HLA-C1 ligands are associated with increased susceptibility to systemic lupus erythematosus

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ABSTRACT

Recently, the role of killer cell immunoglobulin-like receptor (KIR) in autoimmune diseases has received increasing attention. The present study was undertaken to determine the association of KIR genes and the human leukocytes antigen (HLA) ligands with Systemic Lupus Erythematosus (SLE) and accompanying oxidative stress. Presence or absence of 17 KIR and 5 HLA loci was performed using the polymerase chain reaction-sequence specific primer (PCR-SSP) method by case-control study. A total of 45 SLE patients, and 60 healthy controls, all of Sicilian descent, were enrolled. Plasma values of the anti-oxidant molecule Taurine were determined in all subjects by capillary electrophoresis UV detection. The carrier frequency of the KIR2DS2 gene was significantly increased in SLE patients compared to healthy controls (73.3 versus 45.0%; OR = 3.36; 95% CI = 1.46–7.74; p = .005) suggesting a role of KIR2DS2 gene in the susceptibility to disease. We also observed a strong positive association between the presence of HLA-C1 ligands group and the disease (82.2% in SLE patients versus 41.7% in controls; OR = 6.47, 95% CI = 2.58–16.26; p < .0001). Stepwise logistic regression analysis supported the effect of the HLA-C1 ligands in SLE patients (OR = 7.06, 95% CI = 0.07–2.19; p = .002), while the KIR genes were no longer significant. Interestingly, we found that SLE patients HLA-C1 positive showed significantly decreased plasma levels of antioxidant activity marker Taurine (69.38 ± 28.49 μmol/L) compared to SLE patients HLA-C1 negative (108.37 ± 86.09 μmol/L) (p = .03). In conclusion, HLA-C1 ligands group was significantly associated with an increased risk of SLE as well as an increased oxidative stress status overall in SLE patients.

1. Introduction

SLE is a chronic inflammatory disease, which mostly affects young women, characterized by an overproduction of autoantibodies to nuclear antigens. Some of these autoantibody assemble in immune-complexes and affect skin, kidneys, haematological tissues, joints, and serosal membranes, causing different clinical manifestations [1,2].

SLE is considered as a multi-factorial disease with strong contributions from genetic and environmental factors. In particular, genes located in the HLA region as HLA-DRB1∗03:01 (DR3 allelic group) and HLA-DRB1∗15:01 (DR2 allelic group) confer most of the genetic susceptibility for the development of the disease in all Caucasian population, as previously reported [3,4]. Nevertheless, numerous genes outside the HLA region also contribute to increased genetic risk, getting more difficult to understand the aetiology of the disease [5,6]. Accordingly, a common functional variant in the promoter of human Uncoupling Protein-2 gene ($-866G > A$), confers susceptibility for SLE (unpublished observations).

Recently, Spada et al. [7] have suggested the possible role of NK cells in SLE pathogenesis, showing a deregulation of NK cell activity

and altered cytokine production from these cells. It is well known that KIRs are crucial for NK regulation through their interaction with HLA Class I molecules [8]. According to their function, KIR can be divided into inhibitory KIR (KIR2DL1-4, KIR2DL5A, KIR2DL5B, and KIR3DL1-3) and activatory KIR (KIR2DS1-5, and KIR3DS1). KIR2DL4 is involved in both inhibitory and activatory signals. KIRs bind specifically defined alleles of HLA-C, HLA-B, or HLA-A [9].

KIR and HLA are highly polymorphic molecules, with some HLA-KIR combinations or KIR haplotypes having a propensity toward higher activation or lower levels of inhibition of NK cells, affecting immune response. Accordingly combinations of HLA alleles and KIR genes have been associated with several diseases such as infectious diseases, inflammatory disorders, cancer [10–12].

In addition, a growing number of studies report that KIR expressed on NK cells may play an important role in autoimmune disorders including SLE [13–23]. However, current results are inconsistent and population specific, as reported by a recent meta-analysis [24] and only a paper reported data on HLA ligands [18].

The aim of this study is to verify if KIR polymorphisms and their known HLA ligands influence the susceptibility or resistance to SLE in our homogeneous population. Moreover, we performed meta-analyses of the genes, which we found associated with SLE to validate our results.

2. Materials and methods

2.1. Patients and controls

Forty-five Caucasoid Sicilian patients with SLE (40 females and 5 males), age range 21–63 years (41.46 ± 10.89), were consecutively enrolled (so due to chance the female/male ratio was slightly different from the expected) at Rheumatology Unit of the Palermo University Hospital according to the American College of Rheumatology 1997 revised criteria [25]. SLE activity was calculated at the time of blood sampling. Baseline information including age, involved organs, duration and severity of the disease, smoking, body mass index and hypertension were collected by face-to-face interviewing. The control group consisted of 60 healthy individuals, age range 22–64 years (39.25 ± 11.48) with no history of autoimmune diseases. Both patients and controls were born in West Sicily as their parents and grandparents, so our population was genetically homogenous. The suitability of SLE sample size was checked (http://ps-power-and-sample-size-calculation.software.informer.com/) on the basis of the results of a previous study on KIR and SLE [15,26]. The Ethic Committee of Palermo University Hospital approved the study protocol, conducted in accordance with the Declaration of Helsinki and its amendments. Informed consent was obtained for collection of samples from all patients and controls.

2.2. Typing

Peripheral whole blood samples were collected, and genomic DNA was extracted from leukocytes by a commercial kit (PureLink® Genomic DNA, ThermoFisher Scientific, Waltham, MA, USA). KIR and HLA profiles were obtained by PCR-SSP, performing 28 reactions for each individual according to the manufacturer's instructions. The KIR genotyping was performed using KIR-TYPE kit (BAG Health Care GmbH). Fourteen KIR genes (2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1) and 2 pseudogenes (2DP1, 3DP1) were investigated. For HLA class I ligands, a KIR/HLA ligands kit was used (Epitop-TYPE kit; BAG Health Care GmbH, Lich, Germany). DNA of cases and controls was genotyped for the presence of the following KIR ligands groups: HLA-C1, HLA-C2, HLA-B-Bw4-80T (threonine at position 80), HLA-B-Bw4-80I (isoleucine at position 80) and HLA-A-Bw4. KIR gene profiles were determined by the presence or absence of each KIR gene.

The HLA-Cw genotypes were determined using the commercially available HLA Class I C Locus DNA Typing Tray kit (One Lambda, Thermo Fisher Scientific Brand, California, USA). The kit is based on the PCR-SSP method. To detect the specific HLA-Cw alleles, primer mixes are already available in the kit, and PCR amplification is performed according to the manufacturer's instructions .

2.3. Taurine analysis

It was conducted in collaboration with the University of Sassari. Plasma values of the anti-oxidant molecule taurine were determined by capillary electrophoresis UV detection as previously described [27].

2.4. Meta-analysis

The primary source of studies addressing the role of KIR genes in SLE was the PUBMED database limited to English language literature. The medical subject headings used for PUBMED search were: "systemic lupus erythematosus" or "SLE," killer cell immunoglobulin-like receptors" or "KIR". Last search was updated on May 31, 2017. The abstracts found were read to identify papers reporting of the frequencies in Caucasoid controls and patients of KIR genes found positive in our study. The papers were read in their entirety to assess their appropriateness for inclusion in the meta-analysis. Extraction of data was performed independently by CC, CMG and DDB who compared results and agreed on a consensus; disagreements were settled by discussion.

2.5. Statistics

The comparisons of frequencies of KIR genes and haplotypes between case and control groups were tested by contingency tables (χ^2 test) constructed to determine statistical differences of the two groups analysed. The data were tested for goodness of fit between the observed and expected genotype and haplotype values and their fit to the Hardy–Weinberg equilibrium. The magnitudes of risk associations are reported by odds ratios (OR) and confidence intervals (95% CI). Statistical analysis was performed by using Graphpad Prism Software (Sand Diego, CA, USA). The p value of = 0.05 was adopted as the significance limit. A logistic regression model was also carried out, in order to derive a reduced and easily interpretable model for predicting onset. The unpaired Welch's correction of Student's *t* test was used for analysis of two nonparametric quantitative data.

For meta-analysis the data were analysed using Review Manager, version 5.1, a statistical software package for managing and analysing all aspects of a Cochrane Collaboration systematic review (The Cochrane Collaboration, Oxford, UK, 1999). The overall OR between the frequencies of genes in both cases and controls was estimated with models based on both fixed-effects and random-effects assumptions. The fixed effects model considers only within-study variability. The random effects model uses weights that include both the within-study and between-study variance. Because of the high heterogeneity between the populations of most of the studies included in this meta-analysis, we have presented the results of random-effects models that are the most conservative ones [28]. The 95% CI of the OR was also calculated.

3. Results

3.1. Genetic analysis

To assess if genetic variants of KIR and their HLA ligands play a role in SLE we first compared the frequencies of these genes in patients with SLE and controls (Table 1). We observed a significantly higher frequency of the activating KIR2DS2 gene in patients compared to healthy controls (73.3% vs. 45.0%, p = .005; OR = 3.36, 95% CI = 1.46–7.74) suggesting a role of KIR2DS2 gene in the susceptibility to disease. A

Table 1
Comparison between KIR and HLA genes frequencies in SLE patients and healthy controls.

KIR gene	SLE (n = 45)		Control (n = 60)			
	%	n	%	n	p	Odd Ratio (95% CI)
Inhibitory						
2DL1	95.5	43	96.7	58	ns	
2DL2	71.1	32	60.0	36	ns	
2DL3	80.0	36	76.7	46	ns	
2DL4	100	45	100	60	ns	
2DL5A	44.4	20	55.0	33	ns	
2DL5B	48.9	22	25.0	15	= 0.01	2.87 (1.25–6.56)
3DL1	86.7	39	93.3	56	ns	
3DL2	100	45	98.3	59	ns	
3DL3	100	45	98.3	59	ns	
Activating						
2DS1	44.4	20	48.3	29	ns	
2DS2	73.3	33	45.0	27	= 0.005	3.36 (1.46–7.74)
2DS3	46.7	21	45.0	27	ns	
2DS4*001	26.7	12	45.0	27	ns	
2DS4*003-007	64.4	29	66.7	40	ns	
2DS5	42.2	19	43.3	26	ns	
3DS1	44.4	20	48.3	29	ns	
HLA gene						
HLA-A-Bw4	28.9	13	10.0	25	ns	
HLA-B-Bw4-80I	48.9	22	36.7	22	ns	
HLA-B-Bw4-80 T	28.9	13	20.0	12	ns	
HLA-C1^{Asn80}	82.2	37	41.7	25	< 0.0001	6.47 (2.58–16.26)
HLA-C2^{Lys80}	68.9	31	60.0	36	ns	

significant trend was also observed for KIR2DL5B gene. In particular, we found higher frequency of KIR2DL5B in SLE patients compared to controls (48.9% vs. 25.0%, p = .01; OR = 2.87, 95% CI = 1.25–6.56) (Table 1). No statistical differences were found for other KIR genes (Table 1).

Two broad haplotypes of KIR genes have been defined on the basis of gene content. The A haplotype contains a single surface activating KIR gene, KIR2DS4, which is present as a null allele in 80% of cases, and 5 inhibitory KIR genes (KIR2DL1, KIR2DL3, KIR3DL1, KIR3DL2, and KIR3DL3). In contrast, the B haplotype is characterized by variable numbers of activating and inhibitory genes [29]]. No significant differences in the frequency of the two haplotypes (haplotype B included both homo- and heterozygotes, i.e. BB or AB) were observed between the two groups (data not shown).

The combinations of HLA class I and KIR variants contribute to both innate and acquired immune responses, hence they might influence susceptibility to autoimmune diseases [30]. To this end, we analysed all known HLA class I KIR ligands. We observed a strong positive association between the presence of HLA-C1 ligands and the disease. Particularly, we found that the HLA-C1 ligands were present at the ratio of 82.2% in SLE patients versus 41.7% in controls (p < .0001; OR = 6.47, 95% CI = 2.58–16.26) (see Table 1).

Regarding the HLA-KIR interaction, alleles of HLA-C1 group are the ligands of KIR2DL2, KIR2DL3, and KIR2DS2 receptors [8]. The combination of KIR2DL2/2DL3 genes and HLA-C1 ligands was detected significantly higher in patient group compared to controls (60.0% vs. 28.3%, p = .001, OR = 3.79, 95% CI = 1.67–8.60 and 62.2% vs. 30.0%, p = .001, OR = 3.84, 95% CI = 1.70–8.70, respectively). Moreover, the presence of both KIR2DS2 and HLA-C1 ligands also exhibited a higher frequency in SLE group than KIR2DS2 separately (62.2% vs. 21.7%, p < .0001, OR = 5.95, 95% CI = 2.52–14.08), as reported in Tables 1 and 2. Similar findings were reported for the

combination of KIR2DS2 gene and HLA-A-Bw4 in SLE patients compared to controls (24.4% vs. 3.3%, p = .002, OR = 9.38, 95% CI = 1.96–44.89) (Table 2).

All genotypes are shown in Supplementary Tables 1 and 2.

To determine which Cw allele in C1 group is involved in the susceptibility, we determined the frequencies of HLA-Cw alleles in patients and controls. No significant difference between SLE patients and healthy controls was observed (Supplementary Table 3). This suggests that the increased frequency of HLA-C1 ligands in lupus is probably due to the synergic presence of all alleles belonging to this group showing the same epitope.

3.2. Meta-analysis

Due to the discordant results present in literature [13–23] we decided to perform a meta-analysis of the papers that reported the gene frequencies in patients and controls of KIR and HLA genes shown to be associated with SLE in the present report.

Three studies on the association between 2DSD2 gene and SLE were identified in Caucasoid populations by our search strategy, i.e. the studies of Akhtari et al. [18], Pellet et al., [16] and Tozkir et al., [15]. The pooled summary, including data of the present report, OR for the genotypic comparison between the patients versus the controls is 1.30 (95% CI = 0.80–2.12.) with no statistical significance using the random-effects model.

Two studies on the association between 2DL5B gene and SLE were identified in Caucasoid populations by our search strategy, i.e. the studies of Akhtari et al. [18] and Tozkir et al., [15]. The pooled summary, including data of the present report, OR for the genotypic comparison between the patients versus the controls is 1.83 (95% CI = 0.67–4.96) with no statistical significance using the random-effects model.

Unfortunately, as previously stated, only a previous study has been performed on the frequencies of HLA ligands [18], so it was not possible to perform a meta-analysis.

3.3. Logistic regression

However, as a next step a logistic regression model was carried out, in order to derive a reduced and easily interpretable model for predicting the onset of the disease. By logistic regression analysis, the only significant association remained with HLA-C1 ligands (p = .002; OR = 7.06, 95% CI = 0.07–2.19).

3.4. Taurine and KIR/HLA

As further analysis we investigated the implication of HLA-C1 ligands in oxidative stress in SLE patients. To this end, we compared the mean values of the anti-oxidant molecule Taurine [27] according to HLA-C1 ligands, using Student's *t*-test with Welch correction.

Taurine is a sulphur amino acid present at high concentration in tissues exposed to elevated levels of oxidative stress. It is now accepted that taurine plays an important antioxidant activity in the immune system, protecting cells from oxidative stress [31,32].

In our study the plasma levels of antioxidant activity marker Taurine were found significantly increased in SLE patients HLA-C1 negative (108.37 ± 86.09 μmol/L) compared to SLE patients HLA-C1 positive (69.38 ± 28.49 μmol/L) (p = .03) (Fig. 1). No significant differences were observed, analysing data according to Cw alleles (data not shown).

4. Discussion

SLE is a chronic inflammatory disease, affecting mostly young women, characterized by a deregulated immune response. This determines an overproduction of autoantibodies, some of which assemble

Table 2
KIR genotypes detected in SLE patients and healthy controls.

| Genetic Factor	SLE n = 45 (%)	Controls n = 60 (%)	P	Odd Ratio (95% CI)
HLA-C1C1	14 (31.1%)	8 (13.3%)	= 0.03	2.93 (1.10–7.79)
HLA-C1C2	23 (51.1%)	17 (28.3%)	= 0.025	2.64 (1.18–5.95)
HLA-C2C2	8 (17.8%)	19 (31.7%)	Ns	
2DL2 + HLA-C1	27 (60.0%)	17 (28.3%)	= 0.0014	3.79 (1.67–8.60)
2DL3 + HLA-C1	28 (62.2%)	18 (30.0%)	= 0.0014	3.84 (1.70–8.70)
2DS2 + HLA-C1	28 (62.2%)	13 (21.7%)	< 0.0001	5.95 (2.52–14.08)
2DL2 + HLA-C1C1	10 (22.2%)	6 (10.0%)	ns	
2DS2 + HLAC1C1	11 (24.4%)	3 (5.0%)	= 0.007	6.15 (1.60–23.61)
2DL1 + HLA-C2	29 (64.4%)	35 (58.3%)	ns	
2DS1 + HLA-C2	15 (33.3%)	19 (31.7%)	ns	
2DS2 + HLA-C2	22 (48.9%)	18 (30.0%)	ns	
2DS2 + HLA-C2C2	5 (11.1%)	9 (15.0%)	ns	
HLA-B-Bw4-80 T – HLA-B-Bw4-80 T	9 (42.2%)	9 (15%)	ns	
HLA-B-Bw4-80 T- HLA-B- Bw4-80I	18 (40.0%)	19 (31.6%)	ns	
HLA-B-Bw4-80I-HLA-B- Bw4-80I	4 (8.8%)	3 (5.0%)	ns	
3DL1 + HLA- B-Bw4-80 T	12 (26.7%)	11 (18.3%)	ns	
3DL1 + HLA-B- Bw4-80I	20 (44.4%)	21 (35.0%)	ns	
3DS1 + HLA- B-Bw4-80I	8 (17.8%)	12 (20.0%)	ns	
2DS2 + HLA-A-Bw4	11 (24.4%)	2 (3.3%)	= 0.002	9.38 (1.96–44.89)
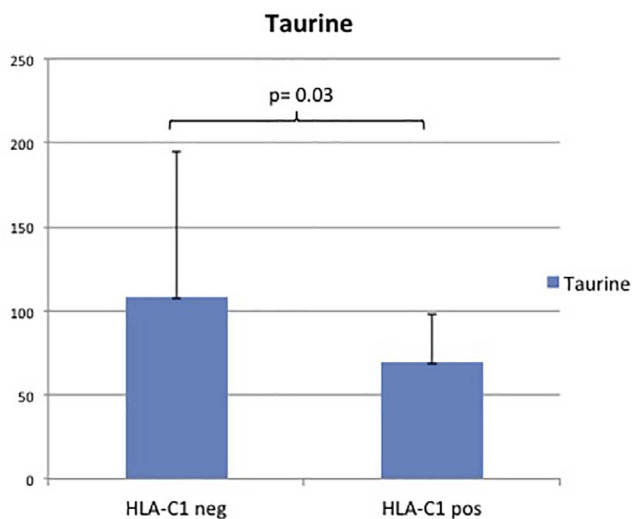
Fig. 1. Analysis of Taurine levels according to the presence or absence of HLA-C1 ligands. SLE patients HLA-C1 negative exhibit significantly higher levels of plasma Taurine compared to SLE patients HLA-C1 positive (p = .03).

in immune-complexes and affect multiple organs, resulting in a wide spectrum of different clinical manifestations. Multiple predisposing factors including hormonal factors, environmental, and, genetics are responsible for the onset of the disease [1,2].

Our genetic association study shows that the KIR2DL5B inhibitory and KIR2DS2 activating receptor genes, as well as the KIR ligand group HLA-A-Bw4 and HLA-C1 are associated with the disease. Activating KIR2DS2 gene was found to be significantly higher in SLE patients (OR = 3.36, p = .005), suggesting a role in the susceptibility to disease, as previously reported by Toloza et al. [33] in SLE patients with vascular arterial events. Similar results have been obtained by Pedroza et al. [19] reporting activating KIR gene-based KIR2DS2 + /KIR2DS5 + / KIR2DS1 + profiles in patients with SLE. However, our data are different from the report by Pellet et al. [16] and Hou et al. [17] which observed, on the contrary, a significant increase in the frequency of KIR2DS1 in the absence of KIR2DS2 gene in Caucasian and Asian patients with SLE, respectively.

A significant association was also observed between KIR2DL5B gene and SLE susceptibility. KIR2DL5B is the last functional KIR identified in the human genome, for which no ligands have yet been recognized. Since its discovery, it was defined as an inhibitory "orphan" KIR

receptor [34] and its function and importance in human health are poorly understood. Recently, Tozkir et al. [15] suggested that the presence of KIR2DL5B might have a role in the pathogenesis of autoimmune disorders such as systemic sclerosis and SLE. In line with these data, we found that KIR2DL5B is mainly expressed in SLE patients respect to controls (OR = 2.87, p = .01).

The results of our analysis comparing SLE to healthy controls showed that the frequency of HLA-C1 ligands group was more frequent in case (82.2%) than in control group (41.7%), suggesting a detrimental role in the pathogenesis of SLE.

Moreover, our stratification analysis on combination of KIR and their ligand suggested that the some receptor–ligand combinations were positively associated with SLE. The KIR2DL2/HLA-C1, KIR2DL3/ HLA-C1 and KIR2DS2/HLA-C1 combinations were reported to be positively associated with SLE. The study by Tozkir et al. [15] presented the combination of KIR2DS2/HLA-C1 as a strong significant risk factor in SLE (this combination existed in 30% of the controls and 53.3% of the patients, p = .03). Our results, in agreement with data from Tozkir et al. [15], may suggest that the activating function is probably the major factor interfering in the pathogenesis of SLE.

However, analysing pooled data with results of previous studies we failed to find correlations between KIR2DL5B and KIR2DS2 genes with susceptibility to SLE (P > .05). As previously mentioned, only a study has been performed on the frequencies of HLA ligands [18], so it was not possible to perform a meta-analysis.

Stepwise logistic regression analysis supported the effect of the HLA-C1 ligands in SLE patients (OR = 7.06, p = .002), while the KIR genes were no longer significant.

To best of our knowledge, in addition to the strong association of SLE susceptibility with HLA class II, the present study is the first reporting significant association between this disease and the HLA class I locus C.

Some report suggests that KIR genes might play a role in disease susceptibility in autoimmune diseases such as rheumatoid arthritis through their interaction with HLA class I molecules[35]. KIR and HLA interaction is crucial for the NK cell regulation. These cells participate in the immune response and T cell activation. Recently, it has been suggested that NK not only exert cell-mediated cytotoxicity against infected or cancer cells, but are also able to promote or suppress functions of other immune cells by secretion of cytokines and chemokines [36]. Thus, it is reasonable suppose that their over activation or dysfunction might be associated with pathogenesis of autoimmune diseases.

Our results showed that KIR and, in particular, HLA-C1 ligands

might to be associated with the onset of the disease. The mechanism by which HLA-C1 is involved in SLE pathogenesis is not known. Nevertheless, we can suppose that HLA-C1 induces NK cell activation through its KIR and thereby promote the autoimmune process.

The detrimental effects of the activating HLA-C1 ligands are also confirmed by data of oxidative stress. We found that SLE patients HLA-C1 negative showed higher levels of antioxidant activity marker Taurine compared to SLE patients HLA-C1 positive.

Taurine is the most abundant sulphur amino acid involved in many fundamental biological functions such as anti-oxidative and anti-inflammatory effects [37]. Studies in animal models and in humans reported that modification in Taurine levels plays a major part in the development of chronic inflammatory and degenerative diseases as diabetes, cardiovascular diseases and ageing [38–41], nevertheless, a few studies have investigated this molecule in autoimmune diseases. However, according to a recent work, Taurine attenuates oxidative stress and alleviates cardiac failure in type I diabetic rats [42]. So, it is reasonable to suppose that it may be equally involved in oxidative stress in SLE, contributing to pathogenesis of the disease.

No significant association has been observed with the other parameters considered in the analysis (data not shown).

In conclusion, we used a genetic approach to provide the first evidence for a direct association of HLA-C1 ligands with SLE pathogenesis in Sicilian population, suggesting its possible role as a strong risk factor marker of the disease. The binding of HLA-C1 ligands to their receptors might induce NK cells activation and an increase of oxidative stress status that translate into SLE progression. Further studies on the genotyping, expression, and function of KIR receptors and their HLA ligands should be done to fully determine the role of these ligands and receptors in the onset of the disease.

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Disclosure

The authors state that they have no disclosure to declare.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.humimm.2018.01.005.

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