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ERAP, *KIR* and *HLA-C* gene interaction in susceptibility to recurrent spontaneous abortion in the Polish population

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ABSTRACT

Endoplasmic reticulum aminopeptidases ERAP1 and ERAP2 trim peptides to generate stable antigenic epitopes for their presentation by HLA class I (HLA-I) molecules to T cell receptor. By influencing the peptide repertoire of HLA-I molecules, they affect also the interactions of HLA-I with killer cell immunoglobulin-like receptors (KIRs) of natural killer (NK) cells. HLA-C is the only polymorphic HLA-I molecule present on the trophoblast.

In this study we investigated the role of *ERAP1* and *ERAP2* polymorphisms in the context of *KIR* and *HLA-C* genes in women suffering from recurrent spontaneous abortion (RSA) in the Polish population. We used TaqMan genotyping assays for *ERAP1* rs27044, rs30187, rs2287987, rs26618, rs2665 and ERAP2 rs2248374; PCR-SSP methods for *KIR* and *HLA-C* genotyping. We tested 285 women who experienced recurrent spontaneous abortion (RSA) and 319 fertile women.

We observed a significant association of *ERAP1* rs30187TT genotype with RSA ($p = 0.02$, OR = 1.89, 95%CI = 1.11–3.21), however the most striking association was found in comparison of patients and controls with *ERAP1* rs30187TT and *KIR* Bx genotypes ($p = 0.006$, $p_{corr.} = 0.036$, OR = 2.40, 95%CI = 1.27–4.52). Moreover, this effect was even stronger in *HLA-C2* positive patients ($p = 0.0031$, $p_{corr.} = 0.019$, OR = 3.46, 95%CI = 1.48–8.11). Other weaker associations of the remaining tested ERAP single nucleotide polymorphisms with RSA were also presented.

In conclusion, *ERAP1* rs30187TT genotype itself increased susceptibility to RSA but this effect was much stronger in patients positive for *HLA-C2* and *KIR* Bx genotypes.

1. Introduction

Reproductive success depends on the immunological tolerance of a mother to her semi-allogenic fetus. It is believed that the recognition of human leukocyte antigen (HLA) molecules by the receptors of uterine NK or T cells leads to a number of mechanisms that determine the immune tolerance of a mother to embryo and later to fetus. The interaction between parental HLA class I (HLA-C or/and HLA-G) expressed by invading placental trophoblast cells and maternal killer cell immunoglobulin-like receptors (KIRs) localized on NK cells, which are a prevalent lymphocyte population in the decidua, could have an influence on pregnancy outcome [1–3]. In turn, inadequate interaction of maternal KIRs and trophoblastic HLA-C causes insufficient invasion of the extravillous trophoblast cell leading to limited blood flow and abnormal placental development by "starving" of the fetus, resulting in retarded fetal growth, still birth or even spontaneous abortion [4]. KIR-HLA-C interactions are affected by HLA-C-bound peptides, therefore proper peptide trimming by endoplasmic reticulum aminopeptidases ERAP1 and ERAP2 could also have a significant impact on gestation.

As mentioned above, uterine NK cells have been shown to express inhibitory (KIR2DL, KIR3DL) and activating killer immunoglobulin-like receptors (KIR2DS, KIR3DS) which, upon recognition of respective HLA molecules on trophoblast cells, can induce either an inhibitory or activating function [1]. Indeed, the function of the NK cell depends on

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balance between the activating and inhibitory signals from all receptors expressed by this cell.

KIR genes are encoded by chromosome 19 and show extensive polymorphism. This means that people differ in both the number and kinds of KIRs; therefore populations exhibit different distribution of these genes. *KIRs* can be organized in two haplotypes: A and B. Both haplotypes consist of four framework genes: *KIR3DL3* at the centromeric end, *KIR3DL2* at the telomeric end and *KIR3DP1* and *KIR2DL4* in the middle of the *KIR* gene cluster. Group A haplotypes are generally non-variable in their gene content possessing *KIR2DL1, KIR2DL3, KIR3DL1, KIR2DS4* and *KIR2DP1*. Group B haplotypes show variation in the number and combination of *KIR* genes present and are characterized by the presence of one or more of *KIR2DL2*, *KIR2DL5A/B*, *KIR2DS1*, *KIR2DS2*, *KIR2DS3*, *KIR2DS5* and *KIR3DS1* genes [https://www.ebi.ac.uk/ipd/kir].

HLA-C gene is enormously polymorphic. Up to December 2018, 4654 alleles were discovered which encode 3070 proteins [5]. These alleles can be divided into two allotypes: C1 and C2 based on the presence of asparagine or lysine at position 80 of the HLA-C α-domain, respectively. HLA-C C1 allotypes bind inhibitory KIR2DL2/3, while HLA-C C2 allotypes bind KIR2DL1 and KIR2DS1. However, the interaction of KIR2DL2/3 with HLA-C is weaker than that of KIR2DL1 [6].

ERAP1 and ERAP2 are encoded by genes, which are polymorphic and single nucleotide polymorphisms (SNPs) can result in differential expression of many products with different multiple amino acid changes and this in turn, can alter the enzymatic activity and substrate binding [7–10]. The *ERAP1* and *ERAP2* genes are located on chromosome 5. Moreover, biological functions of some SNPs of *ERAP1* could be deduced from their location in the ERAP1 structure. The rs2287987 (M349V) polymorphism is located in the active site and could have an impact on interactions with the substrate. In the inner surface of the C-terminal cavity, rs27044 (E730Q) could have influence on substrate sequence or length specificity. At domain junctions there are rs26653 (R127P) and rs30187 (R528K), which could alter the conformational change between open and closed and therefore impact on specificity and enzymatic activity [11]. For instance, *in vitro* studies have demonstrated that 528R has lower enzymatic activity and protein expression in contrast to 528 K [9,10,12].

In contrast to *ERAP1*, *ERAP2* shows limited polymorphism. There are two major SNPs, which could play a role in the biological function of this enzyme: the coding polymorphism rs2549782 (N392K) and the non-coding rs2248374. The non-synonymous SNP rs2549782 causes an amino acid substitution of lysine (K392) to asparagine (N392) near the catalytic center of the enzyme. It affects both enzymatic activity and substrate specificity. Moreover, the N392 variant results in higher trimming efficiency of antigenic peptide precursors than K392 [12,13]. However, *ERAP2* SNPs are grouped into haplotypes (A and B), thus N392 is almost never expressed in humans, because of its strong linkage with SNP rs2248374. This latter SNP (rs2248374) G allele leads to alternative splicing and production of instability in mRNA. The further nonsense-mediated decay occurs, the more it degrades aberrant mRNA including in-frame stop codon that results in truncated protein and a lack of ERAP2. Hence AG heterozygotes and AA homozygotes produce full-length ERAP2 in contrast to GG homozygotes (about 25% of white Europeans) where ERAP2 is not expressed [10–12,14–16].

The aim of this study was to investigate the role of endoplasmic reticulum aminopeptidases *ERAP1* and *ERAP2* polymorphisms in the context of *KIR* and *HLA-C* genes in women suffering from recurrent spontaneous abortion (RSA) in the Polish population; a pregnancy complication defined as the loss of at least three consecutive pregnancies with the same partner. Because we also have access to samples from women with two miscarriages we decided to include this group in the analyses. *ERAP* polymorphisms and correlation of these variants with *HLA-C* and *KIR* polymorphisms allowed us to assess whether each of them separately constitutes a risk factor for RSA or the risk is rather due to their interactions. To date, there are no studies on the role of *ERAP* polymorphisms with RSA. According to our knowledge, no one has published any studies on the combined association between *HLA-C, KIR* and *ERAP* polymorphisms with susceptibility to RSA.

2. Material and methods

2.1. Study design

All patients were recruited from the Department of Surgical, Endoscopic and Oncologic Gynecology and Department of Gynecology and Gynecologic Oncology, Polish Mothers' Memorial Hospital–Research Institute, Poland. Two hundred and eighty five women, who had experienced spontaneous abortion (SA, 2–8 miscarriages, mean age 32.71 ± 3.98, range 24–46) but were free from chromosomal aberrations, uterine anomalies, hormonal disturbances, and infections with *Toxoplasma*, *Chlamydia*, *Listeria*, and *Brucella*, were originally selected for our study. Among them 202 women belonged to the recurrent spontaneous abortion (RSA) category with 3 or more first trimester spontaneous abortion incidents with the same partner. Two hundred and sixty four patients were primary aborters, forming 97.78% of all patients. Two hundred and twenty three women miscarried before the 12th week of gestation (83.52%). Moreover, in 157 patients (57.72%) no autoantibodies were detected. The remaining women (42.28%) possessed a different set of autoantibodies, such as anticardiolipin, antinuclear, antithyroid, anti-β-glycoprotein, and factor LA. In addition, twenty five patients were diagnosed with antiphospholipid syndrome.

The control group was recruited from the 1st Department of Obstetrics and Gynecology, Medical University of Warsaw and from the Disctrict Hospital Strzelce Opolskie. This group consisted of 319 healthy couples with at least 1 healthy-born child and no history of miscarriage or endocrinological or immunological disorders: women had a mean age 32.36 years ± 5.80, (age range 22–68). Thus, both control and spontaneous abortion groups were age-matched. All tested individuals were of Polish origin. Experimental protocols were approved by Local Ethics Committees (the agreement of Medical University of Wroclaw and Polish Mothers' Memorial Hospital–Research Institute in Lodz) and informed consent was obtained from all individual participants included in the study.

2.2. DNA preparation and genotyping

Genomic DNA was isolated from venous blood using the Invisorb Spin Blood Midi Kit (Invitek, Berlin, Germany) following the manufacturer's instructions.

ERAP genotyping was performed using the TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, USA) as described in more detail previously [17]. Characteristics of the ERAP single nucleotide polymorphisms (SNPs) examined in this study were shown in Table 1.

KIR genotyping was performed using KIR Ready Gene kits (Innotrain Diagnostics, Germany) following the manufacturer's instructions, or multiplex PCR described elsewhere [18,19]. 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

Table 1
Characteristics of the SNPs examined in this study.

Chr.	Gene/Locus	SNP	Variation	Assay ID
5	*ERAP1*	rs26618	C > T, M276I	C_3056894_10
5	*ERAP1*	rs27044	C > G, E730Q	C_3056870_10
5	*ERAP1*	rs30187	C > T, R528K	C_3056885_10
5	*ERAP1*	rs2287987	T > C, M349V	C_3056893_20
5	*ERAP1*	rs26653	G > C, P127R	C_794818_30
5	*ERAP2*	rs2248374	G > A, intron	C_25649529_10

Chr. – chromosome.

California, Los Angeles. *KIR* AA genotype means the presence of *KIR2DL1, KIR2DL3, KIR2DS4, KIR3DL1* and the absence of *KIR2DL5*, *KIR2DS1*, *KIR2DS2*, *KIR2DS3*, *KIR2DS5* and *KIR3DS1*, which may be found in KIR Bx genotype. *KIRs* were also divided according to presence in the centromeric or telomeric part of the *KIR* gene cluster: CenA-KIR2DL3, CenB-KIR2DL2 and KIR2DS2, TelA-KIR3DL1 and KIR2DS4, TelB – KIR2DS1 and KIR3DS1 [20].

HLA-C gene fragments determining the C1 and C2 allotypes were established according to a PCR-SSP method described in detail elsewhere [19,21].

2.3. Statistical analysis

The frequencies of alleles and genotypes were estimated by direct counting. Comparison of allele and genotype frequencies between the spontaneous abortion group and the fertile group were calculated using two-tailed Fisher's exact test (GraphPad InStat ver. 3.06, San Diego, CA, USA). A p value < 0.05 was considered significant. The odds ratio (OR) and its 95% confidence interval (95% CI) were computed as the measure of effect size. Bonferroni correction was done in a case of multiple comparisons. Hardy-Weinberg equilibrium was checked using the chi-square test with one degree of freedom. All genotype frequencies were in Hardy-Weinberg equilibrium both in control and in RSA group.

3. Results

Summarized effect of all significant associations is shown in Table 2. Detailed information concerning allele and genotype frequency of the examined genes, as well as comparisons between patients and controls are shown in Supplementary Tables 1–11.

3.1. Distribution of ERAP1 and ERAP2 polymorphisms in RSA patients and fertile controls

We observed a similar distribution of alleles and genotypes between patients and fertile controls in *ERAP 1* rs27044, rs26653, rs2287987 and *ERAP2* rs2248374 SNPs. However, we found significant association of rs30187TT genotype with RSA ($p = 0.02$, OR = 1.89, 95%CI = 1.11–3.21) and rs26618TC ($p = 0.049$, OR = 0.71, 95%CI = 0.50–1.00) with protection against disease (Supplementary Table 1).

3.2. Combination of ERAP1/2 and HLA-C C1/C2 genotypes in women with miscarriages and fertile controls

Women positive for rs30187TT and *HLA-C2* genotypes were strongly predisposed to miscarriage ($p = 0.004$, $p_{corr.} = 0.024$, OR = 2.79, 95%CI = 1.37–5.70), however less significant in rs3018TT/C1C2 patients ($p = 0.02$, $p_{corr.} > 0.05$, OR = 2.63, 95%CI = 1.16–5.99) and the association was lost in rs3018TT/C2C2 positive patients ($p = 0.09$, OR = 3.50, 95%CI = 0.83–14.70). Significance was lost probably from a lower HLA-C2 than HLA-C1 frequency in the Polish population, but an increase in the odds ratio from 2.63 for TT/C1C2 to 3.50 for TT/C2C2 indicates that an increase in the number of patients and/or controls tested could affect the significance of this analysis (Supplementary Table 2).

We also found an association of rs27044GG genotype with RSA, independent of *HLA-C* or *KIR* genotype (Supplementary Tables 2, 5 and 6).

Moreover, patients positive for rs26618TT/HLA-C1 were more frequent in the miscarriage group than in controls ($p = 0.04$, $p_{corr} > 0.05$, OR = 1.44, 95%CI = 1.02–2.04; Supplementary Table 2).

Also, patients carrying rs2248374GG of *ERAP2* and *HLA-C1* ($p = 0.06$, OR = 1.46, 95%CI = 0.99–2.16) and GG/C1C2 combination ($p = 0.03$, $p_{corr} > 0.05$, OR = 1.79, 95%CI = 1.07–2.99) were more

Table 2
Summarized effect of *ERAP, HLA-C* and *KIR* polymorphisms on susceptibility to recurrent spontaneous abortion.

ERAP, HLA-C, KIR combination	Associated combination	p	$p_{corr.}$	OR	95% CI	Effect	Table
ERAP1 rs30187 C > T	TT	0.02	–	1.89	1.11–3.21	↑	Suppl.1
ERAP1 rs30187 C > T/HLA-C	TT/C2+	**0.004**	**0.024**	**2.79**	**1.37–5.70**	↑	Suppl.2
	TT/C1C2	0.02	NS	2.63	1.16–5.99	↑	Suppl.2
ERAP1 rs30187 C > T/KIR	TT/Bx+	**0.006**	**0.036**	**2.40**	**1.27–4.52**	↑	Suppl.3
	TT/cen AB tel AA	0.022	NS	4.53	1.20–17.11	↑	Suppl.3
ERAP1 rs30187 C > T/HLA-C/KIR	TT//C1+/Bx+	0.02	NS	2.24	1.16–4.32	↑	Suppl.4
	TT/C2+/Bx+	**0.0031**	**0.019**	**3.46**	**1.48–8.11**	↑	Suppl.4
	TT/C1C2/Bx+	0.01	NS	3.26	1.31–8.10	↑	Suppl.4
ERAP1 rs27044 C > G/HLA-C	GG/C2+	0.02	NS	2.69	1.14–6.36	↑	Suppl.2
	GG/C1C2	0.04	NS	2.90	1.10–7.70	↑	Suppl.2
	CG/C1C1	0.03	NS	1.84	1.05–3.23	↑	Suppl.2
ERAP1 rs27044 C > G/KIR	CC/Bx+	0.042	NS	0.67	0.46–0.98	↓	Suppl.5
	CC/cen AB	0.034	NS	0.58	0.36–0.95	↓	Suppl.5
	CC/cen AB tel AA	0.05	NS	0.47	0.23–0.96	↓	Suppl.5
ERAP1 rs27044 C > G//HLA-C/KIR	GG/C2+/Bx+	0.01	NS	3.82	1.35–10.84	↑	Suppl.6
	GG/C1C2/Bx+	0.01	NS	4.09	1.30–12.85	↑	Suppl.6
ERAP1 rs26618 T > C	TC	0.049	–	0.71	0.50–1.00	↓	Suppl.1
ERAP1 rs26618 T > C/HLA-C	TT/C1+	0.04	NS	1.44	1.02–2.04	↑	Suppl.2
ERAP1 rs26618 T > C/KIR	TC/Bx+	0.018	NS	0.62	0.42–0.91	↓	Suppl.7
	TT/Bx+	0.02	NS	1.58	1.08–2.31	↑	Suppl.7
	TC/cen AA tel AB	0.01	NS	0.28	0.12–0.67	↓	Suppl.7
	TT/cen AA tel AB	0.02	NS	2.91	1.24–6.79	↑	Suppl.7
ERAP1 rs26618 T > C/HLA-C/KIR	TT/C1+/Bx+	0.02	NS	1.61	1.07–2.42	↑	Suppl.8
	TC//C1+/Bx+	0.02	NS	0.60	0.40–0.92	↓	Suppl.8
	TC/C2+/Bx+	0.03	NS	0.56	0.34–0.91	↓	Suppl.8
	TC/C1C2/Bx+	0.03	NS	0.53	0.30–0.91	↓	Suppl.8
ERAP2 rs2248374 A > G/HLA-C	GG/C1C2	0.03	NS	1.79	1.07–2.99	↑	Suppl.2
	GG/C2C2	0.02	NS	0.23	0.07–0.78	↓	Suppl.2
ERAP2 rs2248374 A > G/HLA-C/KIR	AG/C1C2/AA+	0.02	NS	0.34	0.13–0.86	↓	Suppl.9

↑ susceptibility, ↓ protection; The most striking associations were bold; NS not significant after Bonferroni correction.

prevalent in the miscarriage group, whilst GG/C2C2 carriers were more prevalent in the fertile group ($p = 0.02$, $p_{corr} > 0.05$, OR = 0.23, 95%CI = 0.07–0.78; Supplementary Table 2).

3.3. Combination of ERAP1/2, HLA-C1/C2 and KIR genotypes in women with miscarriages and fertile controls

The most striking association was found in the comparison of patients and controls with *ERAP1* rs30187TT and *KIR* Bx genotypes ($p = 0.006$, $p_{corr.} = 0.036$, OR = 2.40, 95%CI = 1.27–4.52). The odds ratio increased to 4.53 in centromeric AB and telomeric AA patients ($p = 0.022$, $p_{corr} > 0.05$, 95%CI = 1.20–17.11; Supplementary Table 3). This effect was even stronger in *HLA-C2* positive patients ($p = 0.0031$, $p_{corr.} = 0.019$, OR = 3.46, 95%CI = 1.48–8.11; Supplementary Table 4).

In addition, protection against disease was observed in women *ERAP1* rs26618TC and *KIR* Bx positive regardless of the *HLA-C* genotype ($p = 0.018$, $p_{corr} > 0.05$, OR = 0.62, 95%CI = 0.42–0.91; Supplementary Table 7; and $p = 0.02$, $p_{corr} > 0.05$, OR = 0.6, 95%CI = 0.40–0.92 for *HLA-C1* carriers and $p = 0.03$, $p_{corr} > 0.05$, OR = 0.56, 95%CI = 0.34–0.91 for *HLA-C2* carriers; Supplementary Table 8).

Moreover, rs2248374AG carriers of *ERAP2* positive for *HLA-C1* and *KIR* AA genotype were more prevalent in the fertile control group than in the RSA group (51.22% vs 35.48%, respectively), however significant association was detected only for *HLA-C1C2* women ($p = 0.02$, $p_{corr} > 0.05$, OR = 0.34, 95%CI = 0.13–0.86; Supplementary Table 9). On the other hand, rs2248374GG/C1C2 carriers were susceptible to miscarriage ($p = 0.03$, $p_{corr} > 0.05$, OR = 1.79, 95%CI = 1.07–2.99; Supplementary Table 2), particularly in women with *KIR* AA genotype ($p = 0.07$, OR = 2.83, 95%CI = 0.98–8.15; Supplementary Table 9).

Finally, the distribution of *ERAP* haplotypes in patients did not differ from those observed in fertile control (Supplementary Table 10).

4. Discussion

We found for the first time a significant association of *ERAP1* rs30187TT genotype with RSA, even stronger (in terms of both OR and *p* values) in patients positive for *KIR* Bx (particularly in the centromeric AB and the telomeric AA genotypes) and *HLA-C2* in spite of much lower numbers in these subgroups. According to Hiby et al. [4] pregnancy diseases were less frequent in mothers possessing the telomeric end of the *KIR* B haplotype, which contains KIR2DS1, specifically interacting with C2. Indeed, in our study Cen-AB Tel-AA patients lacked KIR2DS1 by definition. When we recalculated frequencies of *KIRs*, divided into centromeric and telomeric genotypes, we again failed to find any association with RSA (Supplementary Table 11). We observed only a trend for higher frequency of the Tel-BB genotype in fertile women in comparison to RSA women ($p = 0.09$, OR = 0.45). Perhaps there were too low numbers of tested patients and controls to observe a *KIR* genotype effect, but there were sufficient numbers of women for the detection of *ERAP* polymorphisms associations with RSA. This undoubtedly indicates that rs30187TT genotype is associated with RSA in patients in the Polish population. The rs30187 C > T (R528K) could alter the change between closed and open conformation and therefore impact on specificity and enzymatic activity [11]. The rs30187TT (528 K variant) genotype encodes enzymes with higher activity and expression [22,23]. High activity of ERAP1 rs30187TT may result in elimination by the overtrimming of these peptides presented by HLA-C which are necessary for stimulation of NK cells to release cytokines ensuring a normal pregnancy outcome.

However, maternal *ERAP* polymorphisms affect only the processing and presentation of these peptides in the context of self – HLA-C, but paternal HLA-C-peptide presentation also appears on the cell surface of the embryo and fetus as was evidenced by the expression of both maternal syngeneic and paternal allogeneic HLA-C at equivalent levels by the trophoblast cells [4]. Therefore, paternal *ERAP* polymorphism could also be important for pregnancy outcome and this study is being performed in our laboratory now.

We observed that the predisposing effect to RSA of *ERAP1* rs30187TT genotype was increased in *HLA-C2* women. The role of maternal, self–HLA-C in regulation of decidual NK cells (dNK) responsiveness was shown in the study of Sharkey et al. [24]. They identified that the expression and function of five inhibitory NK receptors in dNK is influenced by maternal HLA-C. In decidual NK cells isolated from women who carry a HLA-C2 epitope, there is a decreased expression frequency of the cognate receptor, KIR2DL1. In contrast, women bearing a HLA-C1 epitope had an increased frequency of the corresponding receptor, KIR2DL3. Maternal HLA-C had no significant effect on KIR2DL1 or KIR2DL3 in peripheral blood NK cells. This implies mechanisms that determine the repertoire of inhibitory NK receptors, and the effect of self-HLA-C on NK education may differ depending on the tissue [24]. In contrast to peripheral blood, where NK activation with self HLA-I should not occur as it would be dangerous, in the uterus NK cell activation (through KIR-HLA-C interaction) is necessary for the proper development of a pregnancy. Hence, the different roles and phenotypes of NK cells in the uterus and peripheral blood. However, ERAP1 could also regulate NK cell function by controlling the engagement of inhibitory receptors as was evidenced by Cifaldi et al. [25]. Inhibition of ERAP1 expression (by genetic or pharmacological approaches) decreased KIR binding to medulloblastoma DAOY cells and KIR aggregation at the interface between NK and target cells. This resulted in fully functional NK cell activation and killing of this cell line in both alloreactive and nonalloreactive settings, despite of KIR-KIR ligand matching. In the absence of ERAP1, precursor peptides could form unstable peptide-MHC-I complexes, which are not sufficiently conformed to assure the interaction with NK cell inhibitory receptors, or T-cell receptors [25]. In our case, inhibition of ERAP1 expression in patients rs30187TT positive would be favorable.

On the other hand, if *HLA-C2* positive mother transmits the *HLA-C2* gene to the fetus and if the fetus also inherits this gene from the father, this is an unfavorable situation for the outcome of a pregnancy. The lack of KIR2DS1, and the presence of inhibitory KIRs (KIR2DL1, KIR2DL2/3, KIR2DL5, KIR3DL1) in women with genotype Cen-AB Tel-AA indicates a greater likelihood of inhibiting NK activity by the interaction of maternal KIR2DL1 with fetal HLA-C2.

Some other weaker associations of the remaining tested *ERAP* single nucleotide polymorphisms with RSA were also identified. In our study the lack of ERAP2 protein (determined by rs2248374GG genotype) exerted a predisposing effect to RSA, while the presence of ERAP2 protein (rs2248374AG) is associated with protection against RSA in women KIR AA/HLA-C1C2. ERAP1–ERAP2 heterodimers were identified to produce epitopes more efficiently than a mix of the two enzymes which are unable to dimerize. Interaction with ERAP2 changes enzymatic parameters of ERAP1 and improves its substrate-binding affinity. Therefore, dimerization of ERAP1/2 builds up complexes with superior peptide-trimming efficacy. Such complexes are likely to enhance antigen presentation by cells with expression of the two enzymes [26]. A lack of ERAP2 could lead to improper education of NK cells in the uterine microenvironment of the KIR AA/HLA-C1C2 women.

Little is known about associations of *ERAP* polymorphisms with pregnancy disorders. A case-control study in 1103 Chilean maternal-fetal dyads and 1637 African American samples (836 maternal, 837 fetal, 68% of which were paired) was performed to test for associations between two SNPs in *ERAP2*, rs2549782 and rs17408150 with preeclampsia. They found an increased risk for preeclampsia in the African American population, but not in the Chilean population [27]. rs2549782 is in very strong linkage disequilibrium with rs2248374 in most populations, except for Chileans (www.ensembl.org), where this disequilibrium is weaker and therefore rs2549782 cannot be responsible for lack of effect on the risk of preeclampsia in that population. Because ERAP2 is expressed in placental tissue and is involved in

immune responses, inflammation, and blood pressure regulation [28–31], and its expression is altered in the first trimester placentas of women who develop preeclampsia [32], we believe that it could also contribute to development of pregnancy complications such as RSA.

The study reported by Johnson et al. [8] presented a borderline association for *ERAP1*, and a significant association for *ERAP2* with preeclampsia susceptibility in Norwegian and Australian cohorts, however at different loci. In the Australian cohort they identified an association for *ERAP1* gene (rs3734016) and for *ERAP2* gene (rs2549782). In the Norwegian cohort, evidence of a genetic association for *ERAP1* rs34750 and for *ERAP2* rs17408150 was found. However, they did not detect any associations with preeclampsia for rs27044, rs30187, rs26618 as we did for RSA.

In conclusion, rs30187 of *ERAP1* seems to play a role in susceptibility to recurrent miscarriages. This effect was intensified in women with the genotype *KIR* Bx and *HLA-C2* in spite of lower numbers in these subgroups. *ERAP* polymorphism genotyping in paternal samples will allow us to broaden knowledge on the role of ERAP, KIR and HLA in reproductive disorders in humans.

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Conflict of Interest

The authors declare that no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2019.02.010.

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