Sow, O., Lienhardt, C., Sirugo, G., Fine, P., Hill, A.V., 2008. CD209 genetic polymorphism and tuberculosis disease. PLoS ONE 3, e1388.

van Vliet, S.J., Garcia-Vallejo, J.J., van Kooyk, Y., 2008. Dendritic cells and C-type lectin receptors: coupling innate to adaptive immune responses. Immunol. Cell Biol. 86, 580–587.

Wang, L., Chen, R.F., Liu, J.W., Lee, I.K., Lee, C.P., Kuo, H.C., Huang, S.K., Yang, K.D., 2011. DC-SIGN (CD209) promoter-336 A/G polymorphism is associated with dengue hemorrhagic fever and correlated to DC-SIGN expression and immune augmentation. PLoS Negl. Trop. Dis. 5, e934.

Zaykin, D.V., Pudovkin, A.I., 1993. Two programs to estimate significance of χ^2 values using pseudo-probability tests. J. Hered. 84, 152.

Zhou, T., Chen, Y., Hao, L., Zhang, Y., 2006. DC-SIGN and immunoregulation. Cell Mol. Immunol. 3, 279–283.