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Protective effect of the *KIR2DS1* gene in atopic dermatitis

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Keywords: atopic dermatitis; killer cell immunoglobulin-like receptor; HLA-C; genetics

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Abstract

Atopic dermatitis (AD) is a common skin disease of complex etiology including affected humoral and cellular immune responses. The role of NK cells in development of this disease has been recently postulated, but is still poorly documented. The current study was undertaken to determine the impact of genes for the most polymorphic NK cell receptors, known as killer cell immunoglobulin-like receptors (KIR), on the development of AD.

We compared 240 patients suffering from AD with 570 healthy controls. Frequencies of the great majority of *KIR* genes did not differ between patients and controls, except for *KIR2DS1*, whose frequency was significantly (OR = 0.629, CI95% (0.45; 0.87), $p_{corr} = 0.0454$) lower in patients than in controls. These results were confirmed in a second cohort of 201 patients. When both patient groups were combined and compared to the control group, the result for *KIR2DS1* achieved even higher significance (OR = 0.658, CI95% (0.5; 0.86), $p_{corr} = 0.0158$). To the best of our knowledge, this is the first report on *KIR* gene contribution to AD, and to allergy in general.

1. Introduction

Atopic dermatitis (AD) is a common (5-20% of population), chronic or relapsing skin disease of complex etiology, with interactions between susceptibility genes and environmental factors. Numerous immunological disturbances are observed in AD, including humoral and cellular, innate and adaptive responses (Boguniewicz and Leung, 2011; De Benedetto et al. 2009). Although the majority of immunological mechanisms in AD are relatively well known at present, the role of natural killer (NK) cells in development of this disease is still poorly documented (Wollenberg and Klein, 2007). NK cells are involved in innate cytotoxicity as well as in regulation of immune (both innate and adaptive) responses (Boyton and Altman, 2007; Parham, 2005). The most polymorphic NK cell receptors are known as killer cell immunoglobulin-like receptors (KIRs). Originally, these receptors were found on NK cells (Boyton and Altman, 2007; Parham, 2005), but they could also be found on some T cells, especially on memory T cells (Rajagopalan and Long, 2005) and a rare subpopulation of CD4⁺ T cells deprived of CD28 expression (Goronzy and Weyand, 2003).

KIRs exist in four isotypes: inhibitory receptors with two or three extracellular domains and a long cytoplasmic region (KIR2DL and KIR3DL), and activating receptors with two or three extracellular domains and a short cytoplasmic tail (KIR2DS and KIR3DS). *KIR* genes exhibit both allelic (multiple alleles at each locus) and haplotypic (different numbers of inhibitory and activating genes) polymorphism. In addition, some KIRs are known to recognize HLA-C (epitopes C1 or C2), HLA-B (Bw4) or HLA-A (A3 and/or A11, as well as HLA-A allomorphs with Bw4 epitope) molecules. This makes for great variability of reactivities among individuals, depending on the presence or absence of given KIRs and their ligands, and hence differential susceptibility to several diseases (Boyton and Altman, 2007; Goronzy and Weyand, 2003; Khakoo and Carington, 2006; Parham, 2005; Rajagopalan and

Long, 2005), including skin diseases such as psoriatic arthritis and psoriasis (Holm et al., 2005; Łuszczek et al., 2004; Nelson et al., 2004; Suzuki et al., 2004; Williams et al., 2005). However, no data are available regarding associations of *KIR* genes with AD, where inadequate regulation of the immunological response also plays a decisive role. Therefore, this study was undertaken to determine the impact of *KIR* and *KIR* ligand genotype on the susceptibility to AD.

2. Material and Methods

2.1. Subjects. Our study was conducted on two cohorts of AD patients: (i) the first, or discovery, cohort (240 individuals, 142 women, 98 men; mean age, 17.7 ± 12.6 , range, 2-62) consisted of 87 patients from the Department of Dermatology, Venereology and Allergology, Medical University, Wrocław, 81 patients from the 1st Department of Dermatology and Venereology, Medical University of Łódź, and 72 patients from the N. Barlicki Medical University Hospital, Łódź; (ii) the second, confirmatory cohort (201 patients) was from the Department of Prevention of Environmental Hazards and Allergology, Medical University of Warsaw. All the studied subjects were included in the study according to the well-established clinical criteria of AD diagnosis proposed by Hanifin and Rajka (1980). The characteristics of both cohorts are given in **Table 1**.

The clinical severity of AD was estimated with SCORAD (Oranje et al., 2007). The maximum achievable score is 103 points. The mean SCORAD in the first cohort was assessed as 37.9 ± 17.9 points (range, 14 to 93 points). Using the SCORAD index, AD was scored in each patient as mild (<25 points; 32.5% of patients, values for the first cohort), moderate (25-50 points; 48.8%) or severe (>50 points; 18.7%). One hundred and thirty-four patients (55.8%) had a positive AD history in their family. Age at disease onset was 0-1 year for

53.7% of patients, >1-5 years for 32.1%, and above 5 years for 14.2%. All patients with any significant non-atopic co-morbidities or abnormalities that could falsify the results and jeopardize their reliable presentation in the conducted study were excluded from the first cohort, as were patients with incomplete clinical data.

The second (confirmatory) cohort was a subset of individuals described in detail elsewhere (Ponińska et al., 2011).

Five hundred and seventy unrelated healthy volunteers (332 women, 238 men; mean age, 39.3±14.7, range, 19-83) were recruited in the years 2003-2009 by clinics of Wrocław Medical University, the Medical University of Warsaw, and the Pomeranian Medical University, Szczecin. The study was specifically approved by the ethics committees of the participating institutions, and written and signed informed consent was obtained from all subjects.

2.2. DNA isolation and typing of *KIR* and *KIR* ligand (HLA-C C1 and C2 group) genes.

DNA was isolated from venous blood as described (Łuszczek et al., 2004; Nowak et al., 2010). Sequence-specific primers (SSP) as described by Vilches et al. (2007), that enable one to amplify short DNA fragments (96-158 bp), were used for the detection of *KIR* genes in the first cohort of patients by means of PCR-SSP of Vilches et al. (2007) in our modification. Briefly, polymerase chain reaction (PCR) was carried out in 10 µl of solution containing a standard PCR buffer, 100 ng of tested DNA, 0.4 U Taq Polymerase (Invitrogen, Brazil), 1.5 mM MgCl₂, 200 µM dNTPs (Invitrogen, Brazil), and 1 µM of each gene-specific primer except for those specific for *KIR2DL5*, *KIR2DS3* (1.25 µM) and *KIR2DL1* (0.5 µM). All reactions contained 0.02 µM of each primer for an internal positive control (exon 2 of human α-1-antitrypsin; AAT1: 5'-cccaccttcccctctctccaggcaaagg-3', and AAT2: 5'-gggcctcagtcaccaatggctaagagtg-3'). Amplifications were performed under the following

conditions: 95°C, 2 min; 10 cycles of 20 s at 94°C; 10 s at 65°C; 30 s at 72°C, and 20 cycles of 20 s at 94°C; 10 s at 61°C; 30 s at 72°C. The last elongation step was extended to 7 min. All PCR products were electrophoresed on 2.5% agarose gel, and visualized in a UV transilluminator (TFX-20 M, Vilber Lourmat, France) using ethidium bromide staining. The second cohort of patients (201 individuals) was *KIR* typed using multiplex PCR-SSP as described previously (Nowak et al., 2009; Sun et al., 2004). Control DNA samples were *KIR* typed using one of the methods listed above. Our *KIR* typing has been validated three times per year by the International *KIR* Exchange program organized by the Immunogenetics Center of the University of California at Los Angeles, and did not reveal differences between methods mentioned above. Examples of our *KIR* typing using Vilches et al. (2007) and multiplex (Sun et al., 2004) PCR systems are shown in **Figure 1**, including two samples, #104 and #106, differing by *KIR2DS1* presence.

HLA-C groups C1 and C2 were established as described by Frohn et al. (1998) or using a commercial kit (Olerup). This typing could not have been performed in 3 samples from the second cohort and 9 samples from the controls, which did not have a proper amount and/or quality of DNA.

2.3. Statistical methods

A general linear model with binomial errors was used to investigate the relationship between clinical and genetic variables and probability of atopic dermatitis. Frequencies of *KIR* genes and *C1* and *C2* variants of the *HLA-C* gene were explanatory variables. Akaike's information criterion (AIC) was used as a measure of fit of models. The bootstrap approach was employed to estimate the model's coefficients and 95% confidence intervals. Odds ratio (OR) was computed as a measure of effect size. According to linkage disequilibrium results, we calculated the one-side instead of two-side confidence interval for OR in the case of

KIR2DS4 (footnote ^d in **Table 3**). Holm's correction to control the $\text{FWER} \leq \alpha = 0.05$ was used.

The measure for the estimation of pair-wise linkage disequilibrium (LD) was D' (Lewontin, 1988). For two KIRs, D' was obtained as: if p_i and q_j are the population allele frequencies of the i th allele on locus A and the j th allele on locus B , and x_{ij} is the frequency of the haplotype with alleles i and j on loci A and B , respectively, then $D' = \frac{D_{ij}}{D_{\max}}$

Monte Carlo simulations

Two different models were introduced to investigate relationships between frequencies of *KIR2DS4* and *KIR2DS1* in the context of the probability of disease. At each step two haplotypes were randomly selected from the set of haplotypes estimated previously, based on genotyping the control (n=570) individuals. Based on these two haplotypes one diploid with “presence/absence” genotype is created. *KIR2DS4* and *KIR2DS1* are checked. According to the rules specified below, a diploid is ill or not, and odds ratios for both genes are calculated. Two scenarios were considered.

Model 1. In this model we assume that the presence of *KIR2DS4* increases the probability of disease. If *2DS4* is present in the genome, then a diploid is ill with probability $P \in (0.5, \dots, 0.95]$. If *2DS4* is absent, a diploid is ill with probability $P = 0.5$. For each P odds ratios (OR) are calculated for both genes.

Model 2. In this model we assume that presence of *KIR2DS1* decreases the probability of disease. If *2DS1* is present in the genome, a diploid is ill with probability $P \in [0.05, \dots, 0.5)$. If *2DS1* is absent, a diploid is ill with probability $P = 0.5$. For each P odds ratios (OR) are calculated for both genes.

Odds ratios (OR) for each P were calculated based on 75 000 diploids. The range of probabilities of disease, P , was chosen arbitrarily. The aim of these simulations was to

describe changes of odds ratios (for *KIR2DS1* and *KIR2DS4*) when *KIR2DS4* is associated with disease and changes of ORs when *KIR2DS1* is associated with protection from disease.

3. Results

3.1. *KIR* gene frequencies in patients with AD and in controls

There were no significant differences in frequencies of *KIR* genes between AD patients and controls, with one exception (**Table 2**): We found an interesting difference in distribution of the *KIR2DS1* gene: it was significantly lower in patients than in controls (OR = 0.629, p_{corr} = 0.0454). Similarly lower frequency of *KIR2DS1* was observed in the second group of patients (OR = 0.692, p_{corr} = 0.047). *KIR* gene frequencies, including *KIR2DS1*, did not differ significantly ($P > 0.05$) between the two patient populations; therefore the data were pooled (**Table 2**). When both patient cohorts were combined and compared to controls, the difference in *KIR2DS1* frequency was more significant (OR = 0.658, p_{corr} = 0.0158, **Table 2**).

No association of any *KIR* with SCORAD, age at disease onset, or with familial history was found.

3.2. *KIR2DS1* effect in the light of linkage disequilibrium of *KIR* genes

KIR2DS1 is in strong linkage disequilibrium with *KIR* genes located in the telomeric region of the *KIR* gene complex in Caucasians (Gourraud et al., 2010) and most other populations (Gonzalez-Galarza et al., 2011). **Figure 2** shows that this is also the case in our population (*KIR2DL5* was omitted from our analysis because it may be located in the telomeric or centromeric region of the *KIR* complex or both, which was not tested here): *KIR2DS1* was in strong positive LD with *KIR3DS1* and *KIR2DS5*; however, no significant

differences of *KIR3DS1* and *KIR2DS5* frequencies between patients and controls were found. In addition, *KIR2DS1* was in strong negative LD with *KIR2DS4* (**Figure 2**). *KIR2DS4* (“total”, without division into a full-length gene, *KIR2DS4^{fl}*, and a variant with 22 bp deletion, *KIR2DS4^{del}*) was slightly more frequent in patients (OR = 0.155) but did not reach significance ($p_{corr} > 0.05$). Also, differences from controls of the frequencies of two molecular variants of the *KIR2DS4* gene (full length and deletant) were not reproducible in the two groups of patients (**Table 2**). In addition, numerical experiments (Monte Carlo analysis) suggest that the presence of *KIR2DS1* rather than that of *KIR2DS4* affects the susceptibility to disease. Namely, model 1 assumes that the presence of *KIR2DS4* increases probability of disease, and that *KIR2DS1* frequency changes only because of the LD with *KIR2DS4*. **Figure 3** (left panel) shows that increasing the frequency of *KIR2DS4* is not accompanied by *KIR2DS1* frequency decrease equal to values observed experimentally. Model 2 assumes that the presence of *KIR2DS1* protects from disease, and *KIR2DS4* frequency is changed only because of LD. **Figure 3** (right panel) shows that in model 2, decreasing the frequency of *KIR2DS1* is paralleled by an increase of *KIR2DS4* frequency similar to that observed (arrow). Therefore, this model fits our data better.

3.3. HLA-C C1 and C2 epitope frequencies in patients with AD and in controls

The ligand for the *KIR2DS1* molecule is a C2 epitope, i.e., those HLA-C allomorphs which possess a lysine residue in position 80 (Boyton and Altman, 2007; Parham, 2005), although it is not yet known whether this is the physiological ligand of *KIR2DS1*. Therefore, we examined whether C1 or C2 frequency was different in patients as compared to controls. No difference was found for either of the two cohorts of patients or for all patients combined together ($p = 0.234$) (**Table 3**). In addition, no association of C1/C2 genotype with SCORAD, age at disease onset, or with familial history was found.

Moreover, the association of the *KIR2DS1* gene with protection from AD was independent of the presence or absence of C2 ($p = 0.906$). Frequencies of other *KIR-HLA-C* combinations (*KIR2DL1-C2*, *KIR2DL2-C1*, *KIR2DL3-C1*) were not different between patients and controls (data not shown).

Discussion

We found here that *KIR* genes were distributed with similar frequencies in AD patients and controls, with one exception: *KIR2DS1* seemed to be associated with protection from AD. *KIR2DS1* encodes an activating receptor, which binds HLA-C molecules with the C2 epitope. We have not found any contribution of C2 to susceptibility to AD, however. The reason for this may lie in the interaction of the inhibitory *KIR2DL1* with C2-positive HLA-C molecules, which predominates because it is stronger than that of *KIR2DS1*, which binds the same ligand (Parham et al., 2012). The *KIR2DS1* gene was described to be contributing to the susceptibility to disease rather than protection in other skin diseases such as psoriatic arthritis (Nelson et al., 2004; Williams et al., 2005) and psoriasis vulgaris (Holm et al., 2005; Łuszczek et al., 2004; Suzuki et al., 2004). Therefore, a difference in contribution of NK cell or *KIR*-expressing T lymphocyte subpopulations between an atopic disease, AD, where keratinocytes undergo apoptosis (Rebane et al., 2012), and diseases with autoimmune components (psoriasis and psoriatic arthritis), where keratinocytes hyperproliferate rather than being subjected to apoptosis (Albanesi et al., 2007; Kastelan et al., 2009), could be responsible for the apparently opposite roles of the *KIR2DS1* gene in these two clinical situations. Indeed, the Th1- and NK1 pathway is predominant in psoriasis, whereas AD is rather a Th2- and NK2-mediated disease (von Bubnoff et al., 2010; Guttman et al., 2008; Rabin and Levinson, 2008). There are also differences between these two diseases in the incidence of bacterial and viral skin infections (Boguniewicz and Leung, 2011; De Benedetto

et al. 2009), and in the emission rate of T cells from the thymus (Just et al., 2008). All these differences may affect the contribution of KIR2DS1-positive cell subpopulations involved in these clinical situations, resulting in opposite associations of the *KIR2DS1* gene. In support for this thesis, the *KIR2DS1* gene (and other activating *KIRs*) has recently been found to be associated with protection against another Th2-mediated skin disease, pemphigus foliaceus (Augusto et al., 2012).

KIR2DS1 is in positive LD with *KIR3DS1* and *KIR2DS5* and in strong negative LD with *KIR2DS4* in most populations (Gonzalez-Galarza et al., 2011; Gourraud et al., 2010) including ours (see Results). One might then expect an association of *KIR2DS4* with susceptibility to AD, and a protective effect of *KIR3DS1* and *KIR2DS5* similar to that of *KIR2DS1*. The association of *KIR3DS1* and *KIR2DS5* was negligible, and that of *KIR2DS4* was weak and inconsistent. In addition, our Monte Carlo experiments, based on haplotype frequencies for the control group, suggested that increasing the frequency of *KIR2DS4* is not accompanied by a *KIR2DS1* frequency decrease equal to values observed. In contrast, decreasing the frequency of *KIR2DS1* is paralleled by an increase of *KIR2DS4* frequency similar to that observed. Therefore, we assume that we observed the true effect of *KIR2DS1* rather than a result of LD with *KIR2DS4*.

Interestingly, Aktas et al. (2005) described an increased fraction of CD158b (KIR2DL2, KIR2DL3 and/or KIR2DS2)-positive NK cells in AD patients as compared to controls, whereas there was no difference in CD158a (KIR2DL1/KIR2DS1) expression. This might have, however, resulted from the presence of the KIR2DL1 molecule in 95-96% of individuals in our population, masking the binding of monoclonal antibodies to KIR2DS1.

The KIR2DS1 molecule was found to aggregate spontaneously in solution *in vitro*, and was postulated to aggregate also on the cell surface, which would facilitate cell activation upon ligand binding (Hayley et al., 2011). If expressed on NK or T cells regulating an allergic

response by secretion of cytokines or direct killing of effectors, it could contribute to protection from AD. In contrast, in psoriasis and related skin diseases, *KIR2DS1* might be present on effector cells, augmenting the autoimmune response.

In summary, we observed a protective effect of the presence of the *KIR2DS1* gene in atopic dermatitis. To our knowledge, this is the first report on the contribution of the *KIR* gene to this disease, and to allergy in general.

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Legends to figures.

Figure 1. Results of KIR typing by single PCR-SSP of Vilches et al. (2007) and multiplex PCR-SSP (Sun et al., 2004). Single PCR-SSP typing (Fig. 1A and B): **Fig. 1A**, sample #104 (KIR2DS1-positive); **Fig. 1B**, sample #106 (KIR2DS1-negative).

Multiplex PCR-SSP typing (**Fig. 1C** and **D**): for primers, see the table below; lanes 1 and 18, marker; lanes 2-5, group 1; lanes 6-9, group 2; lanes 10-13, group 3; lanes 14-17, group 4.

Fig. 1C: lanes 2,6,10,14, sample #101; lanes 3,7,11,15, sample #102; lanes 4,8,12,16, sample #103; lanes 5,9,13 and 17, sample #104. **Fig. 1D:** lanes 2,6,10,14, sample #105; lanes 3,7,11 and 15, sample #106; lanes 4,8,12,16, sample #107; lanes 5,9,13,17, sample #108.

Fig. 1C

Multiplex group	KIR gene	Amplicon (bp)	101	102	103	104
1	2DL1	437	+	+	+	+
	2DL2	164	-	-	-	+
	2DL4	232	+	+	+	+
	2DS3	279	+	-	+	+
2	2DL3	334	+	+	+	+
	2DS2	204	-	-	-	+
	3DL1	565	-	+	+	+
	3DS1	171	+	-	+	+
3	2DL5	113	+	+	+	+
	2DS1	231	+	-	+	+
	3DL2	142	+	+	+	+
4	2DS4n	130	-	+	-	-
	2DS4d	108	-	-	+	+
	2DS5	194	-	+	-	+
	3DL3	155	+	+	+	+

Fig. 1D

Multiplex group	KIR gene	Amplicon (bp)	105	106	107	108
1	2DL1	437	+	+	+	+
	2DL2	164	-	+	+	+
	2DL4	232	+	+	+	+
	2DS3	279	-	+	-	+
2	2DL3	334	+	+	+	-
	2DS2	204	-	+	-	+
	3DL1	565	+	+	+	+
	3DS1	171	+	-	-	-
3	2DL5	113	+	+	+	+
	2DS1	231	+	-	+	-
	3DL2	142	+	+	+	+
4	2DS4n	130	+	+	-	+
	2DS4d	108	-	+	+	-
	2DS5	194	+	-	+	+
	3DL3	155	+	+	+	+

Figure 2. Pairwise linkage disequilibrium between the presence or absence of 10 *KIR* genes (see Material and Methods). Framework genes (*KIR3DL2*, *KIR3DL3* and *KIR2DL4*) were omitted as virtually always present. *KIR2DL5* was not included either, because it has been typed on the presence-absence level only, without subdivision into *KIR2DL5A* (telomeric) and *KIR2DL5B* (centromeric), so its position was uncertain.

Figure 3. Monte Carlo simulations of the effect of *KIR2DS1* and *KIR2DS4* genes on susceptibility to atopic dermatitis. Left panel: Model 1, assuming that the presence of *KIR2DS4* increases the probability of disease. Right panel: Model 2, assuming that the presence of *KIR2DS1* decreases the probability of disease (for details, see Material and methods). The arrow in the right panel indicates odds ratio values observed for *KIR2DS1* and *KIR2DS4* (see Table 2).

Table 1. Characteristics of atopic dermatitis (AD) patients.

Parameter	Subgroup	First cohort (N = 240)		Second cohort (N = 201)	
		N	%	N	%
Gender	males	98	40.8	91	45.2
	females	142	59.2	110	54.8
Age groups	2-12 years	104	43.3	70	34.7
	13-62 years	136	56.7	131	65.3
Co-morbidities	asthma	72	30.0	15	7.5
	rhinitis	94	39.2	55	27.1
	conjunctivitis	50	20.8	-	-

-, data not available

Table 2. Distribution of KIR genes in control individuals and in AD patients

Group	KIR carrier frequency (%)												
	2DS2	2DL2	2DL3	2DL5	2DS3	2DL1	3DL1	3DS1	2DS5	2DS1	2DS4 fl	2DS4 del	2DS4 total
Controls (N=570)	54.39 (310)	53.86 (307)	90.53 (516)	52.91 (173)	31.58 (180)	97.02 (553)	94.39 (538)	38.25 (218)	28.60 (163)	41.93 (239)	28.07 (160)	82.63 (471)	92.81 (529)
1st group of patients (N = 240)	55.00 (132)	55.83 (134)	91.25 (219)	54.16 (130)	34.02 (82)	95.85 (231)	96.25 (231)	35.00 (84)	31.25 (75)	31.12^a (75)	26.97 (65)	86.25 (207)	95.4 (229)
2nd group of patients (N = 201)	55.22 (111)	47.76 (96)	91.54 (184)	46.77 (94)	24.87 (50)	94.52 (190)	89.05 (179)	35.32 (71)	23.38 (47)	33.33^b (67)	34.32 (69)	82.08 (165)	95.0 (191)
All patients (N=441)	55.10 (239)	52.15 (234)	91.38 (403)	50.79 (224)	29.93 (132)	95.46 (421)	92.97 (410)	35.14 (155)	27.66 (122)	32.19^c (142)	30.38 (134)	84.35 (372)	95.2^d (420)

2DS4fl, 2DS4 full length; 2DS4del, 2DS4 with 22 bp deletion; 2DS4 total, positive for 2DS4fl, 2DS4del, or both; P_{corr} , Holm's correction

^aOR = 0.629, CI95% (0.45; 0.87), P_{corr} = 0.0454

^bOR = 0.692, CI95%(0.49; 0.97), P_{corr} = 0.047

^cOR = 0.658, CI95% (0.5; 0.86), P_{corr} = 0.0158

^dOR = 1.55, CI95% (0.98; ∞), P_{corr} = 0.0562

Table 3. Comparison of HLA-C C1 and C2 frequencies in patient cohorts and controls

Group	Frequency, N (%)	
	C1	C2
Patients 1 st cohort (N = 240)	208 (86.7)	170 (70.8)
Patients 2 nd cohort (N = 198) ^a	159 (80.3)	139 (70.2)
All patients combined (N = 438)	367 (83.8)	309 (70.5)
Controls (N = 561) ^a	470 (83.8)	382 (68.1)

All comparisons gave non-significant results ($P > 0.05$).

^a Three patients and 9 control individuals could not be typed for HLA-C C1 and C2 for technical reasons.

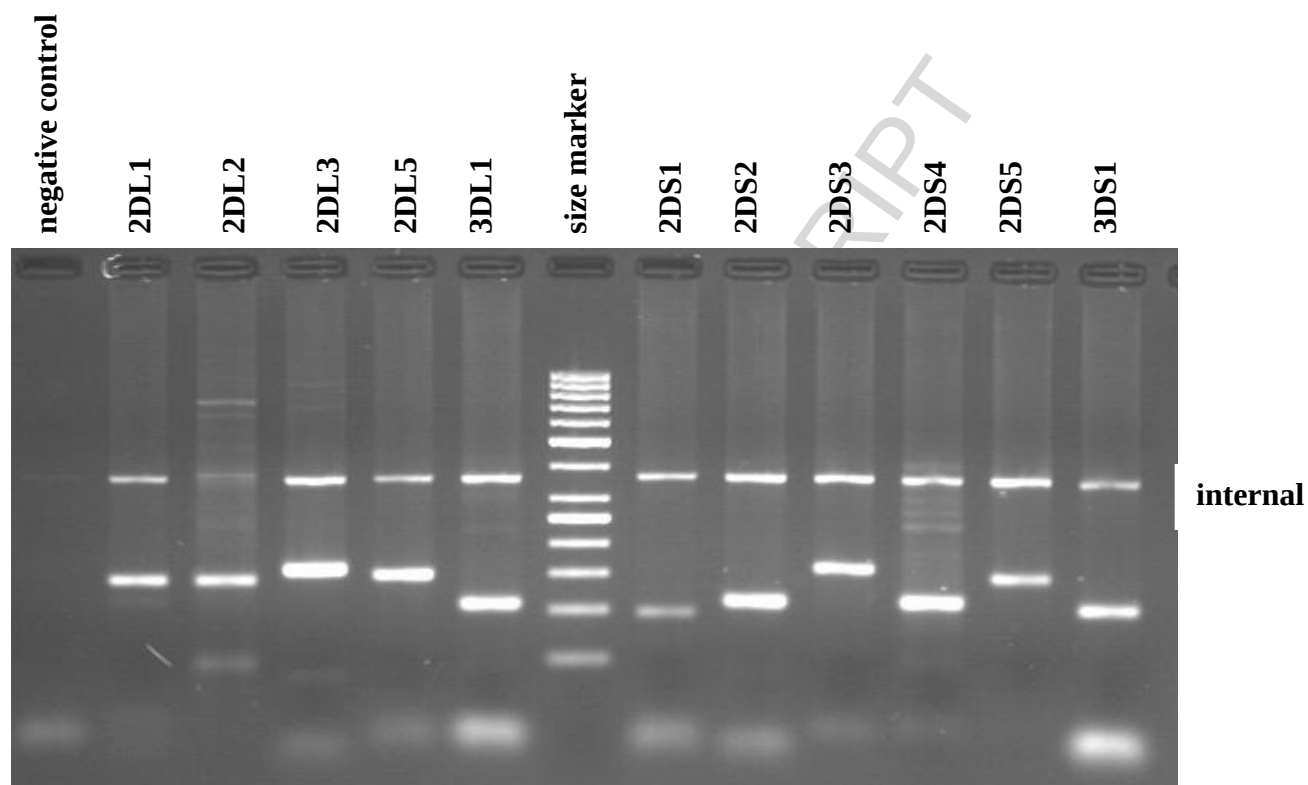


Figure 1a

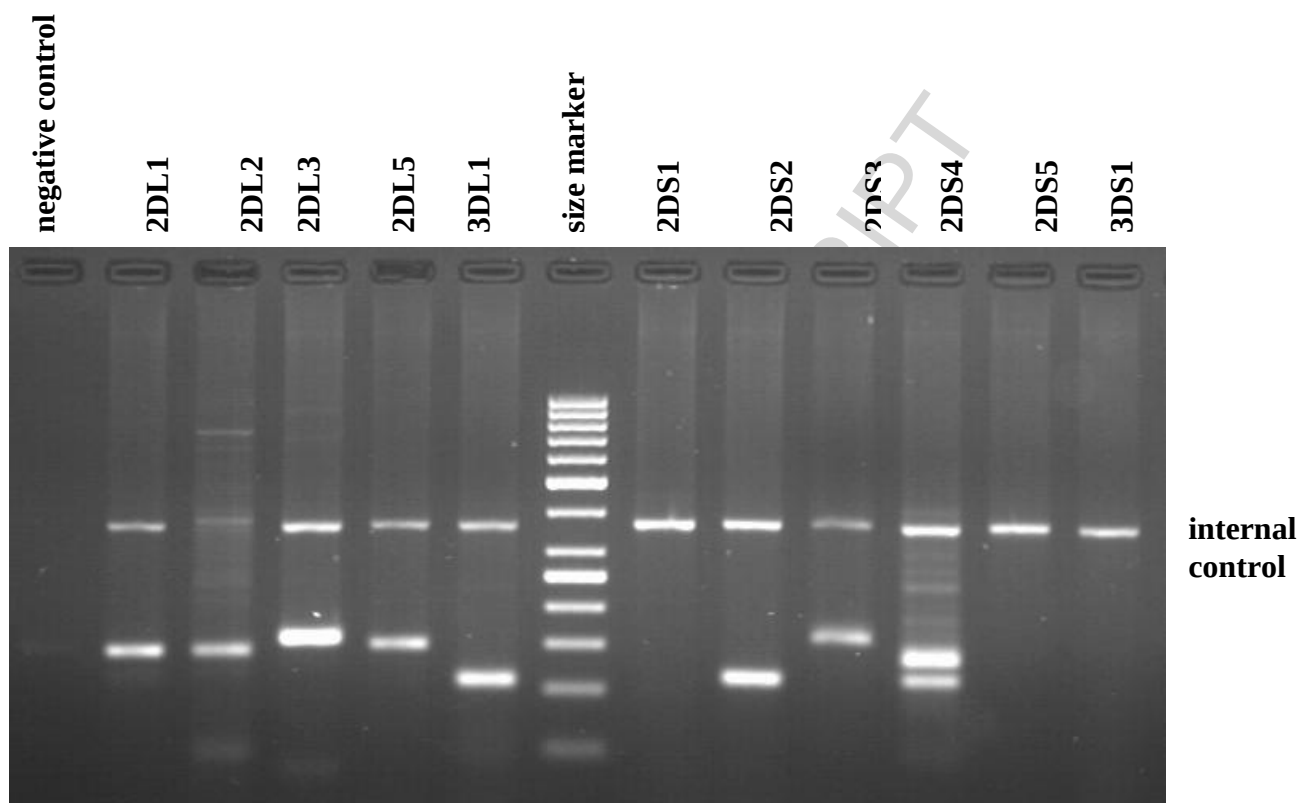


Figure 1b

Fig.1C

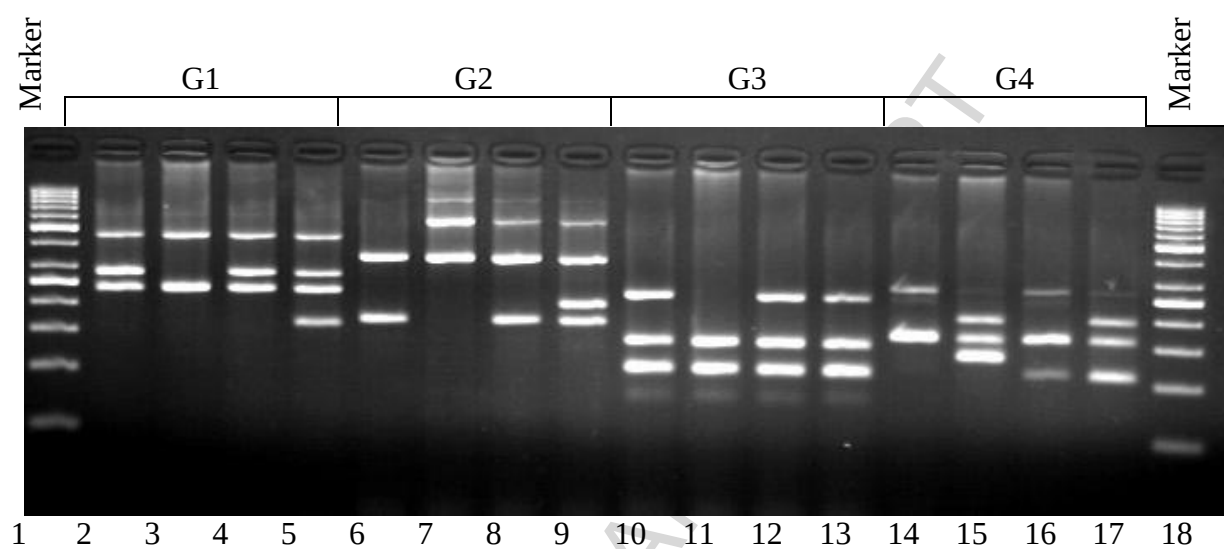
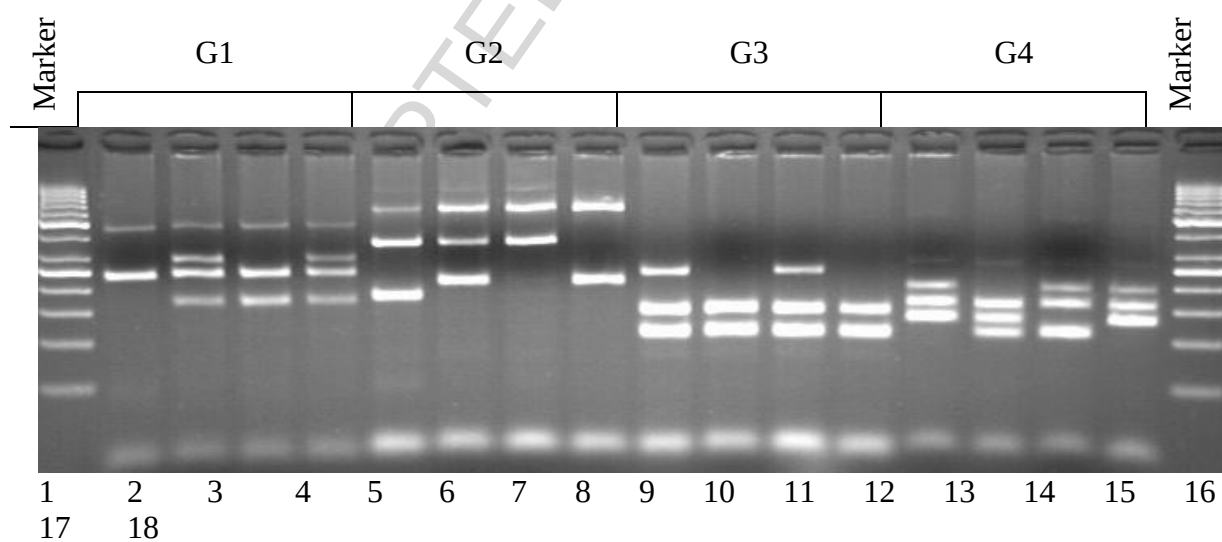


Fig.1D



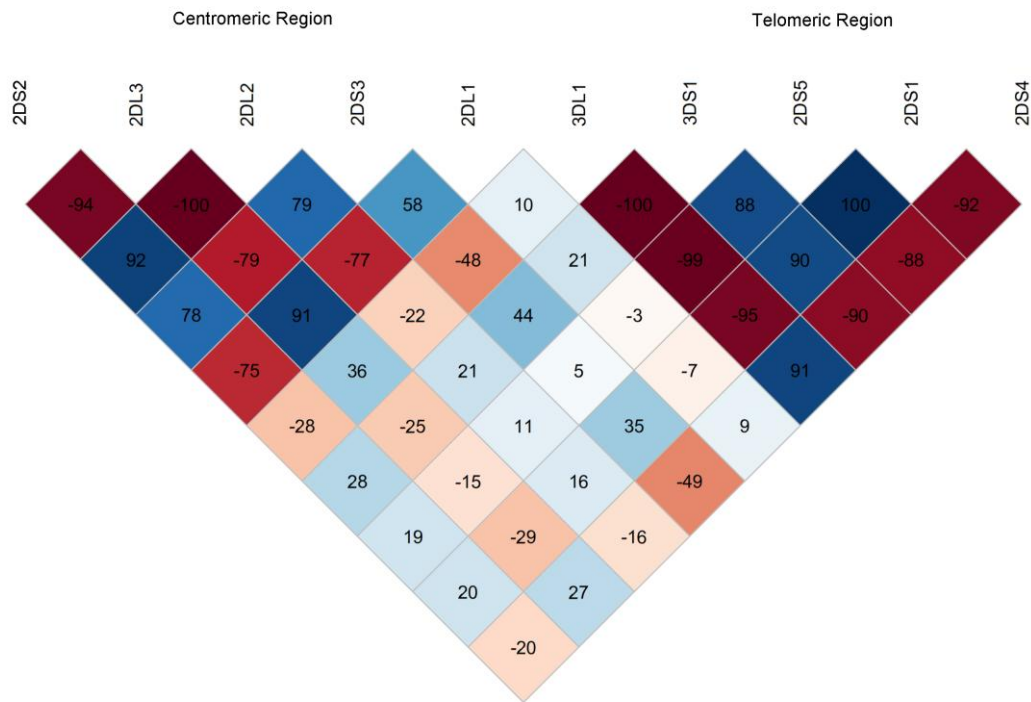


Figure 2

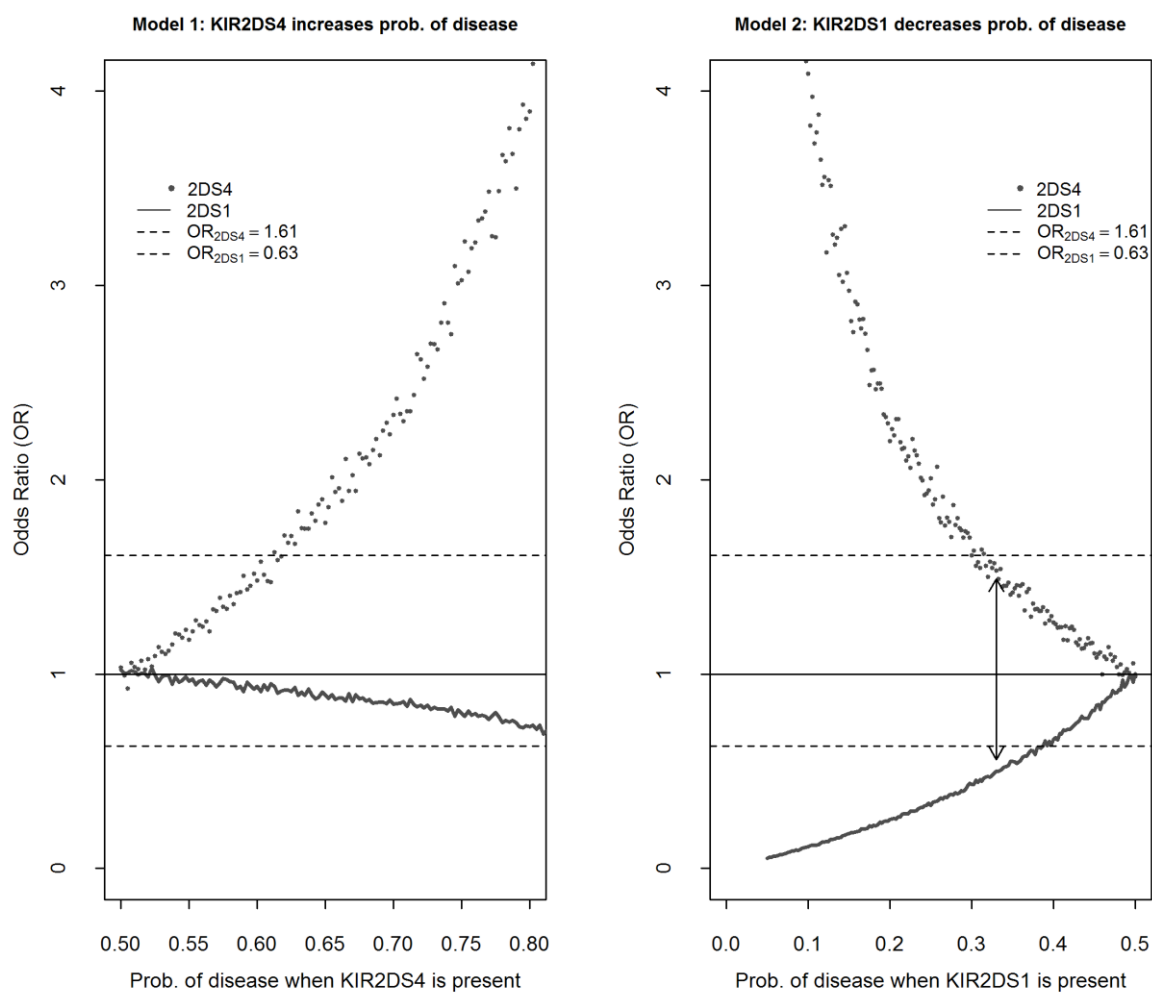


Figure 3

List of Abbreviations:

AD, atopic dermatitis

CD, cluster of differentiation

CI95%, 95% confidence intervals

DNA, deoxyribonucleic acid

HLA, human leukocyte antigen

KIR, killer cell immunoglobulin-like receptor

LD, linkage disequilibrium

OR, odds ratio

p , probability; p_{corr} , corrected p

PCR-SSP, polymerase chain reaction using sequence-specific primers

SCORAD, Scoring Atopic Dermatitis, index of disease severity

UV, ultraviolet

Highlights

- *KIR2DS1* gene seems to be associated with protection from atopic dermatitis
- *KIR2DS4* gene frequency is slightly increased in atopic dermatitis
- *KIR2DS4* is in strong negative linkage disequilibrium with *KIR2DS1*
- *KIR2DS1* rather than *KIR2DS4* affects susceptibility to atopic dermatitis
- This is first report on *KIR* gene contribution to atopic dermatitis