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SHORT COMMUNICATION

Multiple sclerosis associates with LILRA3 deletion in Spanish patients

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*The genetic susceptibility to multiple sclerosis (MS) is only partially explained, and it shows geographic variations. We analyse here two series of Spanish patients and healthy controls and show that relapsing MS (R-MS) is associated with a gene deletion affecting the hypothetically soluble leukocyte immunoglobulin (Ig)-like receptor A3 (LILRA3, 19q13.4), in agreement with an earlier finding in German patients. Our study points to a gene-dose-dependent, protective role for LILRA3, the deletion of which synergizes with HLA-DRB1*1501 to increase the risk of R-MS. We also investigated whether the risk of suffering R-MS might be influenced by the genotypic diversity of killer-cell Ig-like receptors (KIRs), located only ~400 kb telomeric to LILRA3, and implicated in autoimmunity and defence against viruses. The relationship of LILRA3 deletion with R-MS is not secondary to linkage disequilibrium with a KIR gene, but we cannot exclude some contributions of KIR to the genetic susceptibility to R-MS.*
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Keywords: *chromosome 19; gene deletion; killer-cell Immunoglobulin-like receptors; leukocyte immunoglobulin-like receptors; multiple sclerosis; susceptibility*

Introduction

Multiple sclerosis (MS) is a demyelinating disease in which inflammation associates with progressive degeneration of the central nervous system. Its most common clinical form, relapsing-remitting MS (RR-MS), is characterized by episodes of inflammatory lesions in the central nervous system (relapses or exacerbations), followed by the remission of symptoms. In most cases, RR-MS progresses into secondary progressive MS, in which established demyelination and axonal loss cause permanent and progressive disability. RR-MS and secondary progressive MS are considered two stages of the same disease, to which we will refer here as relapsing MS (R-MS). The aetiology of MS is unknown, but this autoimmune disease is believed to be triggered, in a predisposing genetic context, by infection.[1]

The strongest and the best characterized predisposing genetic factor for MS lies, as in many autoimmune diseases, in the major histocompatibility complex of chromosome 6: the HLA-DRB1*1501 allele.[2,3] Polymorphisms of other genes related to the immunological response, namely *IL7R* and *IL2RA*, also contribute, albeit more modestly, to the risk of suffering MS (odds ratio (OR) values: 1.18–1.34).[4–7] In Germans,[8] RR-MS is associated with deficiency of the A3 member of the leukocyte immunoglobulin (Ig)-like receptor family (LILRA3, CD85e), molecule earlier referred to as Ig-like transcript 6 (ILT6), leukocyte Ig-like receptor 4 (LIR-4) or HM43.[9–11]

The *LILRA3* gene maps to the leukocyte receptor complex[12] (LRC, 19q13.4), which encodes multiple polymorphic proteins with Ig-like extracellular domains. Those receptors, expressed in leukocytes of both the lymphoid and the myelomonocytic lineages, belong to three families: the LILR (CD85), the leukocyte-associated inhibitory receptors (LAIRs, CD305) and the killer-cell Ig-like receptors (KIRs, CD158). Members of the LILR and the KIR families also share the capacity of transmitting inhibitory or activating signals upon recognition of human leukocyte antigen (HLA) class I molecules.[13,14]

Among LILRs, which are mainly expressed by myelomonocytic cells, LILRB1 (CD85j) is the best characterized, and it is detected also in T, B and natural killer lymphocytes.[11] LILRB1 modulates leukocyte function and survival upon recognition of self HLA class I molecules in target cells. LILRB1 also recognizes the major histocompatibility complex homologue UL18 protein of human cytomegalovirus with $\sim 10^3$-fold greater affinity than that for HLA molecules. The implications of this putative immune-evasion mechanism in the pathogenicity of cytomegalovirus remain ill defined.[15–17]

Encoded in the central region of the *LILR* gene complex, LILRA3 diverges from the canonical LILR

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structure as it lacks transmembrane and cytoplasmic regions due to a termination codon in the stalk. LILRA3 is likely expressed as a secreted, instead of membrane-anchored, receptor. Some Caucasoids carry a grossly aberrant *LILRA3* allele in which the first seven of its eight exons are deleted,[12] a defect that has been associated with RR-MS in Germans.[8,18] In the same study, French MS patients had an *LILR3* deletion frequency similar to that of German patients, but that frequency was not compared with one of a French healthy control group. Therefore, whether *LILRA3* deletion indeed associates with MS in other populations, and whether its association is primary or secondary to linkage to other polymorphic genes in its vicinity, warrants further studies.

Approximately 400 kb telomeric to *LILRA3*, and only ~10 kb apart from the last *LILR* gene, maps the *KIR* complex, which has an enormously variable content of polymorphic genes.[19–21] Of the 17 *KIR* genes currently recognized, only the two that mark the 5′- and 3′-ends of the *KIR* complex are shared by all human genomes, whereas most individuals lack one or more of the other *KIR* genes. Both the frequency of each *KIR* gene and the manner in which they combine in haplotypes vary substantially among different populations, variations that have been associated with autoimmune and infectious diseases.[22–24]

By means of clonally distributed KIRs that recognize different HLA class I molecules, natural killer cells survey alterations in antigen presentation that often take place in infected cells.[22,25] Best known among pathogens that tamper major histocompatibility complex class I expression are herpesviruses, which can alter T- and natural killer-lymphocyte function by subverting the expression of host major histocompatibility complex molecules, or by encoding viral homologues of these.[26–31] As all of the polymorphisms of the LRC in chromosome 19, natural killer cells, herpesviruses and HLA class I molecules have been implicated in the susceptibility or pathogenesis of MS,[8,32–39] it is also of interest to determine whether the genotypic diversity of KIR is associated with MS and whether such an association could explain, by linkage disequilibrium (LD), the reported relationship between MS and deletion of *LILRA3*.

Results

Association between LILRA3 *deletion and R-MS in Spanish patients*

To determine whether the association between the *LILRA3* gene deletion and MS previously reported in Germans[8,18] is also seen in a Mediterranean genetic context, we studied 126 R-MS patients and 174 healthy unrelated controls of Spanish origin (Hospital Universitario Puerta de Hierro, Madrid, Table 1). To simplify and facilitate the analysis of *LILRA3* genotypes in a clinical context, we designed a single-tube PCR that amplifies both the wild-type and the deleted *LILRA3* alleles, which are then distinguished by their different electrophoretic mobilities in regular agarose gels (Figure 1). This method simplifies further the approach used by Hirayasu *et al*.[41] by using a single reverse PCR primer.

Table 1 Characteristics of the patients[a]

	HUPH	*HdM*	*Total*
Female-to-male ratio	4.25	2.23	3.13
Age at onset			
Mean ± s.d.	26.8 ± 8.3	30.2 ± 9.1	28.4 ± 8.8
Range	11–65	11–58	11–65
Age at time of analysis			
Mean ± s.d.	38.9 ± 9.2	42.0 ± 10.5	40.3 ± 9.9
Range	19–67	23–68	19–68
Expanded disability status scale			
Mean ± s.d.	2.92 ± 2.16	2.99 ± 2.29	2.95 ± 2.21
Range	0–8.5	0–9	0–9
Multiple sclerosis severity score			
Mean ± s.d.	3.30 ± 2.59	3.74 ± 2.81	3.51 ± 2.70
Range	0.05–9.59	0.05–9.79	0.05–9.79
Disease course			
RR	111	79	190
SP	15	20	35
Total	126	99	225

Abbreviations: RR, relapsing-remitting; SP, secondary progressive.
[a]HUPH: Hospital Universitario Puerta de Hierro (Madrid); HdM: replication series from Hospital del Mar (Barcelona). Only patients with definite RR- or SP-MS diagnosis, according to accepted clinical criteria,[40] were included in the study. Controls were healthy unrelated voluntary donors collected from the same geographical region as each series of patients (174 and 157 healthy controls for the HUPH and the HdM series, respectively).

Using such a method, we found that *LILRA3* deficiency is associated with MS also in Spain (Table 2): 31.0% of patients, but only 20.1% of controls, had *LILRA3* deleted in at least one chromosome. To further confirm the association between *LILRA3* deletion and R-MS, we replicated the study in another series of 99 patients from a different centre (Hospital del Mar, Barcelona, Table 1). These showed a similarly higher frequency of the deletion in comparison with 157 healthy controls of the same geographical origin (35.4 vs 26.8%, Table 2), being this increase marginally significant. Taking together both series of patients and controls, deletion of *LILRA3* has a statistically significant OR of 1.62 ($P<0.01$), the aetiologic fraction (percentage of attributable risk[42]) of this genetic marker being 12.6%.

Analysis of genotypes showed that both homo- and heterozygosity for *LILRA3* deletion tended to be more common in R-MS patients than in controls (*LILRA3*-del/del: 4.0 vs 2.1%; *LILRA3*-del/wt: 28.9 vs 21.1%, respectively, in the sum of the two series), although the increase of homozygotes separately was only marginally significant and not seen in the replication series. The global divergence in the distribution of genotypes, however, showed a statistically significant linear trend (Table 2). According to that trend, the risk of suffering MS is highest in individuals lacking *LILRA3* completely in their genome, intermediate in heterozygotes and lowest in individuals having two full-length *LILRA3* alleles. The OR values of homozygosity and heterozygosity for *LILRA3* deletion, in comparison with homozygosity for the wild-type *LILRA3*, were 2.16 and 1.56, respectively,

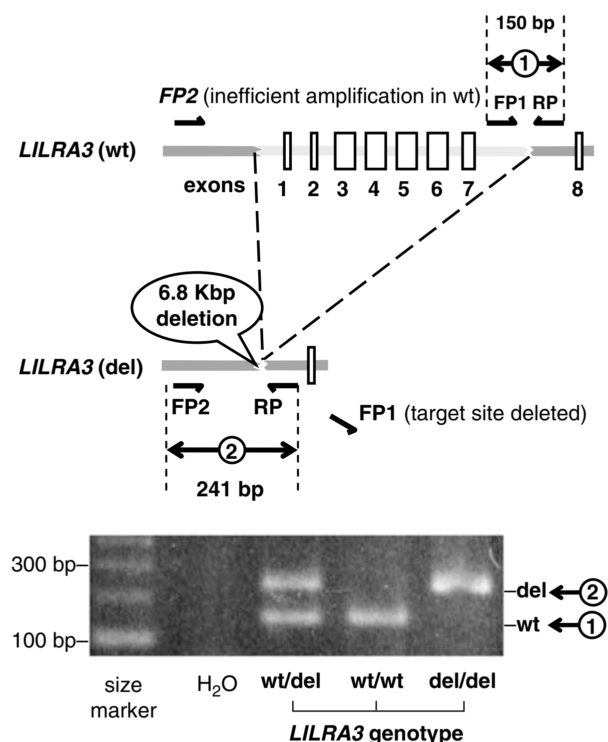

Figure 1 Strategy for *LILRA3* genotyping. Both the complete and the deleted forms of *LILRA3* in homozygous and heterozygous combinations were detected in a single-tube PCR with three primers: two forward (FP1, FILT6i7 + 967, 5′-gacttgtaagggttaaaaagc caa-3′; and FP2, FILT6e2-3638, 5′-catctcgatctgccactgacac-3′), and one reverse (RP, RILT6i7 + 1071, 5′-gacagcagattctaaaacagtgg-3′), oligonucleotides. One hundred nanograms of DNA were amplified with 15 pmol of each primer in 15 μl of PCR buffer (67 mM Tris-HCl, pH 8.8, 16 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 0.01% Tween-20) containing 0.3 U Taq polymerase (EcoTaq, Ecogen, Madrid, Spain) and 100 μM deoxyribonucleotide triphosphates. PCR conditions were 95 °C for 2 min; 10 cycles of 10 s at 94 °C and 40 s at 65 °C; and 20 cycles of 10 s at 94 °C, 20 s at 61 °C and 30 s at 72 °C. In the complete *LILRA3* form, FP1 and RP produce a 150-bp amplicon, whereas FP2 anneals with the DNA, but does not yield a product due to excessive amplicon length (~7 kb). In the deleted form, FP2 and RP yield a 241-bp product, whereas the FP1 target site is lost. The two amplicons were separated by electrophoresis in 2% agarose gels and revealed with ethidium bromide. Exons and introns are not drawn to scale.

greater than those reported for *IL2RA* and *ILR7* polymorphisms.[5,6,35,43] The distribution of genotypes in each series, and in the sum of both, did not diverge significantly from the Hardy–Weinberg equilibrium. The clinical parameters of patients with two complete copies of *LILRA3* did not differ significantly from those who carried the deletion (MS severity score 3.42 ± 2.59 vs 3.70 ± 2.92, respectively), either in homo- or in heterozygosis (not shown).

Analysis of epistatic interaction between deletion of LILRA3 *and HLA-DRB1*1501*

To determine whether there is epistatic interaction between the associations of R-MS with *LILRA3* deletion and the classical risk factor, DRB1*1501, we studied the presence of this HLA allele in patients and controls (41.3 vs 16.6%, respectively, OR 3.54). To enhance the statistical power of all subsequent analyses, the two series of patients and controls derived from each hospital were analysed together (each data set is available upon request). The distribution of positive and negative individuals for each of the *LILRA3* deletion and HLA-DRB1*1501 was then determined (Table 3), and the interaction between the two genetic markers was studied as recommended by Svejgaard and Ryder.[45]

The increase of the *LILRA3* deletion was more conspicuous among DRB1*1501-positive than among DRB1*1501-negative patients (OR 3.08 and 1.40, respectively), but in both cases it lacked significance due to loss of statistical power after stratification and correction for multiple comparisons (Table 3). On the other hand, the association of HLA-DRB1*1501 with the disease is even stronger when *LILRA3* is deleted (OR 6.57 vs 2.99 in the absence of the deletion), and, in isolation, it was stronger than that of the *LILRA3* deletion alone (OR 2.13). Finally, and in line with the earlier findings, having a *LILRA3* deletion and HLA-DRB1*1501 together conferred the highest risk (OR 9.2 when compared with the absence of both markers), indicating that they act synergistically to increase the risk of suffering R-MS.

The severity indexes of HLA-DRB1*1501 patients did not differ from those without this HLA allele (not shown), but patients carrying both a *LILRA3* deletion and DRB1*1501 had significantly worse disability indexes (MS severity score: 4.76 ± 3.04) than patients with other genotypes (MS severity score: 3.32 ± 2.60, $P = 0.0081$), including patients having DRB1*1501, but lacking the *LILRA3* deletion (MS severity score: 3.32 ± 2.56, $P = 0.0248$). The apparent influence of the genotype *LILRA3*^{del}-DRB1*1501 on the prognosis of R-MS warrants confirmation on larger series of patients.

The relationship of R-MS with LILRA3 *deletion is not secondary to an association with a* KIR *gene*

We next searched whether the genotypic diversity of KIRS, which map ~400 kb telomeric to *LILRA3*, could be a primary risk factor for R-MS and whether the association of *LILRA3* deletion with the disease could just reflect LD with a primarily associated *KIR* gene. We studied by PCR[46] the presence or absence of each *KIR* in the genome of 224 R-MS patients and 289 healthy controls, from whom enough DNA was available. Both groups had rather similar frequencies at all *KIR* genes, except for *KIR3DS1*, under-represented among R-MS patients (OR 0.69, $P < 0.05$, Table 4). The lower frequency of *KIR3DS1*, however, lost statistical significance after correcting the *P*-value for the number of comparisons, and the difference was not significant when the two series of patients and controls were compared separately (not shown). No significant LD of *LILRA3* genotypes with *KIR3DS1* was found in either patients or controls; therefore, the deviations in the frequencies of *LILRA3* deletion and *KIR3DS1* are unrelated to each other.

Analysis of LD between *LILRA3* deletion and other *KIR* genes revealed only modestly positive values in healthy controls, but not in patients, for the *KIR2DS2*-*KIR2DL2* pair and for *KIR2DS1* (LD 0.04 and 0.03; relative LD, 0.51 and 0.38, respectively), but they were not significant (corrected *P*-value > 0.05). This is consistent with the lack of LD between *LILRA3* deletion and *KIR* genes observed by Norman *et al*.[47] in British Caucasoids. Furthermore, stratification for the presence or absence of the *LILRA3* deletion showed again no significant difference between patients and controls in the frequency of any *KIR* gene or genotype after

Table 2 Deletion of the *LILRA3* gene is increased in Spanish R-MS patients[a]

LILRA3 deletion	*Patients*	*Controls*	*OR*	*95% CI*	P-*value*
HUPH	31.0% ($n=126$)	20.1% ($n=174$)	1.78	1.01–3.13	0.016
HdM	35.4% ($n=99$)	26.8% ($n=157$)	1.50	0.84–2.67	0.072
Total	32.9% ($n=225$)	23.3% ($n=331$)	1.62	1.09–2.40	0.006

	Patients			*Controls*				
>LILRA3 genotype	*HUPH*	*HdM*	*Total*	*HUPH*	*HdM*	*Total*	*OR*	
wt/wt	87	64	67.1%	139	115	76.7%	1.00[b]	
del/wt	32	33	28.9%	32	38	21.1%	1.56	
del/del	7	2	4.0%	3	4	2.1%	2.16	Linear trend: $P=0.010$

Abbreviations: OR, odds ratio; R-MS, relapsing multiple sclerosis.
[a]Frequencies of individual markers were compared with the χ^2 test or with the Fisher's test when any expected frequency was lower than five individuals. To confirm the association of MS with deletion of *LILRA3*, we used a one-sided *P*-value. The aetiologic fraction indicated in the text (percentage of risk attributable to a given factor) was calculated with the formula $F(\text{OR}-1)/\text{OR}$, where *F* is the phenotypic frequency of the risk factor in patients.[42]
[b]The linear trend between increasing R-MS risk and decreasing *LILRA3* gene dose was evaluated with the Mantel–Haenszel χ^2 test for trend, using EpiInfo-v6. OR values are referred in this case to the *LILRA3-wt/wt* genotype, which is assigned a reference OR of 1.

Table 3 Combined analysis of *LILRA3* deletion and HLA-DRB1*1501 as risk factors for R-MS

LILRA3 deletion	*HLA-DRB1*1501*[a]	*Patients* (n = 225)	*Controls* (n = 331)			
+	+	32 (14.2%)	8 (2.4%)			
+	−	42 (18.7%)	69 (20.8%)			
−	+	61 (27.1%)	47 (14.2%)			
−	−	90 (40.0%)	207 (62.5%)			
				OR	P-*value*	P_C[b]
Is *LILRA3del* associated in the presence of DRB1*1501 (++ vs −+)?[c]		32 vs 61	8 vs 47	3.08	0.0086	0.074
Is *LILRA3del* associated in the absence of DRB1*1501 (+− vs −−)?		42 vs 90	69 vs 207	1.40	0.1477	NS
Is DRB1*1501 associated in the presence of *LILRA3del* (++ vs +−)?		32 vs 42	8 vs 69	6.57	4.8×10^{-6}	4.3×10^{-5}
Is DRB1*1501 associated in the absence of *LILRA3del* (−+ vs −−)?		61 vs 90	47 vs 207	2.99	1.5×10^{-6}	1.3×10^{-5}
Is DRB1*1501 more strongly associated than *LILRA3del* (−+ vs +−)?		61 vs 42	47 vs 69	2.13	0.0057	0.050
Is there a combined association of *LILRA3del* and DRB1*1501 (++ vs −−)?		32 vs 90	8 vs 207	9.20	$<10^{-7}$	$<10^{-6}$

Abbreviations: NS, not significant; OR, odds ratio; R-MS, relapsing multiple sclerosis.
[a]The HLA-DRB1*1501 allele was determined using a sequence-based typing method based on Kotsch *et al*.[44]
[b]Correction of *P*-values for multiple comparisons was performed according to Svejgaard and Ryder.[45]
[c]The symbols ++, +−, −+ and −− refer to the four subgroups of patients and controls indicated in the upper part of the table; the first symbol represents the presence or absence of the *LILRA3* deletion, and the second symbol, that of HLA-DRB1*1501.

correcting *P*-values for multiple comparisons, although deviations of the genes in LD with *LILRA3* in healthy controls were apparent (*KIR2DS2, KIR2DL2* and *KIR2DS1*, Table 4). Finally, no differences between MS patients and controls were appreciated in the distribution of 'A'- and 'B'-type haplotypes (for example, 29.5% 'AA' genotypes in patients vs 24.2% in controls).

In summary, we have confirmed that the deletion of *LILRA3* associates with R-MS in Spain, as reported in German patients, and we have shown for the first time that it synergizes with HLA-DRB1*1501 in increasing susceptibility to the disease. On account of the nature of this genetic trait—a 6.7-kb deletion—it could not be detected by the genome-wide scans for susceptibility loci that focused on micropolymorphisms. Furthermore, among the markers that flank the LRC in chromosome 19 and were studied in earlier genomic scans,[4,5,38,48,49] D19S601 and D19S571 are the closest to *LILRA3*, but they lie more than 1.5 Mb away from it. LD is generally not detectable across such a distance,[47,50–52] which may have prevented detecting the association of *LILRA3* with MS through secondary association of flanking linked markers. For the same reason, previously reported associations of markers in chromosome region 19q13 with MS (reviewed in Pericak-Vance *et al*.[38] and Yeo *et al*.[49]) are possibly unrelated with those of

Table 4 Distribution of frequencies (in %) of variable *KIR* genes in R-MS patients and healthy controls[a]

Total		*2DS2*	*2DL2*	*2DS3*	*2DL3*	*2DP1*	*2DL1*	*3DL1*	*2DS4*	*3DS1*	*2DL5*	*2DS5*	*2DS1*
Patients	N = 224	58.0	58.7	33.5	84.8	95.5	95.5	94.2	94.2	34.8*	53.6	29.0	37.9
Controls	N = 289	59.2	59.5	32.2	88.6	96.2	96.2	97.2	97.2	43.6*	57.1	31.5	44.3
Stratified for deletion of LILRA3													
Positive for LILRA3del													
Patients	N = 74	52.7*	52.1*	37.8	86.5	98.6	98.6	87.8	87.8	37.8	52.7	29.7	37.8*
Controls	N = 67	70.1*	70.1*	40.3	92.4	98.5	98.5	95.5	95.5	53.7	64.2	34.3	55.2*
Negative for LILRA3del													
Patients	N = 150	60.7	62.0	31.5	83.9	94.0	94.0	97.3	97.3	33.3	54.0	28.7	38.0
Controls	N = 222	55.9	56.3	29.7	87.8	95.5	95.5	97.7	97.7	40.5	55.0	30.6	41.0

Abbreviation: R-MS, relapsing multiple sclerosis.
*Uncorrected *P*-value <0.05; *P*-value not significant after correcting for multiple comparisons.
[a]Presence or absence in the genome of each *KIR* gene was determined as described earlier.[46] The gene order is based on that of the *KIR* complex[21,53]—the genes that define the 'A' type of haplotype (*KIR2DL3* through *KIR2DS4*) are represented in the middle, preceded and followed, respectively, by genes characteristic of the centromeric and the telomeric parts of 'B' haplotypes. Framework genes and pseudogenes found in all individuals are not shown. Frequencies were compared using the χ^2 or Fisher's test, as appropriate, and *P*-values were corrected for multiple comparisons according to Svejgaard and Ryder.[45]

LILRA3. Finally, scans of markers within the LRC itself have been complicated by the fact that the complex is constituted by highly homologous duplicated genes (*LILR* and *KIR*) variably arranged in tightly packed tandems.[19]

The relationship of R-MS with *LILRA3* deletion that we have observed is not secondary to association of either with a *KIR* gene. Nevertheless, an apparent under-representation of *KIR3DS1* in patients might indicate a minor and independent protective role of *KIR* haplotypes carrying this gene; or else, it could be a fortuitous result because of the multiple comparisons performed in this study. To circumvent the latter problem and elucidate the possible influence of *KIR3DS1* on the susceptibility to MS, specific studies on that gene in additional series of patients are required.

In favour of a primary role for LILRA3 in the protection from MS is its homology to LILRB1, a receptor expressed in several leukocyte lineages, which recognizes both self-HLA class I molecules and the product of at least one member of herpesviridae,[15–17] family of viruses implicated in the pathogeny of MS.[35,36] Against such a primary role could be a study on Finnish MS patients published during preparation of our manuscript by Bonetti *et al.*,[52] who found only a minor, nonsignificant increase of the *LILRA3-del/del* genotype. This study, however, did not analyse the *LILRA3* deletion in heterozygosity, but only in the homozygous state, which, analysed isolately, was not significantly elevated in our own series. In addition, the Finnish study did not distinguish R-MS patients from those with a non-relapsing disease. Yet, the genetic background of Finnish MS patients might differ from that of Spaniards and Germans. Intriguingly, mutations causing LILRA3 deficiency are common in the Japanese population, who have a low MS incidence rate and a high frequency of non-classical forms.[41] Establishing whether the association of MS with *LILRA3* deletion is primary or secondary to LD of this defect with another, as yet unidentified, risk locus, requires further studies on polymorphisms located in the *LILR* gene complex and functional studies on LILRA3 itself, still hampered by a lack of specific antibodies.

Conflict of interest

The authors declare no conflict of interest.

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References

1 Compston A, Coles A. Multiple sclerosis. *Lancet* 2008; **372**: 1502–1517.
2 Schmidt H, Williamson D, Ashley-Koch A. HLA-DR15 haplotype and multiple sclerosis: a HuGE review. *Am J Epidemiol* 2007; **165**: 1097–1109.
3 Svejgaard A. The immunogenetics of multiple sclerosis. *Immunogenetics* 2008; **60**: 275–286.
4 Haines JL, Bradford Y, Garcia ME, Reed AD, Neumeister E, Pericak-Vance MA *et al.* Multiple susceptibility loci for multiple sclerosis. *Hum Mol Genet* 2002; **11**: 2251–2256.
5 Hafler DA, Compston A, Sawcer S, Lander ES, Daly MJ, De Jager PL *et al.* Risk alleles for multiple sclerosis identified by a genomewide study. *N Engl J Med* 2007; **357**: 851–862.
6 Gregory SG, Schmidt S, Seth P, Oksenberg JR, Hart J, Prokop A *et al.* Interleukin 7 receptor alpha chain (IL7R) shows allelic and functional association with multiple sclerosis. *Nat Genet* 2007; **39**: 1083–1091.
7 Lundmark F, Duvefelt K, Iacobaeus E, Kockum I, Wallstrom E, Khademi M *et al.* Variation in interleukin 7 receptor alpha chain (IL7R) influences risk of multiple sclerosis. *Nat Genet* 2007; **39**: 1108–1113.
8 Koch S, Goedde R, Nigmatova V, Epplen JT, Muller N, de Seze J *et al.* Association of multiple sclerosis with ILT6 deficiency. *Genes Immun* 2005; **6**: 445–447.

9 Arm JP, Nwankwo C, Austen KF. Molecular identification of a novel family of human Ig superfamily members that possess immunoreceptor tyrosine-based inhibition motifs and homology to the mouse gp49B1 inhibitory receptor. *J Immunol* 1997; **159**: 2342–2349.
10 Borges L, Hsu ML, Fanger N, Kubin M, Cosman D. A family of human lymphoid and myeloid Ig-like receptors, some of which bind to MHC class I molecules. *J Immunol* 1997; **159**: 5192–5196.
11 Colonna M, Navarro F, Bellon T, Llano M, Garcia P, Samaridis J *et al*. A common inhibitory receptor for major histocompatibility complex class I molecules on human lymphoid and myelomonocytic cells. *J Exp Med* 1997; **186**: 1809–1818.
12 Torkar M, Haude A, Milne S, Beck S, Trowsdale J, Wilson MJ. Arrangement of the ILT gene cluster: a common null allele of the ILT6 gene results from a 6.7-kbp deletion. *Eur J Immunol* 2000; **30**: 3655–3662.
13 López-Botet M, Bellón T. Natural killer cell activation and inhibition by receptors for MHC class I. *Curr Opin Immunol* 1999; **11**: 301–307.
14 Lanier LL. Face off – the interplay between activating and inhibitory immune receptors. *Curr Opin Immunol* 2001; **13**: 326–331.
15 Cosman D, Fanger N, Borges L, Kubin M, Chin W, Peterson L *et al*. A novel immunoglobulin superfamily receptor for cellular and viral MHC class I molecules. *Immunity* 1997; **7**: 273–282.
16 Chapman TL, Heikeman AP, Bjorkman PJ. The inhibitory receptor LIR-1 uses a common binding interaction to recognize class I MHC molecules and the viral homolog UL18. *Immunity* 1999; **11**: 603–613.
17 Valés-Gómez M, Shiroishi M, Maenaka K, Reyburn HT. Genetic variability of the major histocompatibility complex class I homologue encoded by human cytomegalovirus leads to differential binding to the inhibitory receptor ILT2. *J Virol* 2005; **79**: 2251–2260.
18 Kabalak G, Koch S, Dobberstein B, The YH, Matthias T, Schnarr S *et al*. Immunoglobulin-like transcripts as risk genes for autoimmunity. *Ann N Y Acad Sci* 2007; **1110**: 10–14.
19 Wilson MJ, Torkar M, Haude A, Milne S, Jones T, Sheer D *et al*. Plasticity in the organization and sequences of human KIR/ILT gene families. *Proc Natl Acad Sci USA* 2000; **97**: 4778–4783.
20 Vilches C, Parham P. KIR: diverse, rapidly evolving receptors of innate and adaptive immunity. *Annu Rev Immunol* 2002; **20**: 217–251.
21 Norman PJ, Parham P. Complex interactions: the immunogenetics of human leukocyte antigen and killer cell immunoglobulin-like receptors. *Semin Hematol* 2005; **42**: 65–75.
22 Parham P. MHC class I molecules and KIRs in human history, health and survival. *Nat Rev Immunol* 2005; **5**: 201–214.
23 Khakoo SI, Carrington M. KIR and disease: a model system or system of models? *Immunol Rev* 2006; **214**: 186–201.
24 Estefanía E, Gómez-Lozano N, Portero F, de Pablo R, Solís R, Sepúlveda S *et al*. Influence of KIR-gene diversity on the course of HSV-1 infection: resistance to the disease is associated with the absence of KIR2DL2 and KIR2DS2. *Tissue Antigens* 2007; **70**: 34–41.
25 Moretta A, Locatelli F, Moretta L. Human NK cells: from HLA class I-specific killer Ig-like receptors to the therapy of acute leukemias. *Immunol Rev* 2008; **224**: 58–69.
26 Reyburn HT, Mandelboim O, Vales-Gomez M, Davis DM, Pazmany L, Strominger JL. The class I MHC homologue of human cytomegalovirus inhibits attack by natural killer cells. *Nature* 1997; **386**: 514–517.
27 Farrell HE, Davis-Poynter NJ, Andrews DM, Degli-Esposti MA. Function of CMV-encoded MHC class I homologues. *Curr Top Microbiol Immunol* 2002; **269**: 131–151.
28 Gumá M, Angulo A, Lopez-Botet M. NK cell receptors involved in the response to human cytomegalovirus infection. *Curr Top Microbiol Immunol* 2006; **298**: 207–223.
29 Ressing ME, Horst D, Griffin BD, Tellam J, Zuo J, Khanna R *et al*. Epstein–Barr virus evasion of CD8 and CD4 T cell immunity via concerted actions of multiple gene products. *Semin Cancer Biol* 2008; **25**: 25.
30 Pappworth IY, Wang EC, Rowe M. The switch from latent to productive infection in Epstein–Barr virus-infected B cells is associated with sensitization to NK cell killing. *J Virol* 2007; **81**: 474–482.
31 Guerreiro-Cacais AO, Uzunel M, Levitskaya J, Levitsky V. Inhibition of heavy chain and beta2-microglobulin synthesis as a mechanism of major histocompatibility complex class I downregulation during Epstein–Barr virus replication. *J Virol* 2007; **81**: 1390–1400.
32 Christensen T. Human herpesviruses in MS. *Int MS J* 2007; **14**: 41–47.
33 Morandi B, Bramanti P, Bonaccorsi I, Montalto E, Oliveri D, Pezzino G *et al*. Role of natural killer cells in the pathogenesis and progression of multiple sclerosis. *Pharmacol Res* 2008; **57**: 1–5.
34 Sospedra M, Martin R. Immunology of multiple sclerosis. *Annu Rev Immunol* 2005; **23**: 683–747.
35 Lunemann JD, Kamradt T, Martin R, Munz C. Epstein–Barr virus: environmental trigger of multiple sclerosis? *J Virol* 2007; **81**: 6777–6784.
36 Serafini B, Rosicarelli B, Franciotta D, Magliozzi R, Reynolds R, Cinque P *et al*. Dysregulated Epstein–Barr virus infection in the multiple sclerosis brain. *J Exp Med* 2007; **204**: 2899–2912.
37 Friese MA, Jakobsen KB, Friis L, Etzensperger R, Craner MJ, McMahon RM *et al*. Opposing effects of HLA class I molecules in tuning autoreactive CD8+ T cells in multiple sclerosis. *Nat Med* 2008; **14**: 1227–1235.
38 Pericak-Vance MA, Rimmler JB, Martin ER, Haines JL, Garcia ME, Oksenberg JR *et al*. Linkage and association analysis of chromosome 19q13 in multiple sclerosis. *Neurogenetics* 2001; **3**: 195–201.
39 Yeo TW, De Jager PL, Gregory SG, Barcellos LF, Walton A, Goris A *et al*. A second major histocompatibility complex susceptibility locus for multiple sclerosis. *Ann Neurol* 2007; **61**: 228–236.
40 McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD *et al*. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol* 2001; **50**: 121–127.
41 Hirayasu K, Ohashi J, Kashiwase K, Takanashi M, Satake M, Tokunaga K *et al*. Long-term persistence of both functional and non-functional alleles at the leukocyte immunoglobulin-like receptor A3 (LILRA3) locus suggests balancing selection. *Hum Genet* 2006; **119**: 436–443.
42 Green A. The epidemiologic approach to studies of association between HLA and disease. II. Estimation of absolute risks, etiologic and preventive fraction. *Tissue Antigens* 1982; **19**: 259–268.
43 Consortium IMSG. Refining genetic associations in multiple sclerosis. *Lancet Neurol* 2008; **7**: 567–569.
44 Kotsch K, Wehling J, Blasczyk R. Sequencing of HLA class II genes based on the conserved diversity of the non-coding regions: sequencing based typing of HLA-DRB genes. *Tissue Antigens* 1999; **53**: 486–497.
45 Svejgaard A, Ryder LP. HLA and disease associations: detecting the strongest association. *Tissue Antigens* 1994; **43**: 18–27.
46 Vilches C, Castaño J, Gómez-Lozano N, Estefanía E. Facilitation of KIR genotyping by a PCR-SSP method that amplifies short DNA fragments. *Tissue Antigens* 2007; **70**: 415–422.
47 Norman PJ, Cook MA, Carey BS, Carrington CV, Verity DH, Hameed K *et al*. SNP haplotypes and allele frequencies show evidence for disruptive and balancing selection in the human leukocyte receptor complex. *Immunogenetics* 2004; **56**: 225–237.
48 Sawcer S, Ban M, Maranian M, Yeo TW, Compston A, Kirby A *et al*. A high-density screen for linkage in multiple sclerosis. *Am J Hum Genet* 2005; **77**: 454–467.

49 Yeo TW, Roxburgh R, Maranian M, Singlehurst S, Gray J, Hensiek A *et al*. Refining the analysis of a whole genome linkage disequilibrium association map: the United Kingdom results. *J Neuroimmunol* 2003; **143**: 53–59.
50 Abecasis GR, Noguchi E, Heinzmann A, Traherne JA, Bhattacharyya S, Leaves NI *et al*. Extent and distribution of linkage disequilibrium in three genomic regions. *Am J Hum Genet* 2001; **68**: 191–197.
51 Dunning AM, Durocher F, Healey CS, Teare MD, McBride SE, Carlomagno F *et al*. The extent of linkage disequilibrium in four populations with distinct demographic histories. *Am J Hum Genet* 2000; **67**: 1544–1554.
52 Bonetti A, Koivisto K, Pirttila T, Elovaara I, Reunanen M, Laaksonen M *et al*. A follow-up study of chromosome 19q13 in multiple sclerosis susceptibility. *J Neuroimmunol* 2009; **208**: 119–124.
53 Yawata M, Yawata N, Abi-Rached L, Parham P. Variation within the human killer cell immunoglobulin-like receptor (KIR) gene family. *Crit Rev Immunol* 2002; **22**: 463–482.