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, evidence for any 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, previously associated (lower risk) with activating KIR genes. KIR3DL2*001 was increased in patients (odds ratio (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 nonsynonymous 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 natural killer (NK) cells and also lower intensity of KIR3DL2 on expressing natural killer 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.

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 16256bp. 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 it 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 [14–17], 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 [18]. Many genes, including HLA class II, have been reported to associate with differential susceptibility to PF [19–21]. Activating KIR genes are often associated with protection in infectious diseases and susceptibility to autoimmunity [22]. However, we recently showed that the presence of higher numbers and ratios of activating KIR genes protect from PF [23]. Pemphigus is endemic not only in Brazil, but also in Tunisia and Colombia and the disorder is sporadically seen around the world [24, 25]. 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 [26, 27]. This particularity of PF may explain why activating KIR have 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, 28]. In addition to the fact that all individuals carry this gene, KIR3DL2 is highly expressed on NK cells [29]. 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 PF. We present genetic epidemiological support for an in vivo interaction between KIR3DL2 and HLAA3 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.

Results

KIR3DL2*001 increases the susceptibility to PF

We sequenced KIR3DL2 in patients and controls to test if the polymorphism of this gene plays a role in PF susceptibility. The frequencies of all detected alleles are shown in Supporting Information Table 1 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 (odds ratio (OR) = 2.1, p = 0.015; OR =2.04, p = 0.007, respectively) (Table 1). A statistically nonsignificant 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. An increased risk as also seen when we analyzed presence 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)

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 Afrodescendants. As we reported before [23], 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.

KIR3DL2 exhibits differential expression levels that correlate with 1190T

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 to donors homozygous for 1190T (Fig. 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.

1190T association is apparently independent of gene content haplotypes

There is strong linkage disequilibrium between KIR3DL2 alleles and other KIR genes [30, 31]. Presence of haplotype A has been shown to be associated with increased susceptibility to PF [23]. Thus, we wondered whether haplotype A might exhibit overall higher KIR expression suggesting the linkage disequilibrium 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, Thr/Thr and Thr/Met had a very similar percentage of KIR3DL1+ cells ($p = 0.98$) with similar levels of KIR3DL1 expression per cell ($p = 0.50$) (Fig. 3). We also did not see any correlation between KIR3DL1 and KIR3DL2 expression among these individuals (Fig. 4).

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

In order to test if KIR3DL2*001 and the variant 1190T have distinct effects on PF, 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).

Discussion

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 [22, 32]. In contrast to other autoimmune diseases in which KIR polymorphism has been associated, we have reported that activating KIR genes are protective against PF [23]. Here, we hypothesized that different KIR3DL2 allotypes could be stronger 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.

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 [23]) 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 group of HLA alleles closely associated with ankylosing spondylitis and B27-associated arthritides [33–35]. In our cohort, the frequency of HLA-B*27 is very low (f≤0.03 in patients and controls; Supporting Information Table 2), and therefore, not informative. HLA-A*03, in contrast, is one of the most common HLA-A alleles (Supporting Information Table 2) 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.

The SNP 1190C>T was associated with differential susceptibility to PF (Table 1). This SNP 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 [36]. 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 [37], studying mammalian nuclear genes, estimated that only 17% of the transitions cause nonsynonymous 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 [38]. 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 [39]. 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.

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 [40]. 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 (Fig. 2). 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 proportion of cells expressing KIR3DL2 is lower in 1190T (Met376) homozygotes, in comparison to 1190C (Thr376) homozygotes. This reduces the size of the NK cell subset that may be inhibited by KIR3DL2. In addition, the lower expression levels of KIR3DL2 on the cell surface of positive cells would be predicted to reduce their inhibitory potential. Thus, the NK cell population in 1190T individuals is biased toward lower inhibition in two ways, offering an attractive hypothesis as to why this SNP is negatively associated with PF. Our results also suggest that the differential expression levels of KIR3DL2 are in fact independent of KIR3DL1 (Figs. 3 and 4). Therefore, we conclude that KIR

haplotype gene content is not responsible for the differential expressions levels seen in KIR3DL2.

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 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; Table 1) tells us that there are likely two distinct factors contributing to susceptibility to PF: (i) differential inhibition conferred by KIR3DL2*001 compared to other highly expressed alleles, and (ii) the differential levels of KIR3DL2 expression associated with 1190T.

Our regression analyzes corroborate this hypothesis and 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 the molecules encoded by 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 [41]. 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 PF patients and functional assays to verify the impact of the substitution Glu137Asp on KIR3DL2 binding.

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. Additionally, the amino acid change caused by the SNP 1190C>T is also likely to interfere the receptor function by changing inhibitory signaling, hypothesis that need to be tested. 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.

Materials and methods

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 [42, 43]. Eurodescendants 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.

KIR3DL2 and HLA genotyping

All individuals were genotyped for presence of KIR3DL2 in a former study [23]. 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).

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 [44] was applied for calculating the OR and the 95% confidence intervals. Logistic regression analyzes with stepwise selection were carried out by IBM SPSS Statistics software.

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 (anti3DL2 (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.

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Figure Legends

Figure 1. Carrier frequencies for each KIR3DL2 allele in patients and controls. Sequencing-based genotyping was applied to determine the frequencies of KIR3DL2 in patients and controls. Bars show the frequencies of each allele and only the most frequent alleles are shown. Euro: Euro-descendants (patients, n = 104; controls, n = 90); Afro: Afro-descendants (patients, n = 52; controls, n = 51); Total: total sample (patients, n = 156; controls, n = 141); (C) or (T): presence of the variant 1190C or 1190T (rs3745902).

Figure 2. Position 376 (1190C>T) marks KIR3DL2 expression. KIR3DL2 expression was assessed on a cohort of healthy, predominantly Caucasian, donors (n = 16). (A) Percentage of KIR3DL2 (DX31) positive NK cells versus TT (n =12) and MM genotypes (n = 4) was determined by flow cytometry. Whole blood was stained with anti-CD3, anti-CD56, and anti-3DL2 (DX31; L. Lanier, UCSF). Each dot represents an individual and horizontal bars represent the mean. Mann–Whitney test was used to compare groups. Position 376 is codified by the SNP rs3745902; 1190T: methionine (M); 1190C: threonine (T). (B) KIR3DL2 (DX31) median fluorescence intensity versus genotypes TT (n = 12) and MM (n = 4) was determined by flow cytometry. Whole blood was stained with anti-CD3, anti-CD56, and anti-3DL2 (DX31; L. Lanier, UCSF). Each dot represents an individual and horizontal bars represent the mean. Mann– Whitney test was used to compare groups. Position 376 is codified by the SNP rs3745902; 1190T: methionine (M); 1190C: threonine (T). (C– F) Gating strategy. (C) Forward and side scatter plot of peripheral blood mononuclear cells. 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. KIR3DL1 expression was assessed on a cohort of healthy, predominantly Caucasian, donors (n = 37). Due to 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 (n = 30) and TM (n = 7) genotypes was determined by flow cytometry. Whole blood was stained with anti-CD3, anti-CD56, and anti-3DL1 (DX9, BD Biosciences). Each dot represents an individual and horizontal bars represent the mean. Mann–Whitney test was used to compare groups. Position 376 is codified by the SNP rs3745902; 1190T: methionine (M); 1190C: threonine (T). (B) KIR3DL1 (DX9) median fluorescence intensity versus genotypes TT (n = 30) and TM (n = 7) was determined by flow cytometry. Whole blood was stained with anti-CD3, anti-CD56, and anti-3DL1 (DX9, BD Biosciences). Each dot represents an individual and horizontal bars represent the mean. Mann–Whitney test was used to compare groups. Position 376 is codified by the SNP rs3745902; 1190T: methionine (M); 1190C: threonine (T).

Figure 4. KIR3DL2 expression does not correlate with KIR3DL1 expression. KIR3DL1 and KIR3DL2 expression were assessed on a cohort of healthy, predominantly Caucasian, donors (n = 37). KIR3DL1 (DX9) versus KIR3DL2 (DX31) median fluorescence intensity was determined by flow cytometry. 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)). Each dot represents an individual. KIR3DL1 and KIR3DL2 expression were measured in the same individuals.

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		Patients		F (%)	Controls		F (%)	p	OR	95% CI
		P	A		P	A				
Allele frequencies										
001	Euro	46	160	22.3	22	156	12.4	**0.007**	**2.04**	**1.17–3.54**
	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										
001	Euro	39	65	37.5	20	70	22.2	**0.015**	**2.10**	**1.11–3.97**
	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

Table 1. 3DL2*001 and the SNP 1190T are associated to pemphigus foliaceus

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. 1190T is the variant of the SNP rs3745902; 1190T: methionine (M) in position Thr376Met.

		Patients		F (%)	Controls		F (%)	p	OR	95% CI
		P	A		P	A				
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 3	48	14.3	0.488	0.87	0.32–2.36
	Afro	5	30	14.3		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

Table 2. Association of KIR3DL2 variants and HLA-A ligands with pemphigus foliaceus

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. 1190T is the variant of the SNP rs3745902; 1190T:methionine (M) in position Thr376Met.

Figure 1

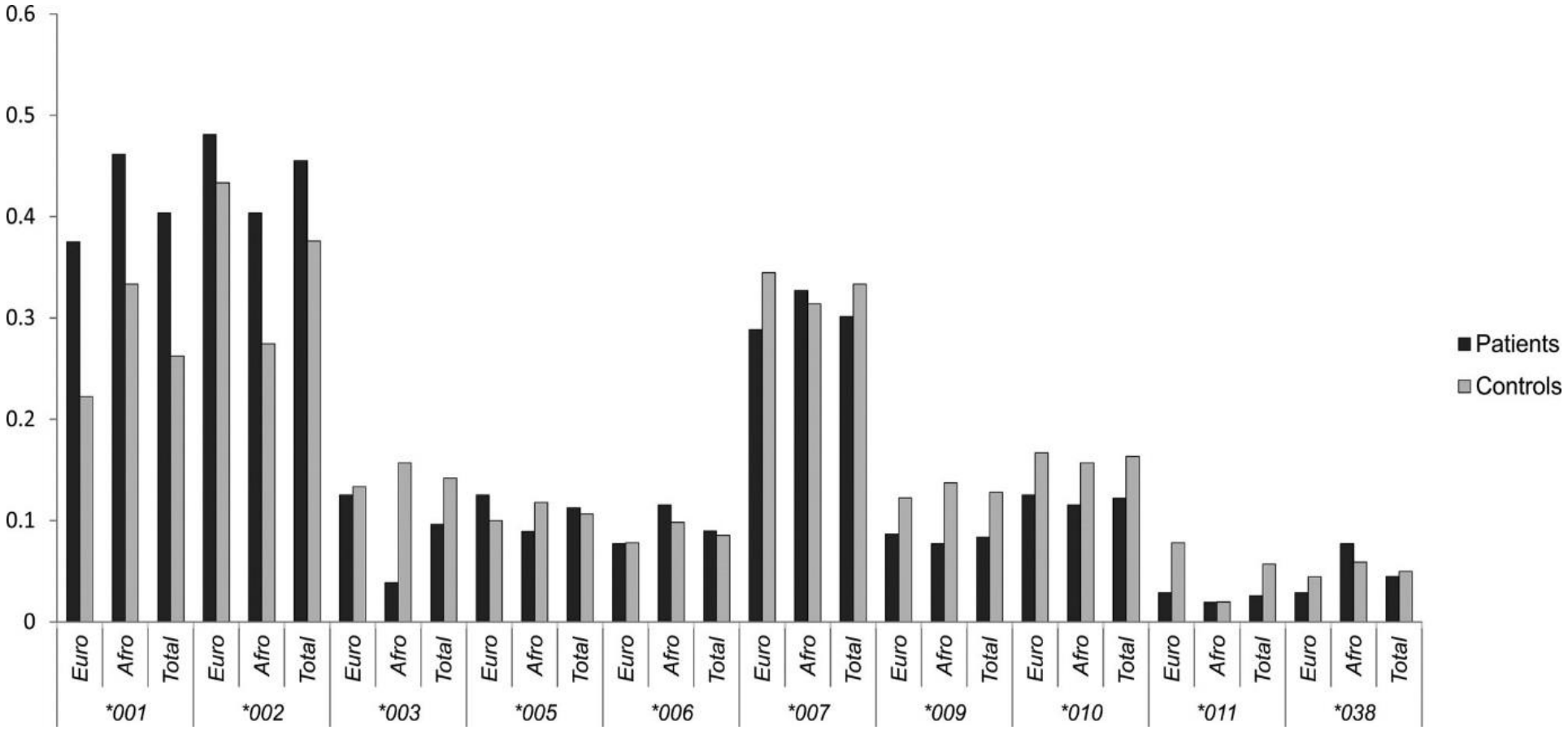

0.6
0.5
0.4
0.3
0.2
0.1
0
Patients
Controls
Euro
Afro
Total
*001
*002
*003
*005
*006
*007
*009
*010
*011
*038

Figure 2

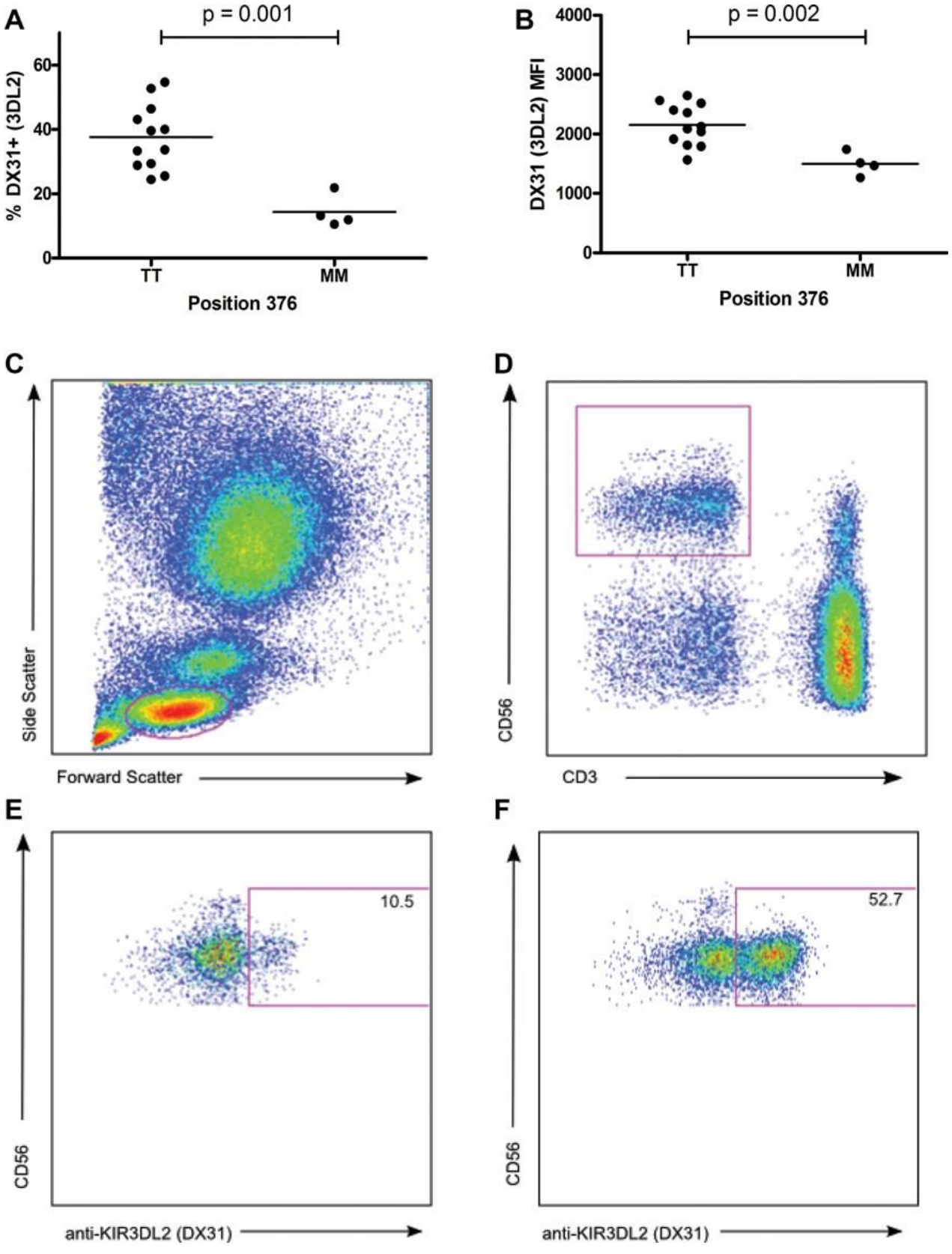

A
p = 0.001
% DX31+ (3DL2)
60
40
20
0
TT
MM
Position 376
B
p = 0.002
DX31 (3DL2) MFI
4000
3000
2000
1000
0
TT
MM
Position 376
C
Side Scatter
Forward Scatter
D
CD56
CD3
E
10.5
CD56
anti-KIR3DL2 (DX31)
F
52.7
CD56
anti-KIR3DL2 (DX31)

Figure 3

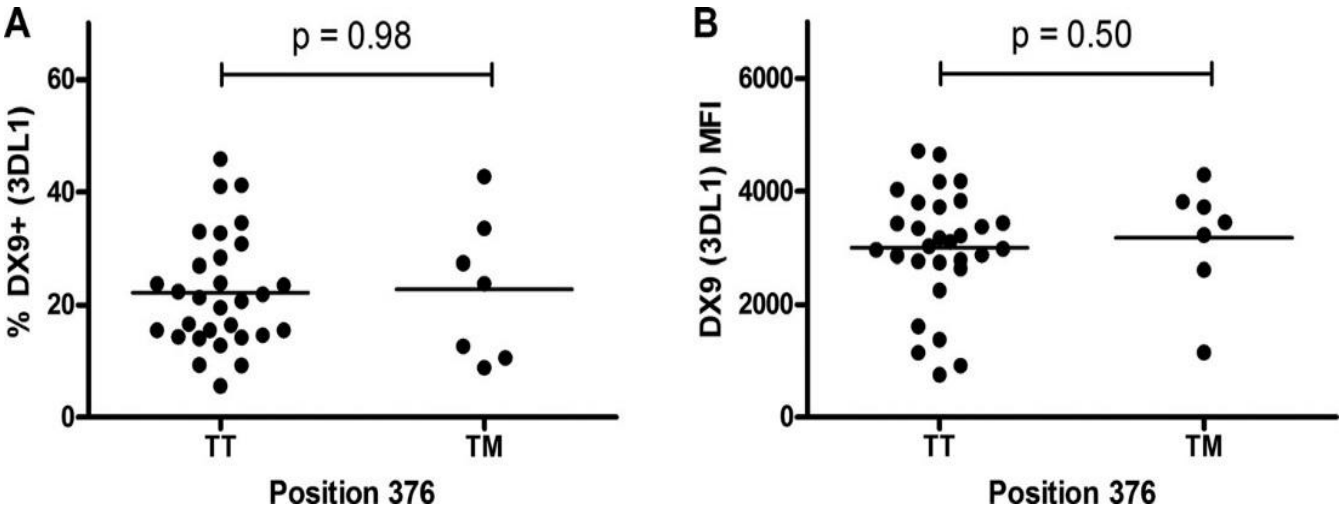
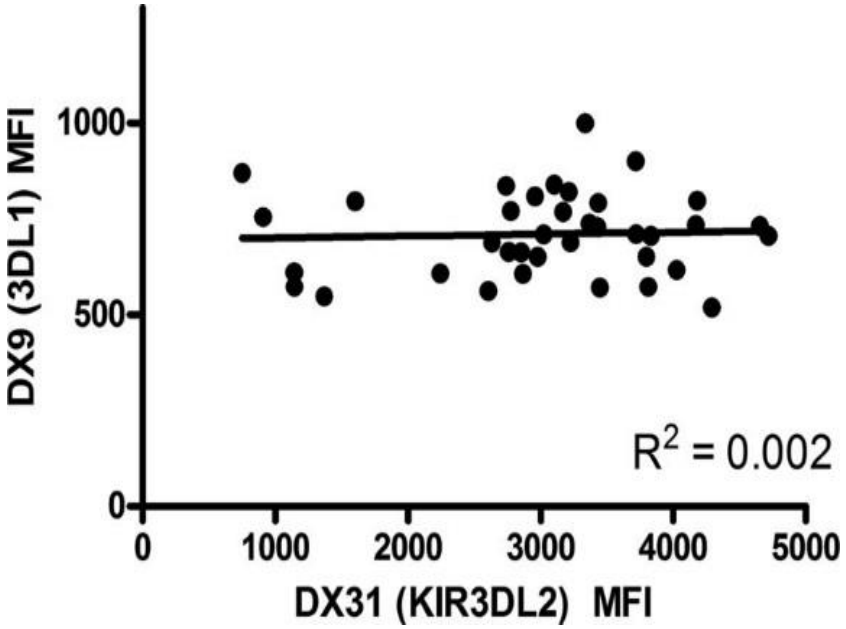

A
p = 0.98
% DX9+ (3DL1)
60
40
20
0
TT
TM
Position 376
B
p = 0.50
DX9 (3DL1) MFI
6000
4000
2000
0
TT
TM
Position 376

Figure 4


DX9 (3DL1) MFI
1000
500
0
0
1000
2000
3000
4000
5000
DX31 (KIR3DL2) MFI
R2 = 0.002