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## Polymorphism in Killer Cell Immunoglobulin-like Receptors and Human Leukocyte Antigen-C and Predisposition to Pre-eclampsia in Ethiopian Pregnant Women Population

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### Highlights

Dear Julia Szekeres-Bartho

Editor-in-Chief

Journal of Reproductive Immunology

We thank you for the positive feedbacks that enriched the document. We have tried to address the comments. We feel privileged for submitting our work in your prestigious journal.

### ABSTRACT

**Introduction:** Pre-eclampsia (PE) is a human specific pregnancy-related syndrome of unknown etiology that affects 2-8% of pregnancies. Polymorphism in maternal Killer Cell Immunoglobulin-like Receptors (KIRs) and the ligand fetal Human Leukocyte Antigen-C (HLA-C) may predispose pregnant mothers for PE due to defective trophoblast invasion into the maternal decidua. Our study aimed to investigate the association between maternal KIR and fetal HLA-C polymorphism and PE in Ethiopian pregnant women. **Methods:** We included a total of 288 (157 controls and 131 PE cases) in a case-controls study at Adama Regional Referral Hospital, Ethiopia. The KIR and HLA-C genotyping was done using traditional polymerase chain reaction on genomic DNA extracted from maternal venous and cord blood followed by 2% agarose gel electrophoresis. **Results:** The statistical associations between variables were evaluated using Pearson's Chi-square test.  $P < 0.05$ , with 95% confidence interval was considered statistically significant. A significant association was observed between the KIR2DS1 and PE, with a higher frequency (60.5%) of the gene in the control group. Similarly, a significant association

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was observed between KIR AA genotype and PE, with a higher frequency (38.2%) of this genotype in the PE group. Ethiopians share the same risk genotype for PE as seen in previous African and European studies, namely homozygosity of a maternal KIR AA genotype. However, Ethiopians differ from other East African populations by sharing the same protective *KIR2DS1* gene as Europeans. Moreover, the frequency of non-self HLA-C2 of paternal origin was associated with PE in Ethiopian women

*Keywords:*

Natural killer cell Trophoblast cell KIR HLA Pre-eclampsia

## 1. Introduction

Pre-eclampsia (PE) only occurs in human pregnancy and is characterized by gestational hypertension accompanied by proteinuria or maternal organ or uteroplacental dysfunctions at or after 20 weeks of gestation (Brown et al., 2018). Globally, the disorder affects 2-8% of pregnant women (Duley, 2009), with high maternal and neonatal mortalities particularly in sub-Saharan African (SSA) countries (Hogan et al., 2010). In Ethiopia, PE affects 5.47% of pregnancies (Berhe et al., 2018). The etiology of PE is not fully understood, but endothelial dysfunction is playing a central role in the pathogenesis of the end stage of the disorder (Roberts and Hubel, 2009). Pre-eclampsia develops in two stages, where reduced placental perfusion caused by defective trophoblast arterial transformation in the first trimester leads to a stressed placenta, triggering the maternal systemic syndrome (Redman and Sargent, 2005, Redman and Staff, 2015).

During early pregnancy, maternal uterine natural killer (uNK) cells, that account for ~70% of the decidual leukocytes (Loke et al., 1995) in the decidua basalis, interact with invading fetal extravillous trophoblast (EVT) cells. Normal pregnancy results from a proper remodeling of the spiral arteries in response to the invading EVTs, a process that is impaired in stage 1 in pregnancies later complicated by PE (Kam et al., 1999). The trophoblast cells home around uterine spiral arteries, followed by the destruction of the smooth muscle media, transforming the arteries into low-pressure vessels that perfuse the fetoplacental unit (Moffett et al., 2015). The uNK cells secrete chemokines that may direct trophoblast invasion into the uterine decidua (Hanna et al., 2006), and they express killer immunoglobulin-like receptors (KIRs) that interact with human leukocyte antigen-C (HLA-C) ligands expressed by EVT. The trophoblast cells express HLA-C products from both maternal and paternal genes at the site of implantation (Hiby et al., 2010). The KIR and HLA-C genes are highly polymorphic; each pregnancy is therefore likely to result in a unique combination of maternal KIR and fetal HLA-C (Chazara et al., 2011). Certain KIR-HLA-C combinations activate cytokine secretion that may help trophoblast invasion, contributing to a normal pregnancy, while other combinations appear to inhibit this process resulting in defective placentation and PE (Moffett et al., 2015). The combination that gives rise to the highest risk is a maternal KIR AA genotype with a paternally inherited fetal HLA-C2 genotype (Hiby et al., 2004).

The KIR gene family consists of 12 genes and two pseudogenes, all arranged in a linear array on chromosome 19. They each encode different receptors with extracellular, transmembrane and intracellular domains. The designation of KIRs, 2D or 3D, and L or S, depends on the number of extracellular Ig-like domains and the length of the cytoplasmic tail, respectively (Campbell and Purdy, 2011). The KIR genes are grouped into A and B haplotypes. The KIR A haplotype consists of inhibitory genes while the KIR B haplotype is more variable in gene content and contains both inhibitory and activating genes. Accordingly, the maternal KIR genotype can be designated as AA, AB or BB (Hiby et al., 2010). The AA increases the risk of PE while BX (AB and BB) has a protective effect (Nakimuli et al., 2015).

HLA-C molecules are major KIR ligands for the HLA-C allotypes that can be further grouped into C1 and C2, based on two distinct KIR epitopes. The specificity of the interaction of HLA-C with cognate KIR receptors is determined by a dimorphism at position 80 of the  $\alpha 1$ -domain of HLA-C. The HLA-C1 has an asparagine at this position while HLA-C2 has a lysine residue (Parham, 2005). The HLA-C1 molecules interact with *KIR2DL2* and *KIR2DL3*, with a neutral effect on outcome of pregnancy. The HLA-C2 molecules strongly interact with inhibitory *KIR2DL1* and activating *KIR2DS1*, resulting in inhibition or activation of uNK cells, respectively. The *KIR2DL1*/HLA-C2 interaction conveys an inhibitory signal, likely contributing to PE while a protective effect from activating *KIR2DS1* (Moffett et al., 2015).

Evidence shows that there is a genetic contribution to PE (Salonen Ros et al., 2000) and that this might explain the variation in prevalence among different populations. Women of African ancestry are at increased risk of developing PE (Nilsson et al., 2004, Skjaerven et al., 2005, Nakimuli et al., 2014, Nakimuli et al., 2015). An Ugandan study showed a diversity of KIR genes and a high frequency of HLA-C2 among African populations (Nakimuli et al., 2013). A South African KIR genotype study showed a lower frequency of *KIR2DS1* and a higher frequency of HLA-C2 than in Europeans (Gentle et al., 2017). In contrast, there are comparable frequencies of most KIR in North African populations similar to what have been reported in Europeans (Meriem et al., 2015).

The growing body of evidences indicate that modern-day Ethiopia is the place where humankind originated (White et al., 2009, Pagani et al., 2012) and then populated the rest of the world by migration via the Eastern route (Lovell et al., 2005, Quintana-Murci et al., 1999). It is argued that events of genetic admixtures with sub-Saharan and Eurasian populations played a role in shaping the gene pool of Ethiopians (Lovell et al., 2005). The genetic heterogeneity and variable spectrum of admixture was shown among different linguistic groups of Ethiopians (Pagani et al., 2015). To our knowledge, genetic studies on KIR and HLA-C genotypes have never been done in Ethiopia. We set out to investigate the polymorphism in maternal KIR and fetal HLA-C in a case-control study of PE in Ethiopian women.

## 2. Materials and Methods

### 2.1. Study Design and Study Participants

We employed a case-control study design. Our study participants were pregnant women delivering at Adama Regional Referral Hospital, situated 99 km South East of Addis Ababa, Ethiopia. The hospital provides 4000-5000 delivery services annually. The hospital is a center for medical residency training in different disciplines including Gynecology and Obstetrics. The midwives and a laboratory technician were involved in the data collection. The data collectors were trained twice on different occasions, one in pre-data collection and the other one was after they started collecting the data to increase the quality of the collected samples and data. We prepared standard operating procedures that the data collectors followed during data and sample collections. The case-control identification was done by the medical residents or specialists in Obstetrics and Gynecology working at the hospital, according to the national protocol (Management Protocol on Selected Obstetrics Topics, Federal Democratic Republic of Ethiopia, Ministry of Health, 2010). The data collection period was from December 2016-August 2017. We included a total of 288 pregnant women, 131 were diagnosed with PE and 157 were normal controls. The participant selection was done in the order of their appearance to the delivery ward. We collected demographic, clinical and delivery data using a designed questionnaire and the COLLECT database (<https://pregnancycolab.tghn.org/collect/>).

### 2.2. Ethical Considerations

The study was ethically approved at different levels: Department of Biochemistry, School of Medicine, College of Health Sciences, Addis Ababa University, Ethiopia (Protocol number 013/16/Boichem Form AAUMF 03-008 May 20, 2016 and May 08, 2018), AHRI-ALERT Ethical Review Committee (Project Reg. No. P015/16; August 02, 2016-August 01, 2017); NRERC, the Federal Democratic Republic of Ethiopia Ministry of Science and Technology (Date: 13/2017 Ref. No. 3.10/186/2018; 3/0/86/2018, Date: 14/05/2018). A signed informed written consent was obtained from all the study participants.

### 2.3. Inclusion criteria

In the PE case group, we included pregnant women with gestational hypertension (two blood pressure (BP) readings of diastolic BP (dBP)  $\geq 90$  mmHg and/or systolic BP (sBP)  $\geq 140$  mmHg four hours apart) with proteinuria (+1 or above on dipstick) at or after 20 weeks of gestation in a previously normotensive woman, or worsening hypertension without proteinuria and signs or laboratory findings of multiple organ involvement or utero-placental dysfunctions such as fetal growth restriction. The participants were 18 years of age or above. In the control group, we included normotensive healthy singleton pregnancies, 18 years and above, with no clinical signs or laboratory findings of organ dysfunction or utero-placental dysfunction.

### 2.4. DNA Extraction and Genotyping

Maternal venous and fetal cord blood samples were collected in EDTA tubes at Adama Hospital delivery ward from PE cases and controls. The blood samples were temporarily stored at  $-20^{\circ}\text{C}$  at Adama Regional Laboratory and then transported to Armauer Hanson Research Institute (AHRI) laboratory, Addis Ababa, Ethiopia on dry ice for storage at  $-20^{\circ}\text{C}$ . Genomic DNA was extracted from 200  $\mu\text{L}$  of maternal and 200  $\mu\text{L}$  of cord blood using QIAGEN DNA Mini Blood Kit (QIAGEN, Germany) at the AHRI laboratory. We genotyped five maternal KIR genes (*KIR2DL1*, *KIR2DL2*, *KIR2DL3*, *KIR2DS1* and *KIR2DS5*), and two HLA-C groups, C1 and C2. The KIR and HLA-C genotyping was done at Department of Obstetrics and Gynecology, Institute of Clinical Sciences Lund, Lund University, Sweden. The KIR genotyping was based on the primers and methods previously described (Martin et al., 2002), with some additional changes. Fifty nanograms (ng) of genomic DNA was amplified in a 20  $\mu\text{L}$  PCR-reaction containing 200  $\mu\text{M}$  dNTP, 200 nM to 1.5 mM of specific primers, 2 mM  $\text{MgCl}_2$ , 2  $\mu\text{L}$  PCR buffer, 4  $\mu\text{L}$  Q solution and 0.5 U *Taq* polymerase (QIAGEN, Germany). Cycling was carried out in a BIO-RAD T100<sup>TM</sup> Thermal Cycler according to a previously described protocol (Hiby et al., 2004). Positive and negative control samples were run together with test samples. PCR products were analysed using 2% agarose gels containing Gel Red Nucleic Acid Stain (BIOTIUM) and visualised under ultraviolet light. A DNA ladder (GeneRuler 100bp Plus DNA Ladder, Thermo Scientific) was used to determine the size of specific bands.

Genotyping of HLA-C1 and HLA-C2 was done by PCR, using primers and methods as described previously (Hiby et al., 2008). Briefly, the HLA-C PCR was performed using 50 ng of genomic DNA per 20  $\mu\text{L}$  PCR-reaction, containing 200 mM dNTPs, 1 mM HLA-C primers, 350 nM DRB internal control primers, 2 mM  $\text{MgCl}_2$ , 2  $\mu\text{L}$  PCR buffer, 4  $\mu\text{L}$  Q solution and 0.5 U Qiagen *Taq* polymerase (QIAGEN, Germany). Cycling was carried out in a BIO-RAD T100<sup>TM</sup> Thermal Cycler according to a previously described protocol (Hiby et al., 2008). The PCR products were analyzed using 2% agarose gel containing GelRed Nucleic Acid Stain (BIOTIUM). The bands were visualized under ultraviolet light. A DNA ladder (GeneRuler 100bp Plus DNA Ladder, Thermo Scientific) was used to determine the size of specific bands.

After completing the genotyping, we designated maternal genotypes KIR AA, KIR AB or KIR BB to each of the study participants. The KIR AA genotype was designated when *KIR2DL3* gene was present and *KIR2DL2*, *KIR2DS1* and *KIR2DS5* genes were absent (Moffett et al., 2015). The remaining KIR gene combinations were designated as BX (AB and BB) genotype. Similarly, the HLA-C genotypes were determined as HLA-C1C1, HLA-C1C2 or HLA-C2C2 based on the presence of specific bands for HLA-C1 and HLA-C2 groups.

### 2.5. Data collection and Analysis

Clinical data was collected using COLLECT, a database developed by CoLaboratory (<https://pregnancycolab.tghn.org/collect/>) with the recommendations for the standardization of minimum clinical data set in low and middle-income countries (Myatt et al., 2017, Myatt et al., 2014). For the clinical data see table 1.

The data were analyzed using IBM SPSS version 25.0 software. The KIR and HLA-C frequency data were compared between the PE cases and the controls using charts and descriptive statistics. We

calculated the gene frequencies for maternal KIR, maternal and fetal HLA-C in both the PE and control groups. We also calculated the KIR haplotype and HLA-C group frequencies in both the PE and control groups. The associations of PE with KIR gene and KIR haplotypes frequencies were tested using Pearson's Chi-square test. P-values at  $< 0.05$ , with a 95% confidence interval, was considered statistically significant. In addition, we determined the P-value using Chi-square table. Since replication of previous KIR gene correlations with PE were being tested, correction for multiple testing was not applied.

### 3. Results

From a total of 345 study participants that were initially recruited in the study, 32 participants were excluded due to lack of blood samples (broken test tubes or samples not collected), lack of matching venous and cord blood samples or lack of supportive clinical data. In total, 313 DNA samples were genotyped. From these, seven samples were excluded from the statistical analysis due to lack of data that supported the diagnosis or matching samples. Genotyping results were excluded due to multiple pregnancy for 9 PE cases and 9 controls. In the end, a total of 288 participants were included (PE cases:  $n = 131$ , controls:  $n = 157$ ). There were missing values for the demographic and clinical characteristics for some participants due to lack of supportive data. The sBP and dBP were missing for 14 PE cases and 21 controls, while GA was missing for 14 PE cases and 14 controls. The maternal age was missing for 9 PE cases and 14 controls.

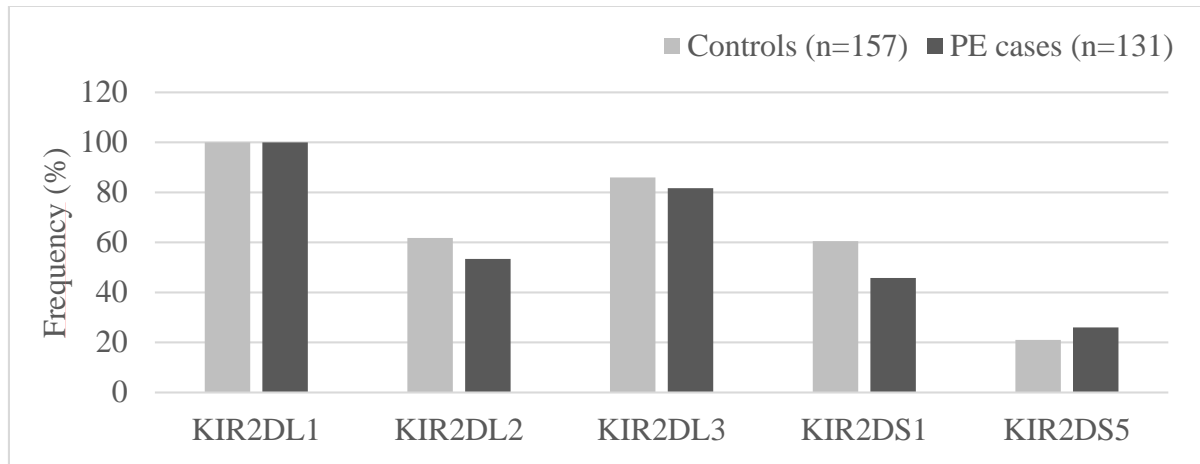
We calculated mean values for continuous variables, including age, sBP, dBP, and gestational age (GA) (Table 1). Among the participants, those mothers in the age range of 20-25 years of age were relatively predominant in both the PE (37.7%) and control (50.3%) groups, while those above 35 years of age accounted for ~4-5% of the total. The mean maternal age of PE ( $26.8 \pm 5.1$ ) cases tended to be higher in comparison with controls ( $25.3 \pm 5.0$ ). The mean sBP and dBP were by definition higher in the PE cases compared to controls. The mean GA at delivery tended to be lower in PE cases (253 days) compared to controls (271 days). Early onset PE was considered as  $GA \leq 238$  days while late onset PE above 238 days. Early onset PE was observed in 26.5% of the PE cases.

The frequency distribution of maternal KIR genotypes was compared between PE cases and controls (Figure 1).



There was a statistically significant difference in KIR genotype frequencies between the PE cases and the controls. A significant association was observed between KIR AA genotype and PE, with a higher frequency (38.2%) of this genotype in the PE group,  $p=0.007^{**}$ ; odds ratio at 95% confidence interval, 2.00 (1.20-3.33).

Next, the frequencies of the five KIR genes were compared among the PE cases and the controls of pregnant Ethiopian women (Figure 2)



Among the five KIR genes analysed, there was statistically a significant difference in activating *KIR2DS1* gene frequency between PE cases and controls,  $p=0.013^*$ ; odds ratio at 95% confidence interval, 0.55 (0.35-0.88). There was no statistically significant difference in frequencies of the inhibitory genes *KIR2DL2*, *KIR2DL3* and the activating gene *KIR2DS5* between PE case and control groups. The inhibitory gene *KIR2DL1* was observed in all individuals.

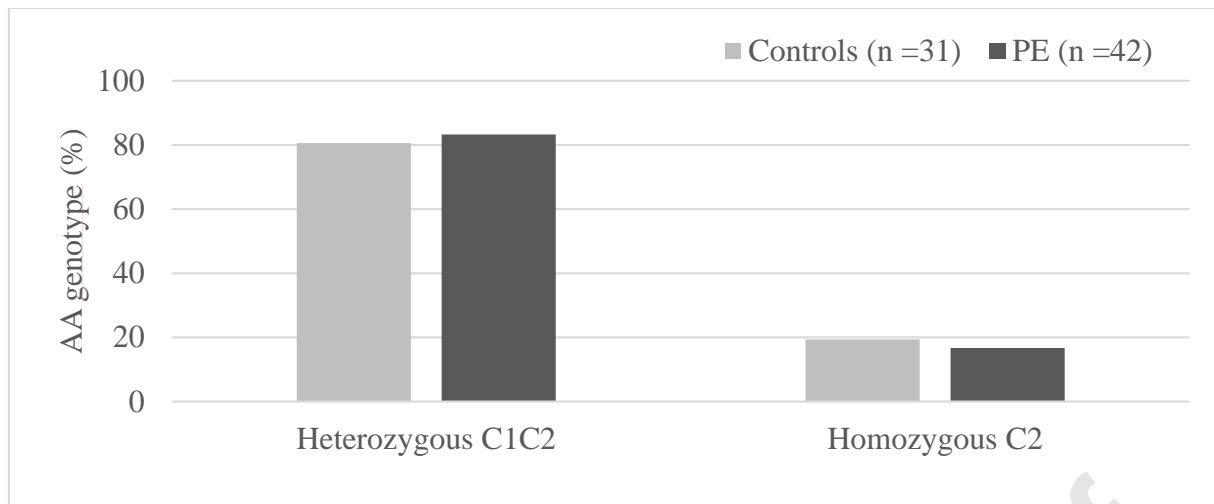
We compared the frequency distribution of the five KIR genes in our study with frequencies found in 10 other populations from Africa, the Middle East, Asia and Europe, extracted from an online database (<http://www.allelefreqencies.net>) (Table 2). The total frequency in our cohort of the activating gene *KIR2DS1* (53.8%), was higher compared to the majority of the other populations but comparable with Western India.

**HLA-C grouping:** we designated HLA-C groups as: HLA-C1 =1, HLA-C2 =2; homozygous C1 (1+1), heterozygous C2 (1+2), homozygous C2 (2+2). The HLA-C groups were subdivided into three subsets depending on the maternal and fetal pair HLA-C groups: non-self= mothers with either homozygous C1 and C2 carrying heterozygous C2 fetuses, missing-self = heterozygous C2 mothers carrying either homozygous C1 or C2 fetuses, and self = homozygous or heterozygous mothers carrying fetuses with same subset of HLA-C.

Then evaluated whether there was a statistical association in frequencies between maternal and fetal subsets in control and PE pregnancies (Table 3). There was a statistically significant association in HLA-C non-self subset frequencies between the mother and fetus in control and PE cases,  $p<0.05$  ( $X^2=4.8$ ) at 3.84 percentage point and 1 degree of freedom, odds ratio at 95% confidence interval, 2.8875 (0.8857-9.4135). There was no statistically significant association in HLA-C missing self-subset frequencies between the mother and fetus in control and PE groups,  $p>0.5$  ( $X^2=0.7$ ) at 1.32 percentage point and 1 degree of freedom, odds ratio at 95% confidence interval, 0.6000 (0.1799-2.0014). There was no statistically significant association in HLA-C self-subset frequencies between the mother and fetus in control and PE groups,  $p>0.5$  ( $X^2=0.2$ ) at 1.32 percentage point and 1 degree of freedom.

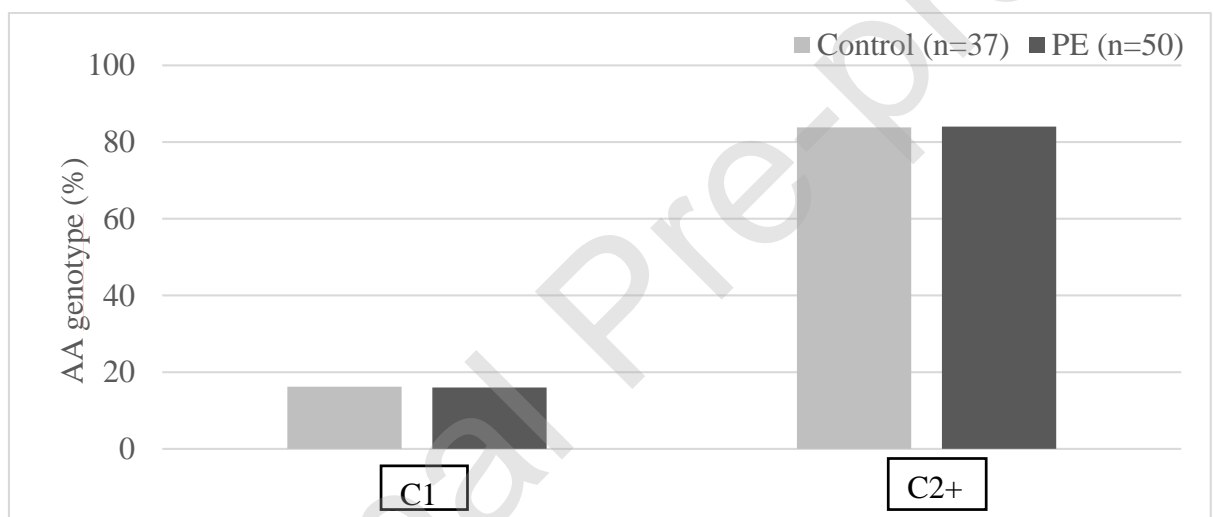
We evaluated whether there was association in maternal KIR AA exposure to fetal heterozygous C1C2 and homozygous C2 HLA-C groups between control and PE cases (Figure 3).





There was no statistically significant association in maternal KIR AA exposure to fetal HLA-C groups between controls and PE cases,  $p > 0.5$  ( $X^2 = 0.1$ ) at 0.45 percentage point and 1 degree of freedom, odds ratio at 95% confidence interval, 1.1657 (0.3488-3.8957).

Finally, we evaluated whether there was association in maternal KIR AA exposure to fetal C1(C1C1) and C2+ (C1C2 and C2C2) HLA-C groups between controls and PE cases (Figure 4).



There was no statistically significant association in maternal KIR AA exposure to fetal HLA-C groups between controls and PE,  $p > 0.5$  ( $X^2 = 0.07$ ) at 0.45 percentage point and 1 degree of freedom, odds ratio at 95% confidence interval, 0.9841 (0.3098-3.1261).

#### 4. Discussion

Our study showed a statistically significant association between *KIR2DS1* gene frequency and protection from PE. The women in the control group showed a significantly higher frequency of *KIR2DS1* compared to women in the PE group. This result was consistent with a previous study in Europeans that showed pregnant mothers possessing *KIR2DS1* to be less predisposed to pregnancy disorders such as PE (Hiby et al., 2010). Our study result was consistent with a SSA PE risk prediction study, that suggested *KIR2DS1* to be protective from PE for South East Africans (Nemat-Gorgani et al., 2019), but in contrast to results from an East African study that showed a lower frequency of *KIR2DS1* in both controls and PE cases (Nakimuli et al., 2015).

Genetic evidence indicates that Ethiopia is the origin of early human migration out of Africa. The early dispersal events of modern humans out of Africa took place via the eastern route as traced by mitochondrial DNA haplotype similarity between Ethiopians and Indians (Quintana-Murci et al., 1999).

The frequency of *KIR2DS1* in Western Indians is 52.2%. Genetic introgression due to back migration from Eurasia to Africa (Pagani et al., 2015) may have contributed to the higher frequency of *KIR2DS1* in Ethiopians compared to some other African populations.

Our study showed a statistically significant association between the KIR AA genotype frequency and PE. The occurrence of PE was associated with a higher frequency of the homozygous KIR AA genotype. Our data was consistent with previous studies that showed an association of PE with high KIR AA genotype frequency (Nakimuli et al., 2015, Hiby et al., 2004).

A previous study showed a significantly increased frequency of maternal KIR AA in the PE cases exposed to fetal HLA-C2 compared to controls (Hiby et al., 2004). Our study showed inconsistent result since there was no difference in the KIR AA frequencies between PE cases and controls exposed to fetal HLA-C2 groups.

Our study also showed statistically significant difference in non-self HLA-C frequencies between control and PE case maternal and fetal pairs. Unlike European study, the frequency of homozygous HLA-C1 mothers exposed to fetal heterozygous HLA-C2 was higher in PE cases compared to control group while frequency of homozygous HLA-C2 mothers exposed to fetal heterozygous HLA-C2 was lower in PE cases compared to the control group. This implies the likely role of paternal HLA-C2 in PE. This finding was inconsistent with an European study (Hiby et al., 2004).

In conclusion, Ethiopians share the same risk genotype for PE as seen in previous African and European studies, namely maternal KIR AA genotype homozygosity. However, Ethiopians differ from other East African populations by sharing the same protective *KIR2DS1* gene as Europeans. Unlike Europeans, the frequency of non-self HLA-C2 frequency was associated with PE in Ethiopian women. Hence, interaction of maternal activating KIR2DS1 on KIRAB/BB genotypes with fetal HLA-C2 facilitates the implantation process and trophoblast invasion into the maternal decidua resulting in proper perfusion of the utero-placental unit with successful pregnancy outcome. However, interaction of fetal HLA-C2 with maternal inhibitory KIR2DL1 on KIRAA genotype will have opposite effect resulting in a pregnancy complicated by PE.

### **Conflicting interests**

The authors declare no conflict of interest.

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#### **Web References and protocol**

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**Figure legends**

Figure 1. Frequency distribution of different KIR haplotypes among pregnant Ethiopian women, of normotensive control and PE pregnancies. There was a statistically significant association of KIR haplotype frequencies between normotensive controls and PE pregnancies,  $p=0.007^{**}$ .

Figure 2. Frequency distribution of five KIR genes among pregnant Ethiopian women, of normotensive control and PE pregnancies. The frequency of KIR2DS1 was significantly lower ( $p=0.013^*$ ) among the PE cases compared to controls, while the other 4 were present at similar frequencies between the groups.

Figure 3. Frequency distribution of maternal KIR AA exposed to fetal heterozygous and homozygous HLA-C subgroups among pregnant Ethiopian women, of normotensive control and PE pregnancies. There was no difference in frequencies between controls and PE cases,  $p>0.5$ .

Figure 4. Frequency distribution of maternal KIR AA exposed to fetal C1 and C2 groups among pregnant Ethiopian women, of normotensive control and PE pregnancies. There was no difference in frequencies between controls and PE cases,  $p>0.5$ .

**Table 1. Demographic and clinical characteristics of participants.**

Characteristics	Controls (n=157)	PE cases (n=131)
Age, mean	25.3 ± 5.0 SD	26.8 ± 5.1 SD
18-19	10.5% (15)	4.9% (6)
20-25	50.3% (72)	37.7% (46)
26-30	26.6% (38)	36.9% (45)
31-35	7.0% (10)	16.4% (20)
>35	5.6% (8)	4.1% (5)
sBP, mean	113.8 ± 8.7 SD	151.1 ± 16.6 SD
dBP, mean	72.4 ± 8.0 SD	98.6 ± 9.8 SD
GA, mean	271 days	253 days
Early onset PE	n.a	26.5% (31)
Late onset PE	n.a	73.5% (86)

GA-gestation age; PE-preeclampsia; sBP-systolic blood pressure; dBP-diastolic blood pressure; n.a-not applicable

**Table 2. Frequency of KIR genes in different populations.**

Population (n)	KIR Gene frequency (%)				
	<i>KIR2DL1</i>	<i>KIR2DL2</i>	<i>KIR2DL3</i>	<i>KIR2DS1</i>	<i>KIR2DS5</i>
Ethiopia (n=288), this study	100	58.0	84.0	53.8	23.3
Israel Ethiopian Jews (n=31)	90.3	80.6	77.4	25.8	n.d
South Africa Black (n=167)	99.4	68.3	82.0	12.6	43.1
South Africa Caucasian (n=97)	99.0	57.7	88.7	39.2	39.2
Tunisia (n=114)	99.1	59.6	91.2	22.8	23.7
Nigeria Benin Yoruba (n=75)	100	38.4	92.0	14.7	n.d
Iran Fars Persian (n=248)	98.0	56.8	91.0	35.0	25.4
Yemen Jews (n=43)	97.7	46.5	95.2	31.0	n.d
India Western (n=161)	94.4	60.2	79.5	52.2	47.5
India Northeast Adivasi (n=101)	95.0	72.3	61.4	24.7	63.4
Sweden (n=102)	98.0	42.2	96.1	43.1	31.4

n.d-no data, Source for data of populations other than our study ([www.allelefreqencies.net](http://www.allelefreqencies.net)).

**Table 3. Frequency distribution of HLA-C subsets between mother and fetus pairs in control and PE cases.**

HLA-C Subgroup		Controls (n=157)	PE cases (n=131)
Mother	Fetus	% (n)	% (n)
Non-self			
1+1	1+2	6.4 (10)	8.4 (11)
2+2	1+2	13.4 (21)	6.1 (8)
Missing self			
1+2	1+1	9.5 (15)	6.9 (9)
1+2	2+2	6.4 (10)	7.6 (10)
Self			
1+1	1+1	9.5 (15)	9.2 (12)
1+2	1+2	51.0 (80)	57.2 (75)
2+2	2+2	3.8 (6)	4.6 (6)

PE-pre-eclampsia