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Pemphigus is associated with *KIR3DL2* expression levels and provides evidence that KIR3DL2 may bind HLA-A3 and A11 *in vivo*

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Abstract

Although HLA-A3 and A11 have been reported to be ligands for KIR3DL2, evidences for *in vivo* relevance of this interaction is still missing. To explore the functional importance of *KIR3DL2* allelic variation, we analyzed the autoimmune disease pemphigus foliaceus, known to be negatively associated with activating *KIR* genes. The frequency of *KIR3DL2*001* was increased in patients (OR=2.04, p=0.007). The risk was higher for the presence of both *KIR3DL2*001* and HLA-A3 or A11 (OR=3.76, p=0.013), providing the first evidence that HLA-A3 and A11 may interact with KIR3DL2 *in vivo*. The non-synonymous single nucleotide polymorphism *1190T* (*rs3745902*) was associated with protection (OR=0.52, p=0.018). This SNP results in a threonine to methionine substitution. Individuals who have methionine in this position exhibit a lower percentage of KIR3DL2 positive cells and also lower intensity of KIR3DL2 on expressing cells; additionally, we show that the expression of KIR3DL2 is independent of other killer cell immunoglobulin-like receptors. Pemphigus foliaceus is a very unique complex disease strongly associated with immune-related genes. It is the only autoimmune disease known to be endemic, showing a strong correlation with environmental factors. Our data demonstrate that this relatively unknown autoimmune disease may facilitate understanding of the molecular mechanisms of KIR3DL2 ligand recognition.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

KIR; autoimmunity; allele polymorphism; ligands; expression; pemphigus foliaceus; MHC; natural killer cells

1. Introduction

Killer cell immunoglobulin-like receptors (KIR) are expressed on the surface of natural killer (NK) cells and T cells, regulating the balance of activating and inhibitory signals [1]. These receptors are important for immune defense [2] and influence placentation during pregnancy [3]. *KIR* genes are located on chromosome 19q13.42 within the leukocyte receptor complex [4],[5]. They vary in number (presence/absence polymorphism) and in allelic polymorphism. Two gene content haplogroups have been reported: haplogroup A is characterized by many inhibitory genes and only one short tailed activating gene (*KIR2DS4*) and is the most frequent in worldwide populations; haplogroup B shows high diversity of combinations of activating genes [6].

The framework *KIR3DL2* is the longest *KIR* gene and spans 16,256bp. It is also one of the most polymorphic *KIR*, with 86 described alleles [7]. The KIR3DL2 receptor comprises a 140-kDa dimeric molecule with three extracellular domains and a long intracellular tail, carrying two immune receptor tyrosine-based inhibitory motifs (ITIM) [8]. A long cytoplasmic tail is normally a hallmark of an inhibitory *KIR*; in contrast, activating *KIR* generally exhibit a short tail and associate with the immune receptor tyrosine-based activating motif (ITAM)-containing signaling chain DAP12 [9],[10] . Thus, the nomenclature of this gene family is based on the number of extracellular domains and the size of the cytoplasmic tail; i.e. KIR3DL, three extracellular domains, long cytoplasmic tail [11].

In vitro studies have suggested that HLA-A3 and HLA-A11 are ligands of KIR3DL2 [12] but these interactions appear to be weak, and highly peptide dependent. Indeed, to date only one peptide (and variants thereof) has been reported to support A3 and A11 recognition by KIR3DL2. Moreover, the *in vivo* significance of this interaction is unclear, especially in light of the finding that this interaction does not lead to fully functional NK cells, in contrast to other inhibitory KIR/ligand pairs [13].

Although *KIR* polymorphism has been studied in many Brazilian populations [14]-[16], *KIR3DL2* allele diversity has not been well characterized. In addition, there is little information about the role of *KIR3DL2* allele polymorphism in diseases. Here, we analyzed the influence of *KIR3DL2* alleles in an autoimmune disease cohort from Brazil. Pemphigus foliaceus (PF) is an autoimmune blistering disease of skin characterized by autoantibodies against desmoglein-1, a molecule important for cell adhesion [17]. Many genes, including *HLA* class II, have been reported to associate with differential susceptibility to PF [18]-[20]. Activating *KIR* genes are often associated with protection in infectious diseases and susceptibility to autoimmunity [21]. However, we recently showed that the presence of higher numbers and ratios of activating *KIR* genes protect from PF [22]. Pemphigus is endemic not only in Brazil, but also in Tunisia and Colombia and the disorder is

sporadically seen around the world [23],[24]. PF is strongly related to environmental factors, possibly due to substances contained in the saliva of hematophagous insects or to infectious microorganisms that trigger the disease in susceptible individuals [25],[26]. This particularity of PF may explain why activating KIR has been associated with protection against the development of this disease.

Allelic polymorphism of inhibitory *KIR* may result in functional differences, shifting the balance of inhibitory and activating signals in NK cells. *KIR3DL2* is highly polymorphic and present in virtually all haplotypes [6],[27]. In addition to the fact that all individuals carry this gene, KIR3DL2 is highly expressed on NK cells [28]. KIR3DL2 is, therefore, a strong candidate for disease association studies as some *KIR3DL2* allotypes could present differential inhibitory effects and affect susceptibility to diseases. Moreover, we previously demonstrated that activating *KIR* protect against PF [22]. Therefore, we hypothesized that stronger inhibitory KIR3DL2 allotypes could confer risk to PF.

Here, we show that the allele *KIR3DL2*001* and the single nucleotide polymorphism *1190T* (*rs3745902*) are associated with differential susceptibility to pemphigus foliaceus. We present genetic epidemiological support for an *in vivo* interaction between KIR3DL2 and HLA-A3 and A11. Moreover we also show that the protective SNP *1190T* marks KIR3DL2 differential expression levels suggesting the necessity for a threshold of inhibition for the development of PF.

2. Results and Discussion

2.1. *KIR3DL2*001* increases the susceptibility to PF

KIR allelic polymorphism and its effect on disease outcome are not well characterized. *KIR* presence/absence polymorphism as well as combinations of KIR-HLA have been associated with several infectious and autoimmune diseases [21],[29]. In contrast to other autoimmune diseases in which *KIR* polymorphism has been associated, we have reported that activating *KIR* genes are protective against PF [22]. Here, we hypothesized that different KIR3DL2 allotypes could be greater inhibitory than others, what could contribute to shift the balance of activating and inhibitory signals on the NK cell surface. Based on previous results, allotypes that show greater inhibition could potentially confer risk to PF.

To test this hypothesis we sequenced *KIR3DL2* in patients and controls. The frequencies of all alleles are shown in Supplementary Table S1 and frequencies of the most common alleles are shown in Figure 1. The allele *KIR3DL2*001* was associated with increased susceptibility to PF for both carrier and allele frequencies in Euro-descendants (OR=2.1, p=0.015; OR=2.04, p=0.007 respectively) (Table 1). A statistically non-significant increase of *KIR3DL2*001* was seen in the Afro-descendants. The risk was increased for homozygotes *KIR3DL2*001/001* (OR=3.83; p=0.025), showing an additive effect. The HLA ligand specificity of KIR3DL2 remains unclear, although HLA-A*03 and A*11 tetramers have been shown to bind to KIR3DL2 when folded with specific EBV peptides [12]. The fact that the susceptibility was increased when we analyzed carriage of *KIR3DL2*001* together with the presence of at least one copy of HLA-A3 or HLA-A11 (OR=3.76, p=0.013 – Table 2) suggests that these HLA-A molecules interact with KIR3DL2 *in vivo*. Furthermore, the

presence of ligands without the receptor was not associated with PF (Table 2). Together with previous data showing that activating *KIR* genes protect from PF, these results suggest that *KIR3DL2*001* may bind HLA-A3 and A11 *in vivo* and that this interaction stronger inhibitory receptor than other alleles. In addition, we tested the combination of *KIR3DL2*001* with HLA-Bw6 (previously associated with increased susceptibility to PF [22]) or other common class I alleles. No additive effect was seen for *KIR3DL2*001* + Bw6 (OR=1.89 p=0.02) or *KIR3DL2*001* + other common *HLA-A, B* or *C* alleles (data not shown), corroborating the hypothesis that A3 and A11 may be functional ligands of KIR3DL2.

In addition to A3 and A11, KIR3DL2 has also been shown to recognize B27, a family of HLA alleles closely associated with ankylosing spondylitis and B27-associated-arthritides [30]-[32]. In our cohort, the frequency of *HLA-B*27* is very low ($f \leq 0.03$ in patients and controls; Supplementary Table S2), and therefore, not informative. *HLA-A*03*, in contrast, is one of the most common *HLA-A* alleles (Supplementary Table S2) and combined with *HLA-A*11*, represents a ligand frequency higher than *B*27* in the majority of worldwide populations, making these alleles the most likely primary KIR3DL2 *in vivo* ligands.

2.2. Cytoplasmic variant *1190T* protects from PF

We next examined if individual SNPs, rather than alleles, were related to the susceptibility to PF. We excluded those SNPs present in low frequency or those that could be explained predominantly by a single allele. Three remaining SNPs were tested (Table 1), two in exon 3 (*322G>A, rs654686*; *337C>G, rs3188286*) and one in exon 9 (*1190C>T, rs3745902*). The variant *1190T* was negatively associated with PF in Euro-descendants (OR=0.52, p=0.018). No significant association for this variant was detected in Afro-descendants. As we reported before [22], the *KIR* relative effect in Euro-descendants is possibly higher than in Afro-descendants as a consequence of differences between ethnicities and the complexity of this disease.

The SNP *1190C>T* causes an amino acid change (Thr376Met) in the long cytoplasmic tail. Although no major changes in mature protein are predicted for this replacement, the physicochemical properties of these two amino acids differ. Methionine is hydrophobic while threonine is polar due to the presence of a hydroxyl group. In the Grantham scale, which measures the physicochemical distance between all amino acid pairs and ranges from 5 to 215, the value for threonine and methionine is 81 [33]. Low values indicate conservative and high values indicate radical replacements. Even though it is an intermediate value, the frequency of amino acid replacements that show such difference is relatively low in mammalian proteins. Zhang [34], studying mammalian nuclear genes, estimated that only 17% of the transitions cause non-synonymous substitutions that alter the polarity of the amino acids.

Another more important characteristic that differs between these two amino acids is that threonine may be phosphorylated by protein kinases in eukaryotic cells. Although tyrosine phosphorylation is critical for both ITIM and ITAM function by facilitating the recruitment of the protein tyrosine phosphatases Src-homology domain containing phosphatase (SHP)-1 and -2, serine/threonine phosphorylation of KIR cytoplasmic domains can also play an

important role in receptor expression and cycling, as has been reported for KIR3DL1 [35]. Importantly, Thr376 lies at position -1 relative to the tyrosine residue predicted to be critical within the KIR3DL2 ITIM. Moreover, experimental evidence supports a role for amino acids neighboring the ITIM tyrosine in controlling the ability of the receptor to interact with downstream molecules such as SHP-1. In particular, substitution at position -2 relative to the tyrosine in the ITIM can prevent its interaction with the protein phosphatases SHP-1 and SHP-2 [36]. Although to date position -1 has not been directly implicated in ITIM function, the Thr376Met it is certainly conceivable that this substitution may alter the receptor's inhibitory function.

2.3. KIR3DL2 exhibits differential expression levels that correlate with *1190T*

In addition to the inhibitory capacity of the ITIM, this motif has also been recently implicated in the endocytosis of KIR in its unphosphorylated form due to interactions with the adaptor protein AP-2 [37]. Thus, modulation of this motif may also influence KIR3DL2 function via control of receptor internalization and/or cell surface expression levels. We tested this possibility by examining if the *1190C>T* SNP might correlate with KIR3DL2 expression. Direct examination of KIR3DL2 expression levels on the NK cells of healthy donors revealed that the percentage of KIR3DL2+ NK cells was 2.6 fold higher in *1190C* homozygotes as compared donors homozygous for *1190T* (Figure 2). In addition to the percentage of positive NK cells, the *1190C>T* SNP also correlated with differential expression levels of KIR3DL2; MFIs of KIR3DL2+ NK cells in *1190T* homozygotes was 1.4 fold lower than in *1190C* homozygotes. Our data are the first to correlate differential expression of KIR3DL2 with a single nucleotide polymorphism and they reaffirm the functional significance of this SNP and corroborate our hypothesis. The lower expression frequency of Met376 allotypes reduces the size of the NK cell subset that is inhibited by KIR3DL2, and the lower level of receptor expression on positive cells would be predicted to reduce their inhibitory potential. Thus the NK cell population in 1190T individuals is biased towards lower inhibition in two ways, offering an attractive hypothesis as to why this SNP is negatively associated with PF.

2.4. *1190T* association is apparently independent of gene content haplotypes

There is strong linkage disequilibrium (LD) between *KIR3DL2* alleles and other genes [38], [39]. Presence of haplotype A has been shown to be associated with increased susceptibility to PF [22]. Thus we wondered whether haplotype A might exhibit overall higher *KIR* expression suggesting the LD between *KIR3DL2* and other genes in the haplotype might be, in fact, responsible for the correlation between *1190T* and differential expression levels. To answer this question, we analyzed KIR3DL1 expression in a subset of individuals in which KIR3DL2 expression was also measured. When we separated them based on amino acid position 376 caused by SNP *1190C>T* genotypes, TT and TM had a very similar percentage of KIR3DL1+ cells (p=0.98) with similar levels of KIR3DL1 expression per cell (p=0.50) (Figure 3). We also did not see any correlation between KIR3DL1 and KIR3DL2 expression among these individuals (Figure 4). These results suggest the differential expression levels of KIR3DL2 are in fact independent of KIR3DL1. Therefore, we conclude that *KIR* haplotype gene content is not responsible for the differential expressions levels seen in KIR3DL2.

2.5. *KIR3DL2*001* and *1190T* possibly have distinct effects on PF susceptibility

Interestingly, the concomitant presence of the variant *1190T* and the ligands HLA-A3 and A11 has no effect on PF disease susceptibility (Table 2). Given the cytoplasmic location of this variant we would not expect it to affect binding affinities, rather our data show these *KIR3DL2* alleles have lower expression levels perhaps reducing KIR3DL2 expression to levels too low to effectively function as an inhibitory receptor. In contrast, the odds ratio of KIR3DL2*001 combined with presence of A3/A11 (OR=3.76) is much stronger than the odds ratio of the presence of KIR3DL2*001 alone (OR=2.04). Therefore, we see a stronger (additive) susceptibility effect when we combined KIR3DL2*001+A3/A11. *KIR3DL2*002* is the most frequent allele in our cohort and like *KIR3DL2*001* does not carry the variant *1190T*. The fact the highly expressed allele *KIR3DL2*002* is not associated with PF (OR=1.21; p=0.507) tells us that there are likely two distinct factors contributing to susceptibility to PF: a) differential inhibition conferred by *KIR3DL2*001* compared to other highly expressed alleles, and b) the differential levels of KIR3DL2 expression associated with *1190T*.

To test this hypothesis, we performed regression analysis with stepwise selection. In our model, we included the presence of variants *KIR3DL2*001*, *1190T*, the concomitant presence of KIR3DL2*001+ligand (A3 and/or A11) and the presence of homozygosity for haplotype A. The logistic regression analysis showed that both the presence of the variant *1190T* (OR=0.46; p=0.02; log likelihood=182.268) and concomitant presence of KIR3DL2*001+ligand (OR=3.2; p=0.04 log likelihood=177.833) retained in the model and explained the result. Similar result was found when we tested a model with all *KIR3DL2* alleles and variants (not shown). In total our data lead us to predict a model where PF associated peptides presented in the context of A3 or A11 are recognized well by KIR3DL2*001 promoting disease. KIR3DL2*002, in contrast, may only recognize these ligands weakly, while the poorly expressed *1190T* alleles fail to effectively inhibit NK cells regardless of their binding affinities. The reason why KIR3DL2*001 and KIR3DL2*002 would exhibit degrees of function is unknown. However, it is worth noting that these two molecules differ by only one amino acid (Glu137Asp) and that nearby residues (138 and 140) have been shown to be HLA contact residues in the related receptor KIR3DL1 [40]. Considering the similarity of KIR3DL2 and KIR3DL1, and assuming these receptors bind similarly to their respective ligands, Glu137Asp would be expected to be very close to HLA binding region of KIR3DL2. Although Glu137Asp is a relatively conservative change between two negatively charged amino acids, its proximity to residues that may contact HLA could substantially change the ability of these KIR3DL2 allotypes to bind ligand. Alternatively, other characteristics of these allotypes may differ, such as alterations in receptor stability or folding. Formally testing these hypotheses will require identification of peptides recognized by KIR3DL2 in pemphigus foliaceus patients and functional assays to verify the impact of the substitution Glu137Asp on KIR3DL2 binding.

3. Conclusion

*KIR3DL2*001* is associated with increased susceptibility to PF in a gene dose and ligand-dependent manner, suggesting that it may be a potent inhibitor as compared to the other

KIR3DL2 alleles. To our knowledge, no other studies have revealed apparent interactions between KIR3DL2 and HLA-A3 and A11 *in vivo*. Moreover, we find that KIR3DL2 exhibits differential expression levels that correlate with the SNP *1190C>T*. Lower expression of KIR3DL2 protects against PF possibly due to an overall decrease in inhibitory signals within NK cells. This effect is independent of KIR3DL1 and apparently independent of gene content haplotypes. This is the first study showing that allele specific *KIR3DL2* differential expression levels are associated with disease. Unfortunately, allelic information is still lacking for the majority of *KIR* studies and these data are crucial in comprehending the role that these genes play in other diseases. Our data demonstrate that even complex diseases such as pemphigus can yield invaluable knowledge regarding KIR-dependent mechanisms that regulate immune responses.

4. Material and Methods

4.1. Samples

A total of 156 patients and 141 controls without history of the disease were analyzed in this present study. Patients were contacted mainly at Hospital Adventista do Pênfigo, Campo Grande, MS, Brazil, a specialized hospital located at the endemic area. All individuals voluntarily agreed to participate and written informed consent was obtained from all participants. In accordance with Brazilian Federal laws, this study was approved by the Human Research Ethics Committee of the Federal University of Parana and the CONEP (Comissão Nacional de Ética em Pesquisa). Because different populations may differ in allele frequencies, the individuals were separated according to their predominant ancestry: Euro-descendants (Euro n=104 patients and n=90 controls) and Afro-descendants (Afro, n=52 patients and n=51 controls). This approach has been validated by previous population genetic studies from our group, which showed that the distribution of alleles known to be restricted to populations autochthonous from one continent follows a gradient among the population strata, as expected if the classification discriminated the strata according to relative contributions of the ancestral populations [41],[42]. Euro-descendants and Afro-descendants were also analyzed as a single population sample when the frequencies between them did not differ statistically; total sample may give us a better representation of the whole population.

4.2. *KIR3DL2* and *HLA* genotyping

All individuals were genotyped for presence of *KIR3DL2* in a former study [22]. Here, we amplified exons 3, 4, 5, 7, 8 and 9 and also intron 7 using gene specific primers and the products were sequenced using the Big Dye terminator kit (Applied Biosystems). Specific PCR-SSP primers were designed to solve the *002/010* or *010/015*, *001/007* or *006/010* ambiguities. All primer sequences are available on request. The *HLA* genotyping was performed using the LABType® SSO reagent kits (One Lambda, USA).

4.3. Statistical analyzes

Tests of population differentiation were performed by analyzes of 2×2 contingency tables, calculating the exact p-value by the metropolis algorithm. The p-value of 0.05 was adopted as the significance limit. The Mantel-Haenszel method [43] was applied for calculating the

odds ratio (OR) and the 95% confidence intervals. Logistic regression analyzes with stepwise selection were carried out by IBM SPSS Statistics software.

4.4. Flow cytometry

KIR3DL2 and KIR3DL1 expression was assessed on a cohort of healthy, predominantly Caucasian, donors. Whole blood was stained with anti-CD3, anti-CD56 and anti-KIR antibodies (anti-3DL2 (DX31; L. Lanier, UCSF) and anti-3DL1 (DX9, BD Biosciences)). After red blood cell lysis, the cells were washed, fixed and analyzed using a BD LSRII flow cytometer using Diva software. Data was analyzed using FlowJo analysis software.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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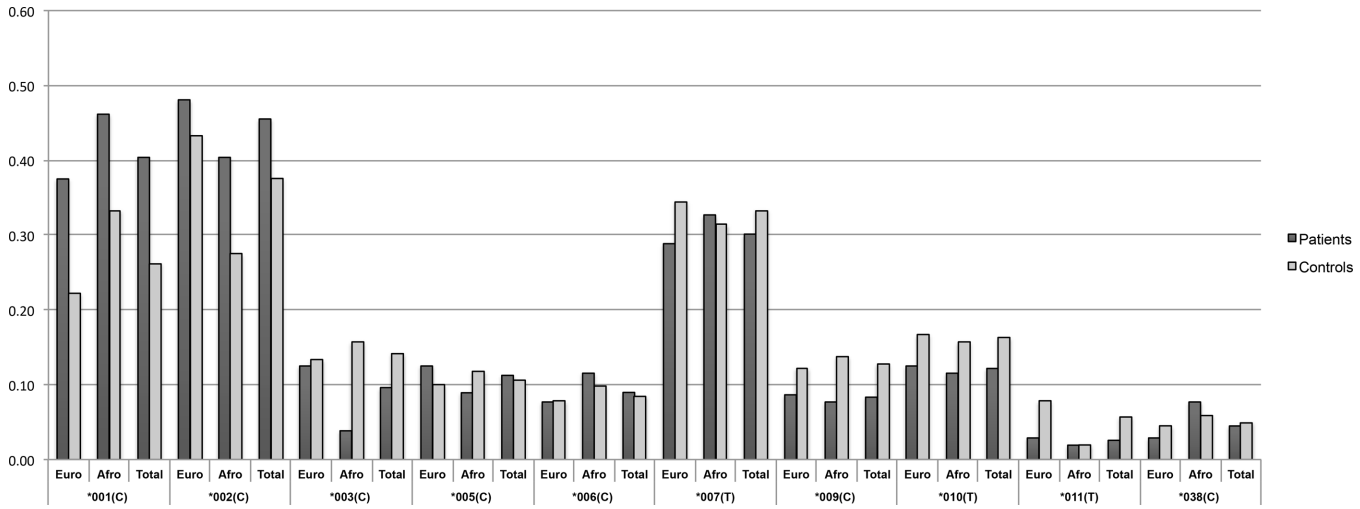

Figure 1. Carrier frequencies for each *KIR3DL2* allele in patients and controls

Only the most frequent alleles are shown. Euro= Euro-descendants; Afro= Afro-descendants; Total=total sample. (C) or (T)= presence of the variant *1190C* or *1190T* (*rs3745902*).

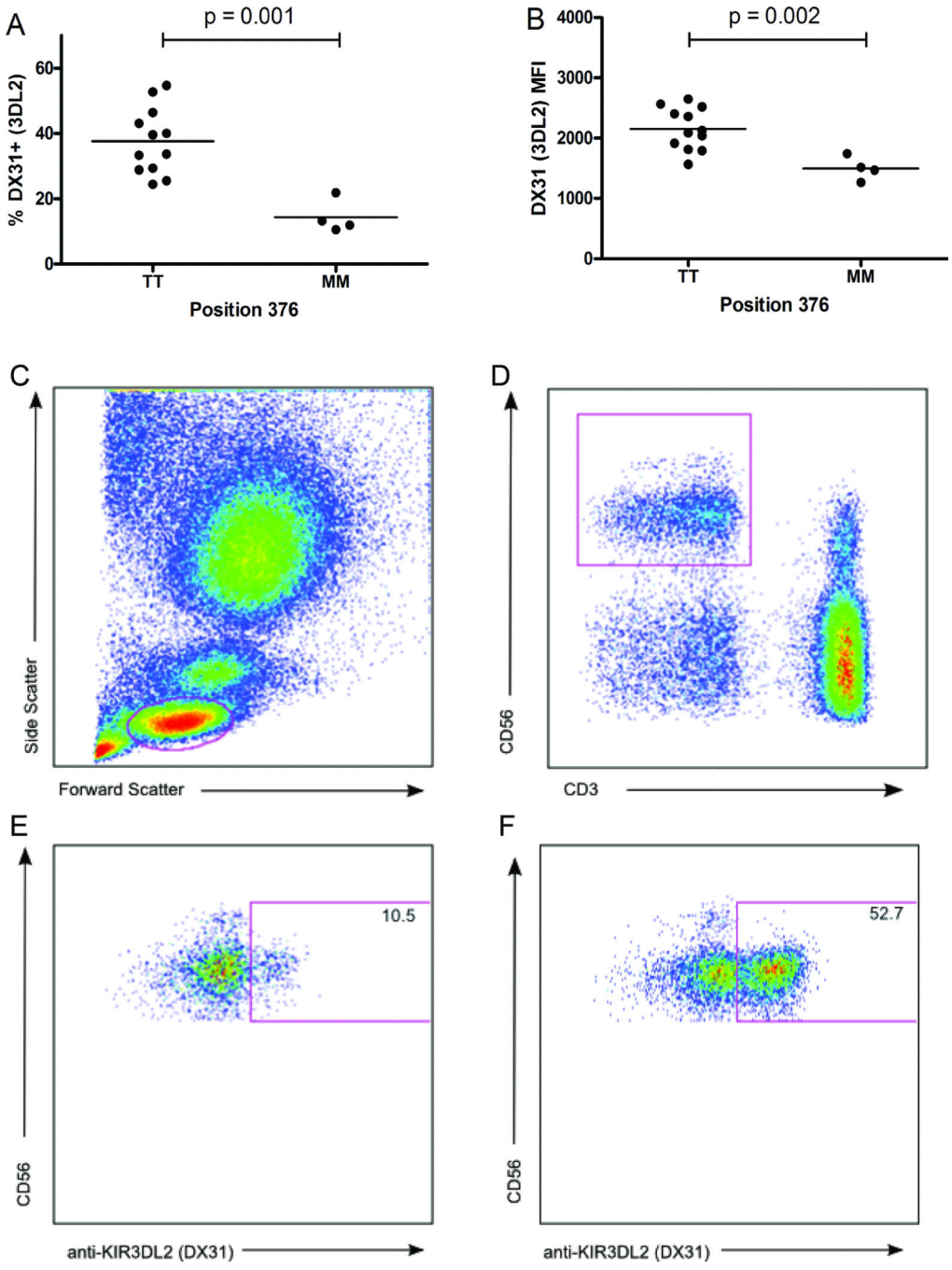

Figure 2. Position 376 (*1190C>T*) marks KIR3DL2 expression

Each dot represents an individual. Position 376 is codified by the SNP *rs3745902*; *1190T*=methionine (M); *1190C*=threonine (T). A) Percentage of KIR3DL2 (DX31) positive NK cells *versus* TT and MM genotypes; B) KIR3DL2 (DX31) median fluorescence intensity *versus* genotypes TT and MM. C) Forward and side scatter plot of peripheral blood mononuclear cells (PBMCs). The lymphocyte population is selected. D) Natural Killer (NK) cells are identified using CD56 and CD3 antibodies. NK positive cells are selected as CD56

positive and CD3 negative cells. Anti-KIR3DL2 (DX31) positive cells are identified with NK positive cells in a donor with E) low expression and F) high expression.

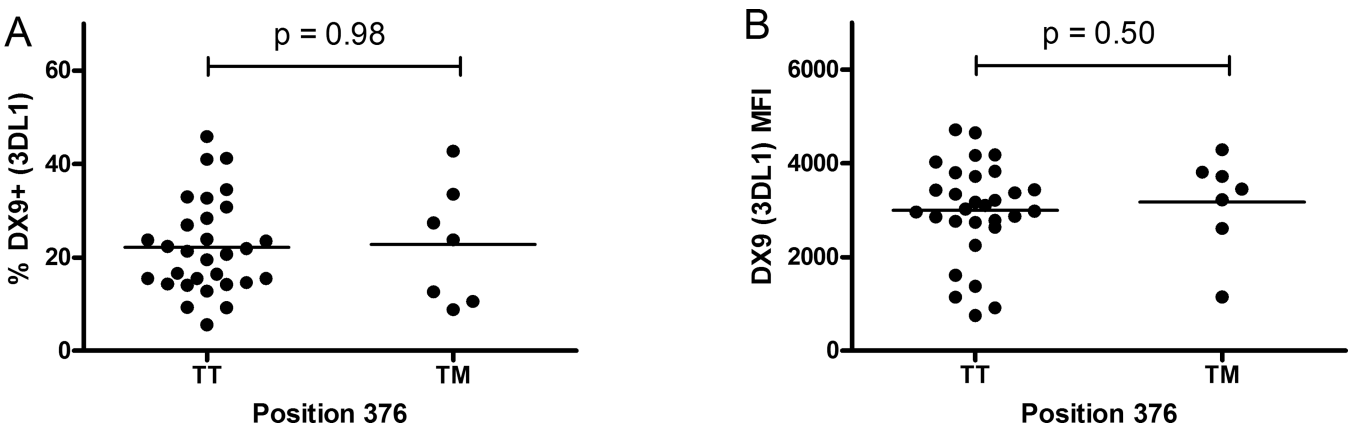

Figure 3. Position 376 (*1190C>T*) does not correlate with KIR3DL1 expression

Each dot represents an individual. Position 376 is codified by the SNP *rs3745902*; *1190T*=methionine (M); *1190C*=threonine (T). Due the linkage disequilibrium between *KIR* genes and alleles, we could not find a representative number of MM/3DL1+ individuals. A) Percentage of KIR3DL1 (DX9) positive NK cells *versus* TT and TM genotypes; B) KIR3DL1 (DX9) median fluorescence intensity *versus* genotypes TT and TM.

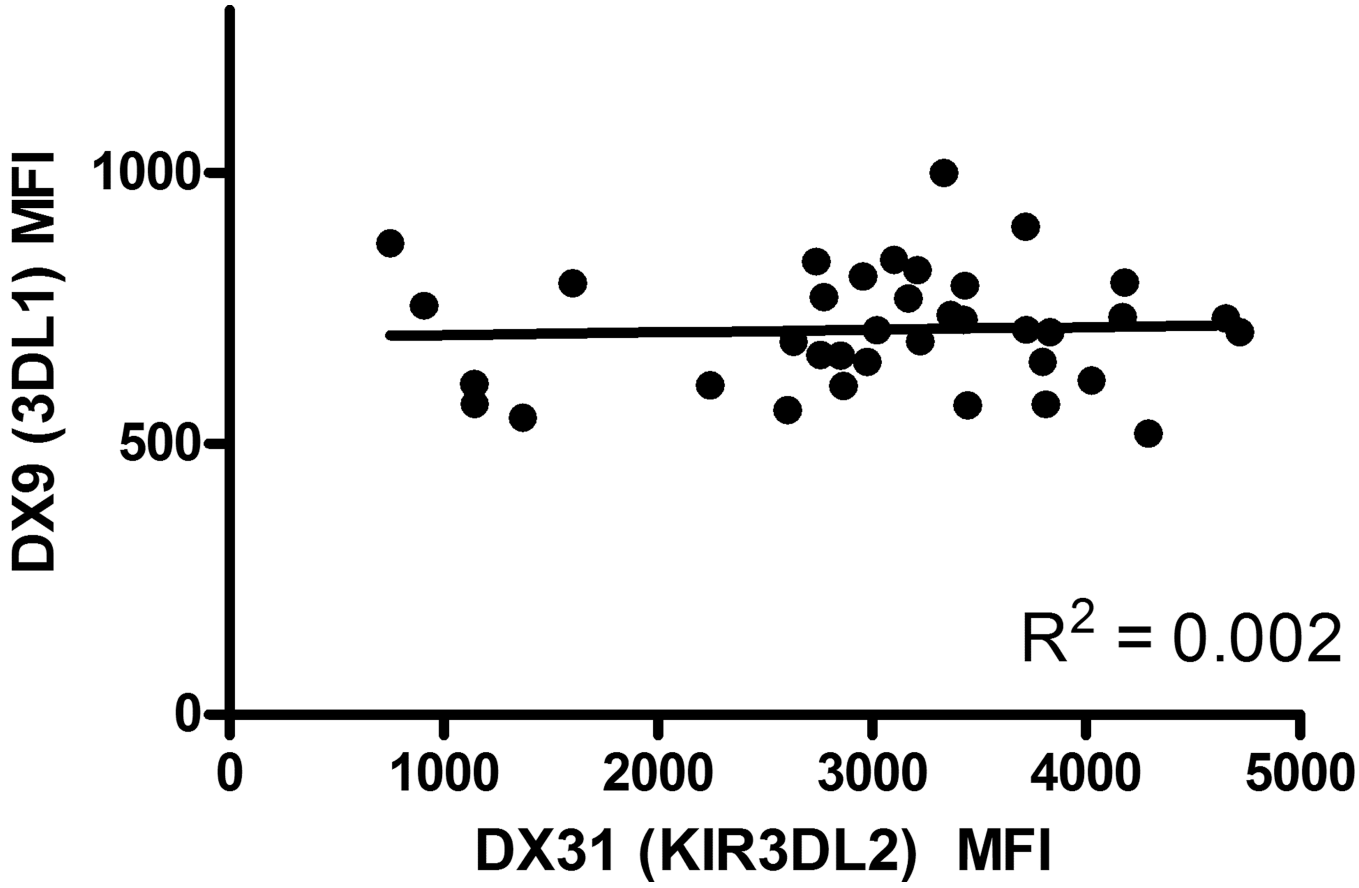

Figure 4. KIR3DL2 expression does not correlate with KIR3DL1 expression

KIR3DL1 (DX9) *versus* KIR3DL2 (DX31) median fluorescence intensity. Each dot represents an individual. KIR3DL1 and KIR3DL2 expression were measured in the same individuals.

Table 1

*3DL2*001* and the SNP *1190T* are associated to pemphigus foliaceus

		Patients			Controls					
		P	**A**	**F (%)**	**P**	**A**	**F(%)**	***p***	**OR**	**95% CI**
Allele frequencies										
	Euro	46	160	22.3	22	156	12.4	**0.007**	**2.04**	**1.17 – 3.54**
001	Afro	29	75	27.9	18	81	18.2	0.070	1.74	0.89 – 3.39
	Total	75	235	24.2	40	237	14.4	**0.002**	**1.89**	**1.24 – 2.89**
Carrier frequencies										
	Euro	39	65	37.5	20	70	22.2	**0.015**	**2.10**	**1.11 – 3.97**
001	Afro	24	28	46.2	17	34	33.3	0.129	1.71	0.77 – 3.81
	Total	63	93	40.4	37	104	26.2	**0.010**	**1.90**	**1.16 – 3.11**
Genotypes										
001/001	Total	12	144	7.7	3	138	2.1	**0.025**	**3.83**	**1.06 – 13.87**
001/other	Total	51	105	32.7	34	107	24.1	0.087	1.55	0.93 – 2.60
SNPs										
322A	Euro	40	64	38.5	45	45	50.0	0.071	0.63	0.35 – 1.11
	Afro	27	25	51.9	24	27	47.1	0.308	0.70	0.35 – 1.38
337G	Euro	29	75	27.9	24	66	26.7	0.490	1.06	0.56 – 2.00
	Afro	9	43	17.3	15	36	29.4	0.146	0.50	0.20 – 1.28
1190T	Euro	40	64	38.5	49	44	54.4	**0.018**	**0.52**	**0.29 – 0.93**
	Afro	23	29	44.2	21	30	41.2	0.752	1.13	0.52 – 2.47

P=presence; A=absent; F=frequency, *p*=p-value; OR=odds ratio, CI=confidence interval. For simplification, *KIR3DL2*001* is represented as 001. Bold highlights the significant associations.

Table 2

Association of *KIR3DL2* variants and HLA-A ligands with pemphigus foliaceus

		Patients			Controls					
		P	**A**	**F (%)**	**P**	**A**	**F(%)**	***p***	**OR**	**95% CI**
Carrier frequencies										
HLA-A ligand	Euro	47	59	44.3	23	46	33.3	0.146	1.59	0.85 – 2.99
	Afro	23	35	39.7	13	21	38.2	0.887	1.06	0.44 – 2.53
	Total	70	94	42.7	36	67	35.0	0.210	1.36	0.83 – 2.31
*3DL2*001+* HLA-A ligand	Euro	18	61	22.8	4	51	7.3	**0.017**	**3.76**	**1.20 – 11.82**
	Afro	6	29	17.1	3	25	10.7	0.360	1.72	0.39 – 7.61
	Total	24	90	21.1	7	76	8.4	**0.016**	**2.89**	**1.18 – 7.09**
1190T+HLA-A ligand	Euro	10	69	12.7	8	48	14.3	0.488	0.87	0.32 – 2.36
	Afro	5	30	14.3	3	25	10.7	0.569	1.19	0.26 – 5.44
	Total	15	99	13.2	11	73	13.1	0.582	1.00	0.44 – 2.32

HLA-A ligand: presence of A3 and/or A11. P=presence; A=absent; F=frequency, *p*=p-value; OR=odds ratio, CI=confidence interval. For simplification, *KIR3DL2*001* is represented as 001. Bold highlights the significant association