Journal of Neuroimmunology xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

Short communication

KIR2DL4 (CD158d) polymorphisms and susceptibility to multiple sclerosis

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ARTICLE INFO

Article history:
Received 28 November 2008
Received in revised form 2 March 2009
Accepted 2 March 2009
Available online xxxx

Keywords:
Multiple sclerosis
Natural killer cells
KIR2DL4
Association
Genetics
Autoimmunity

ABSTRACT

Several lines of evidence implicate CD56^{bright} NK cells in the pathogenesis of multiple sclerosis (MS). This proposed immunoregulatory pathway involves already established susceptibility genes such as interleukin-2 receptor alpha (IL2RA) and interleukin-7 receptor (IL7R). We therefore investigated the CD56^{bright} NK cell effector molecule KIR2DL4 for its involvement in genetic susceptibility to MS in a study population of 763 cases and 967 controls. Whereas 26% of the study population has a genotype corresponding to a lack of any functional membrane-bound form of the molecule, no association of the KIR2DL4 transmembrane alleles with susceptibility to MS was observed.

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1. Introduction

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system, in which immune-mediated demyelination and axonal damage leads to a spectrum of neurological symptoms. MS is a complex disease caused by genetic and environmental factors. The past year has seen substantial progress in unraveling the genetic susceptibility to MS, with the identification of the interleukin-7 receptor (IL7R), the interleukin-2 receptor alpha (IL2RA), CLEC16A/KIAA0350 and CD226 as the first established susceptibility genes outside of the HLA region (Gregory et al., 2007; Lundmark et al., 2007; IMSGC, 2007, 2008a,b).

Natural killer (NK) cells are a subset of lymphocytes which have been alleged to play an immunoregulatory role in the prevention of autoimmune diseases (Segal, 2007). Treatment of MS patients with daclizumab, a monoclonal antibody directed against the IL-2 binding site of IL2RA, resulted in a gradual expansion of CD56^{bright} NK-cells that correlated strongly with a decrease in brain inflammatory activity (Bielekova et al., 2006). A similar expansion of CD56^{bright} NK cells in the peripheral blood and the secondary lymphoid tissues was seen after treatment with interferon-beta (IFN-β) (Saraste et al., 2007) and during the last trimester of pregnancy, which is typically associated with a drop in relapse rate (Airas et al., 2008). Notably, the same subset of NK cells with regulatory properties has recently been reported to be decreased in untreated MS patients and patients with clinically isolated syndrome (CIS) compared to healthy control individuals (De Jager et al., 2008).

KIR2DL4 (CD158d) is a member of the killer cell immunoglobulin-like receptor family (KIR) for which expression is restricted to the CD56^{bright} subset of NK cells and is upregulated after treatment with daclizumab (Bielekova et al., 2006). Six different KIR2DL4 transcripts exist, and it has recently been demonstrated that their relative expression is at least partly controlled by genetic variation in exon 6 (Goodridge et al., 2003, 2007). Given this genetically variable expression of KIR2DL4 as a molecule upregulated in an apparent immunoregulatory pathway, we investigated KIR2DL4 transmembrane alleles for their role in susceptibility to MS.

2. Patients and methods

2.1. Patients

A total of 763 patients and 967 unrelated controls were included in this study. All individuals gave informed consent. Amongst the patients and the unrelated controls, 65% and 61% were female, respectively. Amongst the patients, 654 (86%) had bout-onset MS and 101 (13%) primary progressive MS. Average age at onset was 34 ± 11 years and mean multiple sclerosis severity score (MSSS) (Roxburgh et al., 2005) 5.86 ± 3.21.

2.2. Genotyping

The three KIR2DL4 transmembrane alleles (10A-A, 10A-B and 9A) can be distinguished by polymorphisms at positions 149 (A/C, rs16986028), 177 (A/G, rs34134275) and 440 (C/T) in intron 6, the latter actually being redundant because of linkage disequilibrium

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doi:10.1016/j.jneuroim.2009.03.001

Table 1
Assay details.

SNPID	Position intron 6	FW primer	RV primer	Probe 1 & 2
Rs16986028	149	CAGAGGCAGAGCTTTCTAGAGAGA	GGGACGTGAGGATACAGTTCA	CCAGACAA**A**CCTGCCC CAGACA**C**CCTGCCC
Rs34134275	177	CCTGCCCCTGCCTTCAG	TGGAATCTTCTCCTGGATGTGAGT	TCACAGACC**A**TTGCCT TCACAGACC**G**TTGCCT
nt440	440	TTCATGAAATGAGGACCCAGAAGTG	CTTGGTTCATTACAGCAGCATCTG	CCAGCTGTTT**C**GATTG CCAGCTGTTTT**T**GATTG

Abbreviations: SNPID: Single Nucleotide Polymorphism ID, FW: forward, RV: reverse.

(Goodridge et al., 2007). Because of the highly homologous nature of the many genes of the KIR family, it was not possible to design unique primers and probes located adjacent to the polymorphic sites as required for Taqman chemistry. We therefore performed a primary PCR generating a 650 basepair product using the forward primer in exon 6 and reverse primer in exon 7 as described by Witt et al. (2000). One microliter of this primary PCR product was then used as a template for genotyping each of the SNPs with custom Taqman assays (details given in Table 1). The protocol recommended by the manufacturer was followed, except for a reduction of the number of cycles to 20.

2.3. Sequencing

The following thermal cycler conditions were used: 1 cycle of 5 min at 95 °C, 30 cycles of 20 s at 95 °C/45 s at 65 °C/60 s at 72 °C. For six individuals, two for each genotype, the 650-bp PCR product was purified with GenElute™ Gel Extraction Kit and sequenced with the forward and reverse primer on an Applied Biosystems 3730 sequencer.

2.4. Statistical analysis

Summary statistics including genotyping success rate and Hardy–Weinberg equilibrium were calculated with Pedstats (Merlin). Association analysis was performed with the Unphased package (Dudbridge, 2003).

3. Results

Specificity of the assay was evaluated by sequencing six individuals, two for each genotype. Sequencing confirmed specific amplification of the KIR2DL4 sequence, consistency of genotypes obtained by sequencing with those from Taqman assays and the expected number of A repeats (9A or 10A) at the end of exon 6 for each haplotype. Genotyping success rate was >95% and Hardy–Weinberg P value >0.28. No inconsistencies were observed amongst 92 samples typed in duplicate.

Correlation between the three markers was as expected, with observation of the three common haplotypes corresponding to the 10A-A, 10A-B and 9A KIR2DL4 transmembrane alleles. Neither of these alleles differed in frequency between cases and controls (P>0.15) (Table 2). Similarly, no differences in genotype frequency were observed (P>0.16) (Table 3).

4. Discussion

The past year saw significant progress in unraveling the genetic susceptibility to MS, with the identification of IL7R and IL2RA as susceptibility genes (Gregory et al., 2007; Lundmark et al., 2007; IMSGC, 2007, 2008a,b). Their mechanism of effect has not been unraveled yet. A subset of NK cells, CD56^{bright} NK cells, have been proposed as an immunoregulatory pathway in MS and during expansion of this subset upregulation of molecules known to be regulated by IL-2, such as IL7R, was seen (Bielekova et al., 2006). In this study we considered the NK cell effector molecule KIR2DL4, which was also upregulated during CD56^{bright} NK cell expansion after treatment with daclizumab, as a candidate susceptibility gene.

KIR2DL4 is a divergent member of the KIR family because it is capable of both activation (through the arginin residue in the transmembrane domain) and inhibition (through the cytoplasmic immunoreceptor tyrosin-based inhibitory motif or ITIM domain) of NK cells. The only reported ligand of KIR2DL4 is HLA-G, which is produced by fetal-derived trophoblast cells during pregnancy and interacts with KIR2DL4 expressed on uterine NK cells. Very little is known about the ligand for KIR2DL4 expressed on peripheral NK cells (Witt et al., 2000).

The existence of genetically determined isoforms of KIR2DL4 has recently been demonstrated (Goodridge et al., 2007). Whereas two alleles (10A-A and 10A-B) lead to constitutive or inducible expression of the full-length membrane-bound receptor, respectively, the third allele (9A) produces secreted and truncated forms of the receptor, neither of which is expressed on the cell membrane. Individuals homozygous for the latter allele therefore cannot express KIR2DL4 as a membrane-bound receptor, and in heterozygous individuals this allele appears to act as a negative regulator preventing surface expression from the other allele (Goodridge et al., 2007). Ligation of KIR2DL4 has been shown to result in weak activation of cytotoxicity and strong IFN-gamma secretion. Both responses differ according to KIR2DL4 transmembrane genotype, largely as predicted from surface expression data (Goodridge et al., 2003, 2007). 10A-A alleles, by facilitating IFN-gamma production, may promote a Th1 bias. In contrast, 9A alleles, in addition to lacking an effective membrane-bound receptor, produce a soluble KIR2DL4 that may act to block its ligand, thereby introducing a Th2 bias (Goodridge et al., 2007). Furthermore, the 9A allele cannot mediate any inhibitory function as it lacks the intracytoplasmic ITIM domain (Goodridge et al., 2003).

Neither SNPs differentiating between different transmembrane alleles, nor any highly correlated alternative SNPs, are included on the existing genome-wide micro-arrays, and as such no data was readily available to investigate the hypothesis of KIR2DL4 as a candidate susceptibility gene for MS.

We therefore investigated a Belgian study population consisting of 763 cases and 967 controls. We observed that 26% of individuals are homozygous for the 9A allele, and therefore do not express functional membrane-bound forms of KIR2DL4. However, this lack of membrane-bound KIR2DL4 did not appear to affect susceptibility to MS in our study population. Similarly, the two 10A alleles, 10A-A and 10A-B, differing by the ratios of different membrane-bound isoforms and the constitutive or inducible expression of the full-length isoform, had no observed effect on susceptibility to MS.

Table 2
KIR2DL4 transmembrane alleles and susceptibility to MS.

Tansmembrane allele	Haplotype intron 6 nt149-nt177-nt440	Cases	Controls	P
9A	C-G-T	0.52 (762)	0.49 (926)	0.15
10A-A	C-A-T	0.26 (389)	0.27 (510)	0.68
10A-B	A-A-C	0.22 (321)	0.24 (446)	0.20

Overall p ($2df$) = 0.30.

Table 3
KIR2DL4 9A genotype and susceptibility to MS.

Genotype	Cases	Controls	P
9A/9A	0.26 (195)	0.24 (228)	0.16
9A/X[a]	0.50 (372)	0.50 (470)	0.33
X/X	0.23 (169)	0.26 (243)	reference

[a] X = 10A-A and 10A-B alleles.

Please cite this article as: Goris, A., et al., KIR2DL4 (CD158d) polymorphisms and susceptibility to multiple sclerosis, J. Neuroimmunol. (2009), doi:10.1016/j.jneuroim.2009.03.001

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We conclude that despite the involvement of KIR2DL4 in an immunoregulatory pathway in which two established MS susceptibility genes are involved, neither lack of expression nor more subtle variation in expression of this gene appear to contribute to MS susceptibility.

Acknowledgements

RD is supported by a PhD grant from the Wetenschappelijk Onderzoek Multiple Sclerose (WOMS). AG is a Postdoctoral Fellow and BD a Clinical Investigator of the Research Foundation Flanders (FWO-Vlaanderen). BD holds the *Bayer Chair on fundamental genetic research regarding the neuroimmunological aspects of multiple sclerosis* at the KULeuven, Leuven, Belgium.

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Please cite this article as: Goris, A., et al., KIR2DL4 (CD158d) polymorphisms and susceptibility to multiple sclerosis, J. Neuroimmunol. (2009), doi:10.1016/j.jneuroim.2009.03.001