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To cite this article: Immaculate Mbongo Langmia, Yamunah Devi Apalasamy, Siti Zawiah Omar & Zahurin Mohamed (2015): Interleukin 1 receptor type 2 gene polymorphism is associated with reduced risk of preterm birth, The Journal of Maternal-Fetal & Neonatal Medicine, DOI: 10.3109/14767058.2015.1125466

To link to this article: http://dx.doi.org/10.3109/14767058.2015.1125466
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Abstract

Objective: Interleukin 1 receptor type 2 (IL1R2) regulates the inflammatory pathway that results in preterm delivery. We aim to investigate the impact of interleukin 1 receptor type 2 (IL1R2) gene polymorphisms on the risk of preterm delivery.

Method: A total of 664 women with spontaneous preterm and term deliveries were genotyped for IL1R2 gene polymorphisms (rs2072476A/G, rs2071008G/T, rs2072474C/T) using Sequenom MassARRAY platform.

Result: Ethnic-specific analysis revealed a significant association between the G allele of IL1R2 rs2072476 polymorphism and reduced risk of PTB in the Indian ethnic subgroup (OR: 3.7, 95% CI: 1.3 – 11.3, P = 0.017). The odds of G allele occurring among Indian women with term delivery (>37 weeks) was three times higher than those with preterm delivery (<37 weeks). Genotype analysis showed a significant association between the GG genotype of IL1R2 rs2072476 polymorphism and term delivery in the Indian women.

Conclusion: This study shows disparity in the occurrence of preterm birth due to the differences in the genotype of the women. Particularly, Indian women with the minor allele of IL1R2 rs2072476 polymorphisms were more likely to deliver at term (>37 weeks). These findings suggest the possible influence of maternal IL1R2 gene polymorphism on the risk of preterm delivery.
Interleukin 1 receptor type 2 gene polymorphism is associated with reduced risk of preterm birth

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Key words: Genetic polymorphisms, preterm birth, Interleukin 1 receptor type 2, ethnic disparity, reduced risk.
Introduction

Preterm birth (PTB) which is defined as parturition before 37 weeks of gestation is a global health crisis affecting a total of 1.1 million preterm born babies every year [1,2]. The aetiology of PTB is multifactorial and still remains largely unknown. It has been considered as the highest cause of infant mortality especially in the first few months of life and in children less than 5-years of age [2]. Children born preterm are vulnerable to short and long term complications such as sleep apnea, bronchopulmonary dysplasia, hypotension, intracerebral haemorrhage, necrotizing enterocolitis, anemia, jaundice and retinopathy of prematurity [3-5]. Although environmental and lifestyle factors are known to influence the susceptibility to preterm birth, maternal genetic factors contribute up to 20% risk for developing PTB [1]. Many studies have explored the relationship between preterm delivery and genetic factors related to intrauterine infection, inflammatory cytokines and uterine contraction [6-9]. Interleukin 1 is an important pro-inflammatory cytokine which plays a vital role in the inflammatory pathway of preterm birth [10-13]. Interleukin-1 binds to type 1 receptor (ILR1) resulting in signaling and activation of inflammatory cascade to fight against infection and inflammation. This process has been shown to result in preterm birth in some women (10). However, the role of interleukin-1 in initiating inflammation is controlled by interleukin 1 type 2 receptor (IL1R2) which acts as a decoy in inhibiting the binding of interleukin-1 to interleukin-1 type 1 receptor thereby preventing signaling and inflammation [14-18]. One study carried out in an American population has revealed that genetic disparity between Caucasians and African Americans could explain the differences in the incidence of preterm birth between the two ethnic groups (8). Malaysia is a multi-ethnic country consisting of three major ethnic groups, namely the Malays, Chinese and Indians, each of which are of different genetic background, thus presenting a good opportunity to study the genetic disparity of preterm birth with regards to interleukin 1 receptor type 2 gene.
polymorphisms. In this study we aim to explore the genetic influence of interleukin 1 receptor type 2 gene polymorphisms on the risk of PTB. This may lead to a better understanding of the mechanisms involved in the pathology of PTB and may aid in development of novel management strategies to prevent preterm delivery.
Materials and method

Study Subjects

A total of 664 women with spontaneous preterm and term deliveries (132 and 532 respectively) were prospectively enrolled in this study from 2011 to 2013. The prospective case-control study was approved by the University of Malaya Medical Centre (UMMC) Ethics Committee and written informed consent was obtained from all participants. The participants were classified into two groups; the preterm group consisted of mothers who delivered their baby between 24 to 36 weeks while the control group consisted of mothers with uncomplicated pregnancy who delivered their baby between 38 to 41 weeks. During sample collection, each participant was given a written consent form, patient information sheet and a data collection sheet. Participants’ information such as demographic details, obstetric history, family history and lifestyle were recorded in the data collection sheet. Ethnicity of the participants was obtained from the data collection sheet in which participants self reported their ethnicities as Malays, Chinese or Indians and declared that there has been no mixed marriage for at least three generations. Medical records of each participant were also review for more confirmation of the information provided by the participants. All forms and documentation used in this investigation were approved by the UMMC ethics committee. The physical well-being of the participants was assessed by trained gynecologists. The pregnancy age was calculated from the first day of the last menstrual cycle or obtained from ultrasound results of the patients. Eligibility for participation was based on the following inclusion criteria; healthy mothers between the ages of 18-35 years with normal competent cervix and uterus, without metabolic or autoimmune disease, delivered preterm but with normal and healthy fetus. Exclusion criteria included maternal age of less than 18 years or more than 35 years, abnormal fetus and still birth, mothers with history of drug abuse, mothers with known medical problem, multiple gestation, birth delivery by induction due to
fetal distress or placenta abruption or preeclampsia or hypertension and mothers who were not able to sign informed consent form. At the time of sample collection, each participant was given a written consent form, patient information sheet and a data collection sheet. The consent form was to be signed by the participants to approve their participation. The patient information sheet in very simple terms explained the project and its relevance and finally the data collection sheet consisted of all information about patient’s demographic, obstetric history, lifestyle, and any known medical issues. A one on one interview was made and medical records for each participant were also reviewed for confirmation.

**SNP genotyping**

The genomic DNA was extracted from whole blood using the GeneAll® Exgene™ DNA purification kit. Three SNPs in the *IL1R2* gene (rs2072476 A/G, rs2071008G/T, rs2072474C/T) were genotype using Sequenom MassARRAY platform. SNPs with a successful call rate were considered for statistical analysis. For the three *IL1R2* SNPs genotyped, two (*IL1R2* rs2072476 and rs2071008) with call rates of 99% were considered for statistical analysis while the *IL1R2* rs719248 SNP with a call rate of 79% was excluded from statistical analysis.

**Statistical analysis**

Statistical analysis of subjects was performed using SPSS version 18.0 (IBM Corp., Chicago, IL, USA). Hardy-Weinberg equilibrium (HWE) was checked for the SNPs prior to genetic analysis using a goodness of fit $\chi^2$ test. The test for associations of the *IL1R2* rs2072476 and rs2071008 with PTB was performed using Fisher’s exact test with 2 degrees of freedom, while allele-based test was carried out by using Fisher’s exact test with one degree of
freedom. Categorical and continuous variables were compared between groups using Pearson’s $\chi^2$ test, or independent t-test and were presented as percentage or mean ± standard deviation. Logistic regression models were adjusted for a past history of PTB, miscarriage, bacterial infection and gestational diabetes. According to the demographic information, participants in the case and control groups were not smokers or substance abuse, their mothers did not deliver them before completed 37 weeks (preterm), none of them were alcohol consumers. Therefore confounding factors such as smoking, substance abuse, mothers who were themselves born preterm and alcohol consumption were not included for statistical analysis in this paper.
**Results**

The demographic characteristics of participants are shown in Table 1. Past history of preterm birth, miscarriage, presence of bacterial infection and gestational diabetes were significantly different between the preterm and term groups (Table 1). Based on the information provided by the participants, none of them were drug users, alcohol consumers, hypertensive or preeclampsia patients; thus, these factors were not included in the statistical analysis.

The allelic distributions of *IL1R2* SNPs are shown in Table 2. There was no deviation from Hardy Weinberg equilibrium in preterm and term groups both in overall subjects and after stratification by ethnicity. Ethnicity-specific stratification revealed a significant association between *IL1R2* rs2072476 variants and term birth among Indian ethnic subgroup. The *ILR2* rs2072476 G variant appears to be protective against preterm birth in the Indian women. The odds of the G allele occurring among the term group was thrice that of the preterm group (Odds Ratio 3.7, 95% confidence interval 1.3 – 11.3, P = 0.017) (Table 3). The GG genotype of *IL1R2* rs2072476 was also significantly associated with term birth in the Indian ethnic group (Table 3). No significant association was observed between *IL1R2* rs2072476 and rs2071008 polymorphisms with reduced risk of preterm birth in the Malays and Chinese ethnic groups both at allelic and genotypic level (Table 2, Table 3).
Discussion

In this study we showed a significant association between \textit{IL1R2} rs2072476 G variant and reduced risk of preterm birth in the Indian ethnic group. Interleukin 1 receptor type 2 binds to interleukin 1 preventing its inflammatory actions, which then prevents signaling and influx of the inflammatory cascade that can lead to preterm birth (15). Any genetic variation that will result in increased binding of interleukin 1 receptor type 2 (\textit{IL1R2}) to interleukin 1(\textit{IL1}) will prevent IL1 inflammatory function thus allowing the pregnancy to fully mature for term delivery. Muller et al. 2002 reported \textit{IL1R2} as a potential biomarker for anti-inflammatory pathway [15]. After administration of glucocorticoid, an anti-inflammatory hormone in patients with sepsis (sepsis is a condition that involves systemic inflammation), high levels of \textit{IL1R2} were found in plasma of these patients, indicating high expression of \textit{IL1R2} caused by the anti-inflammatory agent [15]. Many anti-inflammatory agents including; glucocorticoid hormone, interleukin 4 (\textit{IL4}), interleukin 13 (\textit{IL13}) and interleukin10 (\textit{IL10}) act by up-regulating the expression of anti-inflammatory proteins which binds to the inflammatory proteins preventing their function [15]. High levels of \textit{IL1R2} have also been detected in uterine and placenta tissues [17]. At both the local and systemic level, \textit{IL1R2} binds to interleukin 1\beta inhibiting its action [17].

Increased pro-inflammatory action due to imbalance between interleukin 1 and interleukin 1 receptor activity is linked to adverse pregnancy conditions [18]. Under normal conditions, binding of IL1 receptors to IL1 prevents pro-inflammatory action thereby inhibiting inflammation. Studies have demonstrated this property in relation to the action of interleukin 1 receptor antagonist (\textit{IL1RN}) which acts as a competitive inhibitor of interleukin 1. Mutation in interleukin 1 receptor antagonist was associated with decrease IL1 beta response to abnormal vaginal flora; therefore consider as an
advantage over infection mediated preterm birth [18]. Ryckman et al., 2011 demonstrated that gene-gene interactions between IL-1R2 and TLR4 are associated with low levels of cervical pro-inflammatory cytokine concentrations in women infected with bacteria vaginosis. The differences observed in allele frequency of IL1R2 and TLR4 between African Americans and European women partially explain population disparity in pregnancy-related outcomes that are cytokine concentration-dependent [17].

This study demonstrates variability in the occurrence of IL1R2 gene polymorphisms among the three ethnic groups in Malaysia. A significant association of ILR2 gene polymorphism with preterm birth was seen in the Indian ethnic subgroup but not in the Chinese and Malay ethnic groups. Women who carry ILR2 rs2072476 G allele were found to be protected against preterm delivery. Malaysia has a multiethnic population which is composed of three different ethnic groups (Malay, Chinese and Indians) with varying genetic background. The present study shows that variability in the occurrence of preterm birth among women in Malaysia is maybe partially due to differences in their genetic components. We therefore suggest that in addition to lifestyle and environmental factors, genetic factors should be greatly considered in regards to preterm birth complication.
Conclusion

In summary, we found that IL1R2 rs2072476 polymorphism was protective against PTB in the Indian ethnic group. Prior information on the genetic composition of women with PTB may help in the identification and management of preterm birth. Genetic factors have been associated with preterm birth in other populations and according to our findings, IL1R2 rs2072476 polymorphism was specific to the Indian ethnic group. Due to the short and long term complications associated with children born preterm, genetic and environmental factors should be considered in prevention and management strategies of PTB regardless of geographical area. This will help to aid in development of novel management strategies to prevent preterm delivery.

Acknowledgement

We would like to thank the patients and staffs of University of Malaya Medical Center (UMMC) for their participation in this study. We appreciate all staffs and members of HLAF for their financial support.

Declaration of interest: The authors report no conflicts of interest.

Funding

Funding for this project was provided by High Impact Research Grant HIR MOHE E000049-20001 of the University of Malaya.
References

Table 1 Overall demographic characteristics of 532 term and 132 preterm subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Term</th>
<th>Preterm</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (mean ± SD)</td>
<td>29.3 ±3.7</td>
<td>29.8 ± 3.0</td>
<td>0.230</td>
</tr>
<tr>
<td>BMI (kg/m2) (mean± SD)</td>
<td>24.5 ±5.4</td>
<td>24.3 ± 5.1</td>
<td>0.690</td>
</tr>
<tr>
<td>Married (%)</td>
<td>98</td>
<td>98</td>
<td>0.720</td>
</tr>
<tr>
<td>GDM (%)</td>
<td>0</td>
<td>11</td>
<td>NA</td>
</tr>
<tr>
<td>Bacterial infection (%)</td>
<td>0</td>
<td>2</td>
<td>0.042</td>
</tr>
<tr>
<td>Previous PTB (%)</td>
<td>0</td>
<td>14</td>
<td>NA</td>
</tr>
<tr>
<td>Previous miscarriage (%)</td>
<td>15</td>
<td>25</td>
<td>0.011</td>
</tr>
<tr>
<td>Household Income</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (%)</td>
<td>2.0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Average (%)</td>
<td>33</td>
<td>38</td>
<td>0.55</td>
</tr>
<tr>
<td>High (%)</td>
<td>64</td>
<td>62</td>
<td></td>
</tr>
</tbody>
</table>

Data are express as mean ± SD for continuous data and as percentage for categorical data

P values were obtained using Mann-Whitney U test or independent t test

P-values <0.05 were considered significant
Table 2 Allele frequency of IL1R2 SNPs stratified by ethnicity

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Pooled Ethnicity</th>
<th>Malay</th>
<th>Indians</th>
<th>Chinese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preterm</td>
<td>Term</td>
<td>Preterm</td>
<td>Term</td>
</tr>
<tr>
<td>rs2072476A/G</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (reference)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>0.16</td>
<td>0.19</td>
<td>0.61</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.84</td>
<td>0.39</td>
<td>0.35</td>
</tr>
<tr>
<td>Adj OR (CI)</td>
<td>0.89</td>
<td>0.39</td>
<td>1.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Adj P</td>
<td>0.369</td>
<td>0.420</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td>rs2071008G/T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G (reference)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>0.19</td>
<td>0.17</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>0.81</td>
<td>0.83</td>
<td>0.85</td>
<td>0.85</td>
</tr>
<tr>
<td>Adj OR (CI)</td>
<td>0.85</td>
<td>1.0</td>
<td>0.27</td>
<td>1.42</td>
</tr>
<tr>
<td>Adj P</td>
<td>0.59</td>
<td>0.64</td>
<td>0.953</td>
<td>0.660</td>
</tr>
</tbody>
</table>

Adj P-adjusted P value, Adj OR-adjusted OR, CI-confidence interval
P values were adjusted for previous PTB, previous miscarriage, bacterial infection and gestational diabetes. P<0.05 was considered significant, rs2072476; reference allele = G, effect allele = A, rs2071008; reference allele = G, effect allele = T.