ORIGINAL ARTICLE

Lack of association between *CD226* genetic variants and inflammatory demyelinating diseases in Korean population

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Abstract

OBJECTIVE: This study was conducted to find the possible association between *CD226* polymorphisms and inflammatory demyelinating diseases in Korean population.
METHODS: A total of 14 *CD226* SNPs were selected based on their linkage disequilibrium, minor allele frequency, and location. Then, the SNPs were genotyped in 178 IDD patients and 237 healthy controls. Subsequently, we conducted logistic analysis to find possible associations
RESULTS: Statistical analyses revealed only a marginal signal for a common SNP *rs1788229* with inflammatory demyelinating disease (p=0.05), while other SNPs failed to show associations with any diseases. However, the significance of *rs1788229* disappeared after a multiple testing correction of the data (p>0.05). Interestingly, *rs763361*, which showed significant associations with multiple sclerosis in several previous studies, did not show any association at all.
CONCLUSIONS: While prior studies have found *CD226* polymorphisms to be significantly associated with inflammatory demyelinating diseases, our results indicate the *CD226* polymorphisms to be not associated with the diseases in Korean population. However, our results suggest that the causal genes for inflammatory demyelinating diseases may vary depending on the population.

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory disorder in which myelin sheaths in brain and spinal cord are damaged by focal lymphocytic infiltration (Compston & Coles 2008). Accumulated damage on the central nervous system caused by the disease leads to a wide variety of symptoms, including loss of sensitivity, hypoesthesia, paresthesia, muscle weakness, muscle spasms, ataxia, and even

psychological symptoms such as fatigue, depression or unstable mood. Furthermore, MS is a complex-trait disease with a combination of genetic, environmental and infectious factors.(Kenealy *et al.* 2003) The most prominent genetic region known to affect MS is *HLA-DRB1* locus, but even this region accounts for less than 50% of MS genetic etiology (Kenealy *et al.* 2003). In order to further investigate the possible genetic factors for MS, a number of studies have looked for the risk genes. As a result, several genome-wide association studies (GWAS) revealed genes such as *IL2RA*, *IL7RA*, *GPC5*, *CD6*, and *TNFRSF1A* as risk genes for MS (Sawcer *et al.* 2011; De Jager *et al.* 2009; Baranzini *et al.* 2009; Hafler *et al.* 2007).

Neuromyelitis optica (NMO) is also an inflammatory disorder which specifically affects optic nerves, leading to loss of vision. The disease is also known to cause spinal cord damage as well. Although its signs and symptoms are similar to MS, several evidences suggest that the pathway of NMO is different from MS (Wingerchuk *et al.* 1999; Wingerchuk *et al.* 2006). While MS is a fairly rare disease in Asian population, NMO is more common in Asian (Fukazawa *et al.* 2000; Lau *et al.* 2002; Das & Puvanendran 1998). Furthermore, although there have been numerous studies conducted between MS and gene polymorphisms, studies on relations between NMO and gene polymorphisms have been far fewer. In our previous study, a GWAS was conducted for NMO and MS, and the results showed that risk polymorphisms for NMO and MS were quite different from each other (Kim *et al.* 2010). The results suggest that although MS and NMO are both autoimmune diseases with similar symptoms, genetic factors that affect NMO may be distinguished from MS.

Cluster of Differentiation 226 (*CD226*) encodes for a protein CD226, which is expressed on the surface of immune cells. Recently, a number of studies investigated for a possible association between genetic polymorphisms of *CD226* and MS. Hafler et al. showed that several tag SNPs of *CD226* were significantly associated with MS in Caucasian population (Hafler *et al.* 2009). A genetic polymorphism of *CD226* has also shown the association with MS in German and Indian populations as well (Wieczorek *et al.* 2009; Pandit *et al.* 2011). However, no study has looked into the possible association between *CD226* and MS or NMO in Asian population yet. In order to investigate how genetic etiologies might differ between MS and NMO in Asian population, we conducted association analysis between *CD226* polymorphisms and the inflammatory demyelinating diseases (IDD). Furthermore, we have also conducted separate association analyses for MS and NMO as well.

MATERIAL & METHODS

Subjects

For genotyping of *CD226* polymorphisms, a total of 415 subjects were included, with 98 NMO patients, 80 MS patients, and 237 controls (Age = 47.3 (38.0–60.0), Female/Male = 156/81). For the biologically homogenous study population, we only included the patients with both optic neuritis and longitudinally extensive myelitis according to the revised diagnostic criteria for NMO (Wingerchuk *et al.* 2006) or limited form of NMO, which were seropositive for aquaporin-4 antibody (Wingerchuk *et al.* 2007). We measured the anti-AQP4 antibodies with an enzyme-linked immunosorbent assay (ELISA) and cell-based assay (CBA) with a commercial slide kit (Euroimmun, Luebeck, Germany) (Jarius *et al.* 2010; Kim *et al.* 2011). Patients were evaluated in the MS center at National Cancer Center (NCC) of Korea. Additionally, 237 healthy and old-age controls of Korean ethnicity were recruited, who do not have IDDs including NMO, classical MS, optic neuritis, and transverse myelitis. The study protocol was approved by the Institutional Review Board of the NCC of Korea. Written informed consent was obtained from each subject before the study. The clinical characteristics of the samples are shown in Table 1. Detailed demographic and clinical characteristics of NMO patients were previously described elsewhere (Kim *et al.* 2012).

Single nucleotide polymorphism (SNP) selection and genotyping

Fourteen SNPs of *CD226* were selected based on linkage disequilibrium (LD) (only one SNP if there were absolute LDs (r^2=1)), minor allele frequency (>0.05), locations (SNPs in exons were preferred) and amino acid changes (non-synonymous SNPs were preferred) from Asian (Chinese and Japanese) population database of International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/). Then, the selected SNPs were genotyped in 178 IDD cases and 237 normal controls subjects using TaqMan assay on the ABI prism 7900HT sequence detection system (Applied Biosystems, USA). Genotyping quality control was performed in 10% of the samples by duplicate checking (rate of concordance in duplicates >99.5%).

Statistics

LD was obtained using the Haploview v4.2 software from the Broad Institute (http://www.broadinstitute.org/mpg/haploview), with examination of Lewontin's D' (|*D*'|) and the LD coefficient r^2 between all pairs of bi-allelic loci (Barrett *et al.* 2005). Haplotypes were first estimated using PHASE software (Stephens *et al.* 2001), and then computed using Statistical Analysis System (SAS). Associations for the IDD, MS and NMO under logistic model were adjusted by age (continuous value) and sex (male = 0, female = 1) as covariates using SAS. In order to correct for the multiple testing error, the SNPSpD program (http://gump.qimr.edu.au/general/daleN/SNPSpD/) was used, with the correction number of 8.6317. Comparisons between races were conducted with SAS, and LD plot of the races were obtained by using the Haploview software.

RESULTS

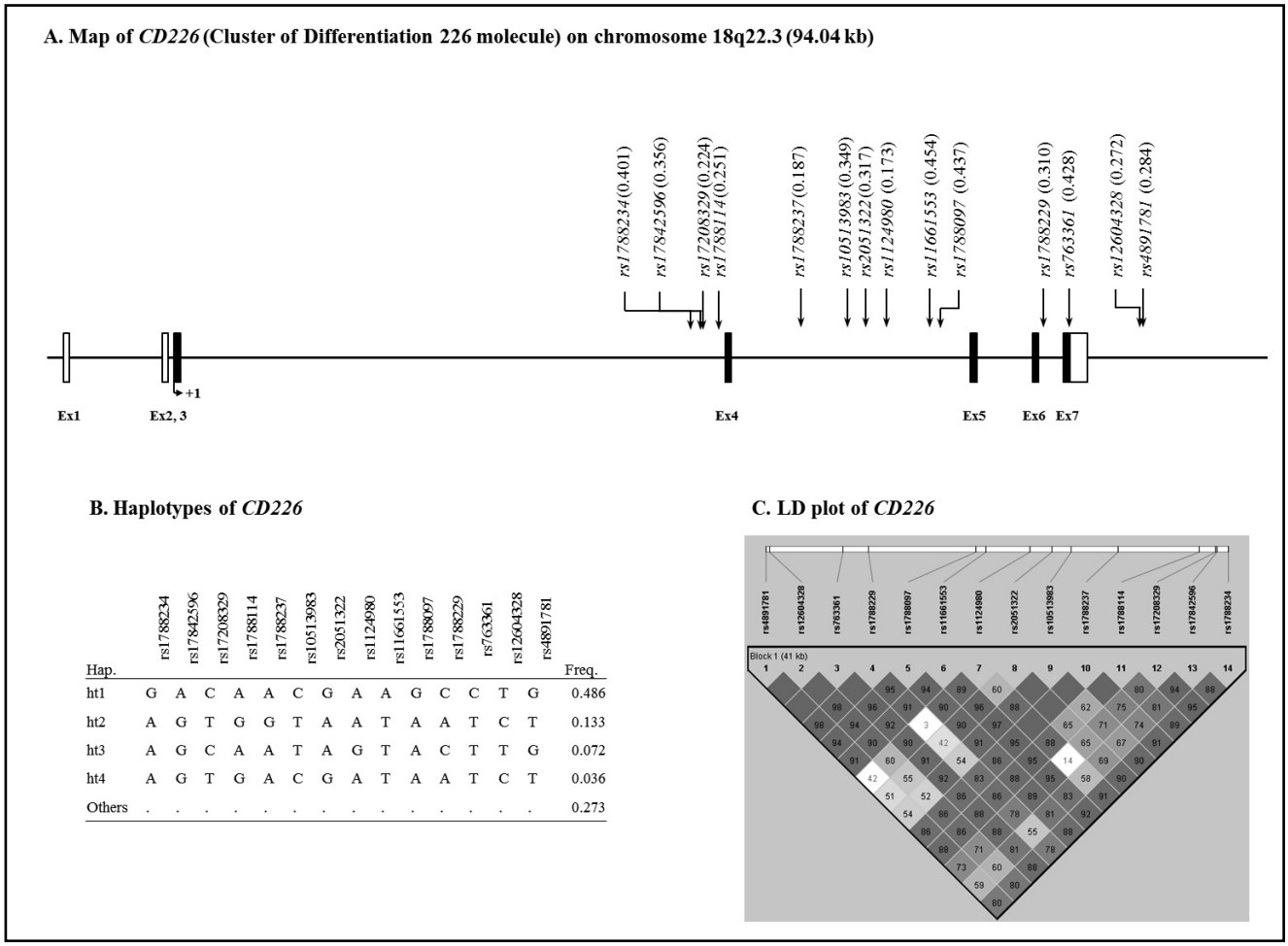
We selected 14 SNPs in *CD226* based on their location, MAF and LD status, then genotyped them in 98 NMO patients, 80 MS patients, and 237 healthy controls. The physical map of *CD226* and selected SNPs are shown in Figure 1A. Haplotype analyses of the *CD226* SNPs using PHASE program yielded 4 haplotypes with significant frequencies (Figure 1B). Furthermore, LD status of the 14 SNPs were analyzed using Haploview program, and the results revealed one LD block formed by the SNPs (Figure 1C). Detailed information of the SNPs, including their positions, genotypes, MAFs, heterozygosity, and Hardy-Weinberg equilibrium (HWE), are listed in Table 2. Among the SNPs, *rs763361* was located in exon region and induced amino acid change from serine at position 307 to glycine. On the other hand, *rs12604328* and *rs4891781* were located in 5' UTR, while the rest of the SNPs were in intronic region. Noticeably, case population for *rs1124980* significantly deviated from HWE (p=0.004). This might be due to the small number of sample sizes since we found no evidence for genotype calling error or allelic loss in the SNP.

We conducted logistic analyses for three different groups: IDD (MS and NMO), MS, and NMO as cases against healthy controls with 14 SNPs and 4 haplotypes (Table 3). Initial results showed that *rs1788229* was marginally associated with IDD in co-dominant model

Tab. 1. Clinical characteristics of study subjects.

	NMO	MS	IDD (NMO+MS)	Controls
N	98	80	178	237
Age (mean (min.-max.))	39.9 (11–67)	34.3 (14–57)	37.0 (11–67)	47.3 (38–60)
Sex (M/F)	10/88	29/51	39/139	81/156
Onset age (mean±Std)	33.5±12.26	30.08±10.23	31.99±11.50	–
Duration (year, mean±Std)	7.0±4.42	4.45±3.59	5.86±4.26	–

NMO, neuromyelitis optica; MS, multiple sclerosis; IDD, inflammatory demyelinating diseases.



Hap.	rs1788234	rs17842596	rs17208329	rs1788114	rs1788237	rs10513983	rs2051322	rs1124980	rs11661553	rs1788097	rs1788229	rs763361	rs12604328	rs4891781	Freq.
ht1	G	A	C	A	A	C	G	A	A	G	C	C	T	G	0.486
ht2	A	G	T	G	G	T	A	A	T	A	A	T	C	T	0.133
ht3	A	G	C	A	A	T	A	G	T	A	C	T	T	G	0.072
ht4	A	G	T	G	A	C	G	A	T	A	A	T	C	T	0.036
Others	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.273

Fig. 1. (**A**) A gene map of *CD226*. Exons are marked with black boxes while 5' and 3' UTR region are marked with white boxes. SNPs are marked along with their MAFs. (**B**) Haplotypes of *CD226*. A total of four haplotypes with frequencies over 0.05 are used for statistical analyses. (**C**) Linkage disequilibrium plot among *CD226* SNPs.

Tab. 2. Genotype distributions and allele frequencies of *CD226* SNPs

SNP ID	Position	Genotypes (n = 415)			MAF	Heterozygosity	HWE		
							Case	Control	Total
rs1788234	Intron	GG(155)	AG(186)	AA(73)	0.401	0.480	0.126	0.678	0.188
rs17842596	Intron	AA(183)	AG(167)	GG(64)	0.356	0.459	0.229	0.029	0.014
rs17208329	Intron	CC(254)	CT(136)	TT(25)	0.224	0.348	0.247	0.584	0.240
rs1788114	Intron	AA(238)	AG(144)	GG(32)	0.251	0.376	0.400	0.221	0.125
rs1788237	Intron	AA(275)	AG(125)	GG(15)	0.187	0.304	0.880	0.976	0.865
rs10513983	Intron	CC(180)	CT(179)	TT(55)	0.349	0.454	0.139	0.986	0.323
rs2051322	Intron	GG(198)	AG(171)	AA(46)	0.317	0.433	0.353	0.625	0.326
rs1124980	Intron	AA(291)	AG(104)	GG(20)	0.173	0.287	0.004	0.387	0.010
rs11661553	Intron	AA(131)	AT(190)	TT(93)	0.454	0.496	0.491	0.169	0.130
rs1788097	Intron	GG(138)	AG(190)	AA(86)	0.437	0.492	0.774	0.119	0.170
rs1788229	Intron	CC(199)	AC(175)	AA(41)	0.310	0.428	0.775	0.626	0.781
rs763361	Exon (S307G)	CC(142)	CT(191)	TT(82)	0.428	0.490	0.942	0.126	0.223
rs12604328	5′ UTR	TT(223)	CT(157)	CC(34)	0.272	0.396	0.980	0.312	0.394
rs4891781	5′ UTR	GG(215)	GT(164)	TT(36)	0.284	0.407	0.965	0.514	0.555

MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium.

Tab. 3. Logistic analyses of *CD226* SNPs with the risk of inflammatory demyelinating diseases in Korean population (n=415).

SNP/Haplotypes	Allele Change	Inflammatory Demyelinating Disease				Neuromyelitis Optica				Multiple Sclerosis			
		MAF				MAF				MAF			
		IDD (n=178)	NC (n=237)	OR (95%CI)	*p*	NMO (n=98)	NC (n=237)	OR (95%CI)	*p*	MS (n=80)	NC (n=237)	OR (95%CI)	*p*
rs1788234	G>A	0.390	0.409	0.91 (0.66–1.26)	0.59	0.399	0.409	0.99 (0.68–1.45)	0.96	0.380	0.409	0.79 (0.48–1.28)	0.33
rs17842596	A>G	0.347	0.363	0.95 (0.69–1.30)	0.72	0.364	0.363	1.02 (0.70–1.49)	0.91	0.327	0.363	0.82 (0.51–1.30)	0.39
rs17208329	C>T	0.205	0.238	0.87 (0.59–1.26)	0.45	0.212	0.238	0.90 (0.57–1.42)	0.64	0.196	0.238	0.80 (0.46–1.37)	0.41
rs1788114	A>G	0.226	0.270	0.80 (0.56–1.15)	0.23	0.224	0.270	0.79 (0.51–1.22)	0.29	0.228	0.270	0.78 (0.45–1.35)	0.37
rs1788237	A>G	0.163	0.205	0.83 (0.55–1.26)	0.39	0.167	0.205	0.78 (0.47–1.31)	0.35	0.158	0.205	0.95 (0.52–1.74)	0.87
rs10513983	C>T	0.325	0.367	0.85 (0.61–1.18)	0.33	0.328	0.367	0.85 (0.57–1.27)	0.43	0.321	0.367	0.85 (0.52–1.39)	0.51
rs2051322	G>A	0.303	0.327	0.95 (0.68–1.34)	0.78	0.313	0.327	0.97 (0.64–1.46)	0.87	0.291	0.327	0.95 (0.57–1.56)	0.82
rs1124980	A>G	0.174	0.173	0.93 (0.62–1.39)	0.71	0.177	0.173	1.00 (0.62–1.59)	0.98	0.171	0.173	0.79 (0.42–1.48)	0.47
rs11661553	A>T	0.435	0.468	0.83 (0.60–1.14)	0.24	0.439	0.468	0.87 (0.60–1.27)	0.47	0.430	0.468	0.75 (0.46–1.22)	0.24
rs1788097	G>A	0.432	0.441	0.90 (0.66–1.24)	0.53	0.423	0.441	0.89 (0.61–1.30)	0.53	0.443	0.441	0.97 (0.60–1.55)	0.88
rs1788229	C>A	0.278	0.333	0.70 (0.49–1.00)	0.05	0.263	0.333	0.67 (0.44–1.03)	0.07	0.297	0.333	0.75 (0.45–1.27)	0.29
rs763361	C>T	0.416	0.437	0.87 (0.63–1.20)	0.39	0.404	0.437	0.85 (0.58–1.25)	0.42	0.430	0.437	0.90 (0.56–1.43)	0.64
rs12604328	T>C	0.249	0.289	0.79 (0.55–1.13)	0.20	0.237	0.289	0.74 (0.48–1.14)	0.17	0.263	0.289	0.88 (0.52–1.48)	0.63
rs4891781	G>T	0.258	0.304	0.77 (0.54–1.10)	0.15	0.247	0.304	0.72 (0.47–1.11)	0.14	0.272	0.304	0.84 (0.50–1.41)	0.51
CD226_ht1	–	0.497	0.477	1.14 (0.84–1.55)	0.41	0.500	0.477	1.12 (0.78–1.62)	0.53	0.494	0.477	1.16 (0.74–1.82)	0.52
CD226_ht2	–	0.101	0.156	0.71 (0.44–1.15)	0.17	0.096	0.156	0.64 (0.35–1.18)	0.16	0.108	0.156	0.89 (0.46–1.73)	0.74
CD226_ht3	–	0.084	0.063	1.42 (0.78–2.58)	0.25	0.091	0.063	1.46 (0.74–2.90)	0.28	0.076	0.063	1.29 (0.53–3.16)	0.57
CD226_ht4	–	0.039	0.034	0.82 (0.33–2.01)	0.66	0.030	0.034	0.76 (0.26–2.24)	0.62	0.051	0.034	0.65 (0.17–2.57)	0.54

Logistic regression models were used for calculating odds ratios (95% confidence interval) and corresponding *P* values with age and sex as covariates. P values shown are all based on co-dominant models. IDD, inflammatory demyelinating disease; NMO, neuromyelitis optica, MS, multiple sclerosis; NC, normal control; MAF, minor allele frequency; OR (95% CI), odds ratio (95% confidence interval).

Tab. 4. Comparison of genetic effect of *CD226* SNPs with MS in previous studies

References	Population (study size)	P [OR]								
		rs1788234	rs17842596	rs17208329	rs1788114	rs1124980	rs11661553	rs763361	rs12604328	rs4891781
Hafler et al. (2008)	UK and USA (2187 MS vs. 9972 controls, and 1275 trios)	**0.008 [1.06]**	0.10 [1.05]	0.39 [0.95]	**3.91×10^{-4} [1.10]**	**0.004 [1.06]**	**0.005 [0.94]**	**4.54×10^{-4} [1.14]**	**0.02 [1.06]**	**0.02 [1.03]**
Wieczorek et al. (2009)	German (422 MS vs. 1226 controls)	–	–	–	–	–	–	**0.01 [1.23]**	–	–
Pandit et al. (2010)	Indian (197 MS vs. 197 controls)	–	–	–	–	–	–	**0.04 [1.35]**	–	–
Johnson et al. (2010)	African American from USA (918 MS vs. 656 controls)	–	–	–	–	–	–	0.57 [1.04]	–	–
This Study	Korean (80 MS vs. 237 controls)	0.33 [0.79]	0.39 [0.82]	0.41 [0.80]	0.37 [0.78]	0.47 [0.79]	0.24 [0.75]	0.64 [0.90]*	0.63 [0.88]	0.51 [0.84]
	Korean (98 NMO vs. 237 controls)	0.96 [0.99]	0.91 [1.02]	0.64 [0.90]	0.29 [0.79]	0.98 [1.00]	0.47 [0.87]	0.42 [0.85]*	0.14 [0.74]	0.14 [0.72]
	Korean (178 IDD vs. 237 controls)	0.59 [0.91]	0.72 [0.95]	0.45 [0.87]	0.23 [0.80]	0.71 [0.93]	0.24 [0.83]	0.39 [0.87]*	0.20 [0.79]	0.15 [0.77]

Bold values indicate P below 0.05. OR, odds ratio. *minor allele is reversed in the case of *rs763361*.

(p=0.05). However, after a multiple testing correction, the SNP lost its significance (p>0.05).

We also compared our results with previously conducted studies for a possible association of *CD226* SNPs and MS (Table 4). Previous studies had found *rs763361* to be significantly associated with MS in Caucasian and Indian population, but not in African American population ($p=4.54\times10^{-4}$, 0.04, and 0.57, respectively). Although our result did not show significance, it did show a similar trend in odds ratio (OR=1.14 for C allele of *rs763361* in Hafler *et al.*, OR=0.90 for T allele of *rs763361* in our result). In order to see whether our results were due to the racial difference, we calculated the ethnic differences between African, Asian, and Caucasian using chi-square test (Supplementary Table 1). We have also analyzed LD status of the selected *CD226* SNPs in different races.

DISCUSSION

Cluster of domain 226, encoded by *CD226*, is a glycoprotein expressed on the surface of immune cells such as natural killer (NK) cells, platelets, monocytes, and T cells (Pubmed). Previously, several reports have suggested that CD226 is an important factor for the various immune cells. In particular, the protein is known to be a mediator for the adhesion between platelet and vascular endothelial cells (Kojima *et al.* 2003). Also, it is involved in the differentiation and proliferation of immune cells such as megakaryocytic cells and T cells (Shibuya *et al.* 2003; Ma *et al.* 2005). Furthermore, a study has found that CD226 expression deficiency in systemic lupus erythematosus patients led to higher rates of NK cells' apoptosis (Tao *et al.* 2005), while another study has reported the expression of CD226 to be an indicator of T helper 1 (Th1) cell differentiation (Dardalhon *et al.* 2005). As such, *CD226* has been associated with various autoimmune diseases; *rs763361*, which causes amino acid change Gly307Ser, was found to be significantly associated with type 1 diabetes (T1D), MS, autoimmune thyroid disease (AITD), and rheumatoid arthritis (RA) (Hafler *et al.* 2009; Douroudis *et al.* 2009). It is theorized that the polymorphism may affect splice site enhancer or silencer, thus altering RNA splicing, which in turn could disrupt immune recognition of pancreatic islet antigens, T cell repertoire development, or immune regulation and ultimately develop T1D (Todd *et al.* 2007).

To date, many risk genes and environmental factors for MS have been reported, the exact cause of the disease is still unknown (Ascherio & Munger 2007). In the present study, we have performed association analyses of *CD226* SNPs with IDD, MS, and NMO in Korean population. Previous studies in other populations showed several SNPs of *CD226* to be associated with MS. Especially, *rs763361* repeatedly showed significant associations with MS in various populations, as previously stated (Hafler *et al.* 2009; Pandit *et al.* 2011; Wieczorek *et al.* 2009). However, our results have shown that *rs763361* may not be significantly associated with IDDs in Korean population. We suspect that the different genetic makeup among races could be a cause for the apparent lack of association. Most of the studies conducted between *CD226* polymorphisms and MS so far had been limited to Caucasian population. Johnson et al. investigated the relation between *CD226* and MS in African Americans, but the results were not significant (Johnson *et al.* 2010). Another study with a similar design was conducted in Indian population, and although *rs763361* was associated with MS, the significance was marginal (p=0.04) when compared with the results from Caucasian populations.

(Pandit *et al.* 2011) In order to confirm the genetic differences among populations, we have compared MAFs of selected SNPs for three different races (African, Asian, and Caucasian) (Supplementary Table 1). The result showed significant deviations existing between each race. Furthermore, we analyzed the LD plots of *CD226* SNPs in the three races, and each race exhibited a unique LD map (Supplementary Figure 1).

Although there was no significant association detected between *CD226* SNPs and IDDs, our results followed the same direction of genetic effects from the previous results. Most notably, in the case of *rs763361*, previous studies have shown that the major allele C is the risk allele for MS, with OR ranging from 1.04 to 1.35 (Table 4). In our results, the minor allele T showed a protective effect, with OR of 0.90, clearly in the same direction with worldwide trend. Therefore, although our results did not show significance, with a larger sample population for the study, *rs763361* might show an association with IDDs in Korean population.

In summary, we conducted association analyses of *CD226* polymorphisms with two IDDs, MS and NMO. Previous studies between *CD226* and MS in other populations, mostly Caucasian, showed significant relations between them. Especially, *rs763361* was significantly associated with MS in various studies. In our results, no SNPs or haplotypes of *CD226* showed an association with MS or NMO after multiple testing corrections. Further analyses showed that there were significant differences of *CD226* SNPs among races, which might explain the apparent lack of association in our results. Additionally, OR of *rs763361* in our results complied with the previous studies' trends, which could mean that with a larger sample size, the results might be different. Our results may prove useful for researchers who study MS or NMO, and could pave the way for further studies.

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