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A Toll-Like receptor 9 (*rs352140*) variant is associated with placental inflammation in newborn infants

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Abstract

Objective—Chorioamnionitis contributes to premature birth and associated postnatal morbidity. The genetic basis of altered immune responses underlying placental inflammation (PI) remains understudied. The aim of this study was to evaluate the relationship among TLR signaling pathway polymorphisms and different patterns of PI.

Methods—Prospective cohort study in infants involving cord blood collection and placental examination for PI. 159 infants enrolled in study out of which 28 were term (8 with PI) and 131 preterm (47 with PI). DNA from blood was genotyped for SNPs in *TLR2*, *4*, *5*, *9*, *NFKB1*, *NFKBIA*, *TIRAP* and *IRAK1* genes using multiplexed single base extension assay.

Results—While there were no differences in BW, GA, gender, race, and SPL among infants with or without PI, there was a higher incidence of PPRM, maternal smoking, drug use and clinical chorioamnionitis among infants with PI. Out of nine TLR variants, only CT and/or TT genotypes of the TLR9 variant (*rs352140*) were significantly associated ($p=0.004$) with any PI and maternal pattern of inflammation ($p=0.012$) both by univariate analysis and logistic regression.

Conclusions—Presence of a variant T allele in a common SNP (*rs352140*) in the TLR9 gene whose product recognizes bacterial DNA is associated with increased PI.

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Declaration of Interest Statement

The authors report no declarations of interest

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Keywords

Chorioamnionitis; Placental inflammation; Toll-Like receptor 9 polymorphisms; rs352140; innate immunity

Introduction

Placental inflammation (PI), both among preterm and term infants, is associated with higher prevalence of postnatal complications such as sepsis, neurologic injury, developmental delay and death (1–4). PI is usually a bacterial infection, often polymicrobial, caused by aerobic and anaerobic organisms originating in the genitourinary tract, gastrointestinal system, or skin. Other causative pathogens include fungi (usually *Candida spp*) and mycoplasma (usually *U. Urealyticum* (5). Histological PI encompasses maternal (deciduitis), fetal (funisitis and chorionic plate vasculitis) or a feto-maternal pattern (chorioamnionitis and villitis) (6) based on which component of the placenta is showing an inflammatory response. Both maternal and fetal inflammatory responses to microorganisms in the placental membranes are predominantly mediated by neutrophil activation for enhanced phagocytosis and bactericidal function. While the mechanisms underlying the causation of PI remain unclear, data show that the innate immune pathway is activated while the adaptive immune pathway is suppressed during the placental response to infection/inflammation (7).

TLR's are phylogenetically conserved, germline encoded pattern recognition receptors that are an integral component of the innate immune pathway necessary for microbial recognition (8). TLRs recognize microbial components known as pathogen associated molecular patterns and trigger a complex signaling cascade, resulting in the activation of multiple transcription factors like nuclear factor-kappa B (NFkB) and activator protein 1 that induce the expression of pro-inflammatory cytokines, notably IL-1, IL-6 and TNF-a, which ultimately lead to distinct anti-microbial responses. The mammalian family of TLRs includes at least 10 distinct receptor proteins (9) and the expression of all 10 TLRs has been described in the human placenta (10,11). At the placental interface, TLRs are expressed not only in immune cells but also in nonimmune cells such as trophoblasts and decidual cells (12).

The genetic basis of altered innate immune responses in the placenta which can contribute to PI are not known. Previous studies from our group and others have shown that TLR sequence variants are associated with altered risk of premature birth (13,14), which is a well-known consequence of PI(15). However, the impact of TLR genetic variants on PI or specific patterns of PI remain understudied. Since multiple TLR's are involved in the placental response to bacteria leading to inflammation/infection at the maternal-fetal interface, we examined the relationships between TLR signaling pathway sequence variants and PI.

Methods

Patient recruitment

This was a prospective, cohort study conducted at two centers (Froedtert Memorial Lutheran Hospital (FMLH) Newborn nursery and the Neonatal Intensive Care Unit of Children's Hospital of Wisconsin (CHW), Milwaukee, Wisconsin) from April 2009 to May 2011. The study was approved by the IRB for Human Research at CHW. Written informed consent, which was approved by the IRB committee, was obtained from parent(s) before enrolling patients into the study. After obtaining consent, the original consent form was kept in a secure cabinet in the research department at CHW and a copy was given to the parents and another copy went into the infant's medical records. From every infant that was recruited, we collected cord blood samples and saved the placenta for histopathological examination. The DNA extraction (from cord blood) and subsequent genotyping were done at the Children's Research Institute. Clinical and demographic data were collected from hospital electronic medical records as well as patient charts, de-identified, and entered into preformatted data sheets and stored in a secure database.

Eligibility criteria

All infants born at FMLH Labor & Delivery (L&D) unit and admitted to either the newborn nursery or CHW NICU were eligible.

Exclusion criteria

All babies with congenital anomalies and products of multiple pregnancy were excluded from the study.

Placental examination

Upon delivery, placentas were bagged and stored in a refrigerator in L&D until transport to the pathology lab, where they were weighed and grossly examined in the fresh state before further refrigeration. Samples were harvested from fresh, refrigerated placentas within 12 hours of delivery. Placentas were then fixed overnight in 10% neutral buffered formalin. Representative histologic sections were taken from (1) proximal and distal umbilical cord, (2) a roll of the fetal membranes, and (3) disk parenchyma (lesions and unremarkable areas, at least three full-thickness pieces). Routine hematoxylin-and-eosin staining was performed. Placental pathology was reviewed by a single perinatal pathologist (J.J) at CHW who was blinded to the genotyping results. Placentae which showed inflammation were classified according to Redline's criteria (16–18) as fetomaternal (chorioamnionitis/villitis), only maternal (deciduitis) and only fetal (funisitis/chorionic plate vasculitis) patterns of inflammation. For the purpose of the study we defined the group 'any PI' as those infants whose placentae showed any inflammation irrespective of the specific pattern. The severity of PI was staged and graded as follows; Stage 1-umbilical phlebitis or chorionic vasculitis, Stage 2-umbilical arteritis and/or early funisitis, Stage 3-necrotizing funisitis and concentric umbilical perivasculitis and grade; Grade 1-mild to moderate and Grade 2-severe. Membrane inflammation, when present, was staged with the same system: subchorionitis and chorionitis (stage 1), chorioamnionitis (stage 2), and necrotizing chorioamnionitis (stage 3) –

and likewise graded according to severity – mild to moderate (grade 1) or severe (grade 2). Also noted but not staged/graded were chronic villitis and acute or chronic deciduitis.

Selection of SNP's

Candidate genes in the TLR pathway that are critical for mediating immune responses against bacterial agents at the fetomaternal interface in the placenta were targeted. Nine TLR SNPs for eight loci (the receptors, 2, 4, 5 and 9, a key kinase [IRAK1], NFKBIA, the adapter TIRAP, and the transcription factor, NFKBI) were genotyped (Table I). SNPs in these genes were identified based on whether:

1. Variants were previously reported to be associated with human infectious disease, and
2. Variants were associated with altered expression or function of the gene.

Laboratory methods

Genomic DNA was extracted from human neonatal blood samples (0.5 to 1 ml obtained from either umbilical cord at birth or by venipuncture or heel stick) using the FlexiGene DNA kit (Qiagen, Valencia, CA, USA) and stored at 4 °C. The SNPs which were investigated in this study are listed in Table I. The 9 SNPs were genotyped by using a multiplexed single-base extension assay essentially as described before by Koukouritaki et al (19) and performed by Sampath et al (20).

Statistical Analysis

The distribution of SNPs between the two groups (PI vs no PI) was analyzed using a 2-way contingency table with Mantel-Haenszel Chi-square and Fisher's exact test. We used a dominant model, i.e. genotypes which were homozygous or heterozygous for the variant allele were grouped into one unit and comparison was made with the homozygous reference allele. This model assumes that presence of a single copy of the variant alters functionality, and is based on studies showing that TLR SNPs are penetrant even in haploinsufficient states (21). The threshold α for significance after the 9 sequential pairwise comparisons with a Bonferroni correction was 0.0055. Our analysis compared infants with 'no PI' with those having 'PI'. We also compared each individual component of the PI group (any PI, fetal, maternal and feto-maternal type) with the no-PI group. For variants which were significantly associated with either group, we did a stepwise logistic regression analysis to correct for confounding variables like birth weight (BW), gestational age (GA), gender, maternal smoking, spontaneous preterm labor (SPL), pregnancy induced hypertension (PIH) and PPRM. Additionally we also looked at interactions between the variants and demographic variables using logistic regression model (MedCalc software, version 13.2, Acaciaaan 22, B-8400 Ostend, Belgium)

Results

Distribution of PI in our cohort

There were 159 infants enrolled, of which 28 were term (>37 weeks gestational age) and 131 preterm. 55 placentae showed evidence of inflammation (PI) while 104 placentae had no evidence of inflammation (No-PI). Among the placentae with PI eight were term and 47 preterm. A maternal pattern of PI was present in 5 term and 16 preterm placentas. A fetal PI pattern was present only in the preterm (n=16). Feto-maternal inflammation was present in 5 term and 33 preterm placentas (Figure 1). Among preterm infants (PI vs. No-PI) (Table II), there were no significant differences in birth weight, gestational age, gender, race or clinical chorioamnionitis between the two groups. However premature infants with PI had significantly higher incidence of PPRM ($P=0.001$), maternal smoking ($P=0.001$) and self declared drug use ($P=0.043$) when compared to the No-PI group. The incidence of PIH was found to be lower in the premature PI group ($P=0.037$) when compared to the No-PI group. There were no significant differences between the two premature groups with respect to incidence of SPL or in the incidence of placenta previa or placental abruption. Epidemiologic and clinical characteristics of term infants was comparable with no significant differences between the two groups. Staging and grading of PI was available and relevant for 33 out of 55 (60%) infants, the remaining infants having deciduitis and/or villitis which could not be graded or staged.

TLR variants and placental inflammation

The results of genotyping for the nine TLR pathway variants stratified by placental pathology (all infants) are illustrated in table III. Hardy Weinberg equilibrium was confirmed at all loci except for IRAK1 which is an X-linked gene. The genotype proportions in females were significantly different from that expected under HWE ($p=0.046$). The distribution of variant allele frequencies were not statistically significant between the No-PI vs PI group for TLRs 2, 4, 5, NFKB1, NFKBIA, TIRAP and IRAK1 SNPs. Presence of CT or TT when compared to CC genotype for the TLR9 SNP (*rs 352140*) was increased with every category of PI in the entire cohort (n=159) but statistically significant only with the any PI (OR 3.8 95% CI 1.4–11, $P=0.004$) and maternal pattern of PI group (OR 9.2, 95% CI 1.1–193, $P=0.012$) when compared to the No-PI group.

A stepwise logistic regression model correcting for confounding variables (BW, GA, gender, SPL, PPRM, PIH, race and maternal smoking) found that presence of the CT or TT genotype of the TLR9 variant (OR 4.2, 95% CI 1.5–11.4, $P=0.0054$), smoking (OR 4.1, 95% CI 1.8–9.4, $P=0.001$) and PPRM (OR 4.3, 95% CI 1.6–11.3, $P=0.0032$) were the only variables associated with PI (especially any PI and maternal pattern).

Relationship between 2848 C/T TLR9 variant and PI in premature infants and Caucasian infants

Among preterm infants (n=131), 35% (47/131) had PI out of which 33 had fetomaternal and 16 infants each had maternal or fetal pattern of inflammation (Figure 1). Among infants who did not have PI, 68% (57/84) had the CT or TT genotypes compared to 89% (42/47) of preterm infants with PI. Presence of the CT or TT genotype of the TLR9 SNP was

associated with PI (No-PI *vs* any PI OR 3.9, 95% CI 1.3–12, $p=0.006$). All preterm with maternal pattern of inflammation had the CT or TT genotypes for the variant SNP (16/16) compared to 68% (57/84) of infants without PI (OR ∞ , 95% CI 1.38– ∞ , $p=0.008$).

Since our population is genetically heterogenous, we analyzed whether the distribution of SNP's is influenced by the ethnicity of the population. Caucasian infants comprised the largest racial cohort (106 infants out of which 33 had PI). Presence of the CT or TT genotype of the TLR9 variant was associated with increased prevalence of PI (Caucasian PI (30/33) *vs*. Caucasian no-PI (54/73), $p=0.048$). There were 44 African American (AA) infants in this study out of which 19 had PI. Among AA infants, we saw a similar trend with the CT or TT genotype of the TLR9 variant enriched among placentas with PI (AA with PI (17/19) *vs*. AA no-PI (14/25), $p=0.016$) although the sample size was limited.

Discussion

Although multiple studies have shown that activation of innate immune system is central to the pathogenesis of PI(22,23), the impact of TLR genetic variants on PI remains significantly understudied. This study takes a pathway approach to examine the association between function altering fetal TLR genetic variants and PI. We report a novel association between presence of the variant T allele in a common TLR9 SNP (*rs352140*) and PI. These data suggest that inherited differences in the fetal immune response can modulate inflammatory responses at the maternal-fetal interface. The lack of maternal genotyping data, a genetically heterogeneous population and the lack of functional expression studies for TLR9 in the placenta are limitations of this study.

These results are the first to demonstrate that a TLR9 variant SNP (*rs352140*) is associated with PI. We found that although the CT and TT genotypes of the TLR9 variant was increased with all patterns of PI, numbers reached significance with any PI and maternal pattern of PI. The same trends were present in the premature and Caucasian infants. Although this study found that the TLR9 variant was more prevalent in infants with PI (figure 2, table IV), it was also found in ~65% infants without PI which suggests that presence of this common variant alone is not enough to cause PI. Because PI involves a complex interplay between genetic, immunologic (both maternal and fetal) and microbiological factors, we speculate that this TLR9 fetal genetic variant is one of the factors which increases inflammatory response and can contribute to PI. While our objective was to compare TLR9 variant distribution between the 'No-PI' group with the individual PI sub-phenotypes, we initially compared the 'Any PI' with the 'No PI' group as the relationship between TLR pathway SNP's with PI has not been examined before. Although the frequency of CT and TT genotypes in the placental fetomaternal ($p=0.06$) and fetal (funisitis) subsets were not statistically different from the no-PI group the prevalence of those genotypes were higher in these subsets of PI too (Figure 2). A larger population for each of these sub-groups would enable us to clarify the association between the TLR9 variant and PI subtype. Because the immunological factors which regulate funisitis and fetomaternal pattern of PI might differ from other sub-phenotypes of PI it is also possible that TLR genotypes might be only associated with certain PI sub-phenotypes (24,25)

TLR9 receptor, found in the immune as well as non-immune cells of the placenta, recognizes unmethylated CpG oligonucleotides that are abundant in bacterial DNA. These CpG motifs are methylated in vertebrate genomes which could be a protective mechanism preventing activation of innate immune system against self-antigens (5,26). TLR9, which is an intracellular endosomal receptor, acts upstream of the adaptor protein Myeloid Differentiation factor 88 (MyD88) and links CpG motif recognition to the Toll-like Interleukin-1 receptor (TLR/IL-1R) pathway which induces cell signaling pathways including the mitogen activated protein kinases (MAPKs) and NF κ B. Cells that express TLR9, which include plasmacytoid dendritic cells (pDCs) and B cells, produce cytokines, chemokines and interferons (27). The TLR9 variant examined in this study has been shown to be associated with chronic inflammatory conditions like Systemic Lupus Erythematosus (SLE), Crohn's (28) disease and cervical cancer (29) where abnormal sensitization of the immune system by microbial agents plays a role in disease evolution.

The functional significance of this 2848 C/T variant in the TLR9 gene on human chromosome 3p21.3, was investigated by Chen et al in the Han Chinese population (30). They demonstrated that presence of the CT or TT genotype of this variant was associated with increased TLR9 mediated TNF α production in peripheral blood leukocytes among patients with sepsis and multiorgan failure suggesting a gain of function. Kikuchi et al's (31) study in adults with primary biliary cirrhosis (PBC) reported that the CT and TT genotype of the TLR9 *rs352140* variant is associated with higher expression of TLR9 following CpG stimulation in peripheral blood mononuclear cells. While the 2848 C/T TLR9 variant is a synonymous coding variant, two other studies (22,30) have suggested that this variant is in linkage disequilibrium with promoter variants which increase TLR9 expression and therefore increase inflammation. So we speculate that infants with the CT or TT genotype of the TLR9 variant (*rs352140*) have increased TLR9 expression at the maternal-fetal interface in the placenta leading to greater recognition of bacterial CpG motifs causing an exaggerated inflammatory response leading to PI. Another potential mechanism might be aberrant activation of TLR9-mediated inflammation in the placenta in response to mitochondrial DNA as suggested by Goulopoulou et al (32). Past data have shown reliable correlation between placental histopathology and placental IL-6, IL-1 β and TNF α levels (23).

In our study we did not find any association between other function altering TLR variants especially TLR4 SNPs and any form of PI. This is in contrast to Simhan et al's prospective cohort study which examined the link between both maternal and fetal genetic variants and PI. They genotyped 13 tag TLR4 SNPs and found a correlation between maternal and fetal TLR4 genetic variants and PI(33). Both these variants are promoter variants which have not been functionally evaluated. Further, Simhan's group have reported an association between the *rs1554973* TLR4 variant and increased cervical cytokines in European women with bacterial vaginosis during pregnancy (34). The TLR 4 SNPs (*rs 4986790* and *rs 4986791*) that were evaluated in this study were chosen because they were exonic and previous studies have shown decreased function (35,36). Unlike Simhan et al's study, the current study did not have maternal genotyping data and the fetal TLR4 SNP that they found to be significant (*rs1554973*) was different from the ones we looked at (*rs 4986790* and *rs 4986791*). In addition our population was predominantly Caucasian when compared to Simhan's study population which was predominantly African American. While our study population

consisted predominantly of preterm infants, the gestational age distribution of study population in Simhan's study was not apparent. This could have also contributed to the differences in the results between the two studies.

Infants with PI had a higher prevalence of maternal smoking, drug use and PPROM which is consistent with reported literature (37–39). Also among infants in the PI group there was a lower prevalence of maternal PIH than those in the No-PI group. This could be attributed to the fact that PI, which can lead to preterm birth, is usually associated with PPROM or SPL whereas PIH is a known maternal cause for premature birth.

Conclusion

We report a novel association between 2848 C/T TLR9 variant (*rs352140*) and increased inflammation at the maternal-fetal interface. The mechanism by which genetic variants in TLR9 alter innate immune responses and contribute to the pathogenesis of PI has to be examined in future studies. Our data lends further support to the hypothesis that genetic variation in the TLR pathway alters susceptibility to PI and prematurity.

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Abbreviations

PI	Placental inflammation
TLR	Toll-like receptor
SNPs	Single nucleotide polymorphisms
BW	birth weight
GA	gestational age
PIH	Pregnancy induced hypertension
PPROM	Preterm premature rupture of membranes
SPL	Spontaneous preterm labor
MAPKs	Mitogen activated protein kinases
NFKBI	Nuclear factor kappa B inhibitor
IRAK1	Interleukin-1 receptor–associated kinase 1
NFKBIA	Nuclear factor kappa B inhibitor alpha
TIRAP	Toll-like Interleukin-like domain receptor–associated protein

IL	Interleukin
TNF-α	Tumor Necrosis Factor Alpha
MYDD88	Myeloid Differentiation factor 88
TLR/IL-1R	Toll-like Interleukin-1 receptor

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Figure 1. Study recruitment

Figure illustrates the categorization of study placenta by presence and pattern of placental inflammation. PI defined per Redlines criteria

*Note that numbers are not additive as a single placenta can have multiple patterns of inflammation

Fig 2. Distribution of 2848 C/T TLR9 variant stratified by placental pathology

Overall 71/104 infants in the No-PI, 49/55 in any PI and 32/38, 20/21 and 13/16 in the fetomaternal, maternal and fetal patterns of PI respectively had the 2848 C/T TLR9 variant. Any PI includes infants with any inflammation irrespective of the pattern. Combined fetomaternal pattern includes chorioamnionitis and/or villitis, maternal pattern includes deciduitis and a fetal pattern includes funisitis and/or chorionic plate vasculitis. Chi-square (Mantel-Haenszel) and Fisher exact tests were used for comparisons between groups and for generating p values. Data labels represent actual number of infants in each group.

*P=0.004 (no PI vs any PI)

** P=0.012 (no PI vs maternal pattern of inflammation)

Table I

TLR SNPs investigated in the current study with their accession numbers and functional annotations.

Gene	Peptide change	SNP	Accession#	Functional annotation ^a
TLR2	p.Arg753Gln	g.2258G>A	rs5743708	Hypomorphic
TLR4	p.Asp299Gly	g.8552 A>G	rs4986790	Hypomorphic
TLR4	p.Thr399Ile	g.8852 C>T	rs4986791	Hypomorphic
TLR5	p.Arg392X	g.1174 C>T	rs5744168	Amorphic
TLR9	p.Pro545Pro	g.2848 C>T	rs352140	Associated with gain of function (26)
NFKB1	None	24519delATTG	rs28362491	Hypomorphic
NFKBIA	None	g.-1004 A>G	rs3138053	Unknown
TIRAP	p.Ser180Leu	g.2054 C>T	rs8177374	Hypomorphic
IRAK1	p.Ser453Leu	g.6435 T>C	rs1059703	Hypermorphic

^aHypomorphic; loss of function, Hypermorphic; gain of function

Table II

Epidemiologic and clinical factors stratified by placental pathology in preterm newborn

Variable	Preterm with PI (n=47)	Preterm no PI (n=84)
BW (gms) ^a	1440±737	1429±600
GA (wks) ^a	29.6±3.7	29.9±3.3
Gender (Male) (%)	45	51
Race (%)		
Caucasians	60	74
Black	36	21
Clinical Chorioamnionitis	3	1
Spontaneous Preterm Labor (%)	55	48
PPROM (%)	30 [#]	11
Maternal factors (%)		
Smoking	38 [#]	13
Drug use	19 [*]	7
PIH	15	31 [*]
Placental factors (%)		
Abruptio	13	13
Previa	2	4

Abbreviations: BW birth weight, GA gestational age, PTL preterm labor, PPRM Preterm premature rupture of membranes, PIH Pregnancy induced hypertension,

^{*} P<0.05,

[#] P=0.001

^a Birth weights and gestational ages are represented as standard deviations of the mean

Table III

Distribution of TLR variants stratified by pattern of placental inflammation

SNP Accession number	Allelic frequency in the cohort (n=159)				
	No PI (n=104)	Any PI (n=55)	Fetomaternal pattern (n=38)	Maternal pattern (n=21)	Fetal pattern (n=16)
TLR2 rs5743708	G/G 98 (94%)	G/G 53 (96%)	G/G 38 (100%)	G/G 20 (95%)	G/G 15 (94%)
	G/A 6 (6%)	G/A 2 (4%)	G/A 0	G/A 1 (5%)	G/A 1 (6%)
TLR4 rs4986790	A/A 96 (92%)	A/A 50 (91%)	A/A 35 (92%)	A/A 19 (90%)	A/A 15 (94%)
	A/G 8 (8%)	A/G 5 (9%)	A/G 3 (8%)	A/G 2 (10%)	A/G 1 (6%)
TLR4 rs4986791	C/C 97 (93%)	C/C 54 (98%)	C/C 37 (97%)	C/C 38 (100%)	C/C 15 (94%)
	C/T 7 (7%)	C/T 1 (2%)	C/T 1 (3%)	C/T 0	C/T 1 (6%)
TLR5 rs5744168	C/C 100 (96%)	C/C 51 (93%)	C/C 36 (95%)	C/C 19 (90%)	C/C 15 (94%)
	C/T 3 (3%)	C/T 4 (7%)	C/T 2 (5%)	C/T 2 (10%)	C/T 1 (6%)
	T/T 1 (1%)	T/T 0	T/T 0	T/T 0	T/T 0
TLR9 rs352140	C/C 33 (32%)	C/C 6 (11%)*	C/C 6 (16%)	C/C 1 (5%)#	C/C 3 (18%)
	C/T 45 (43%)	C/T 30 (55%)	C/T 20 (53%)	C/T 14 (67%)	C/T 8 (50%)
	T/T 26 (25%)	T/T 19 (34%)	T/T 12 (32%)	T/T 6 (28%)	T/T 5 (31%)
NFKB1 rs28362491	Ins/Ins 37 (35%)	Ins/Ins 15 (27%)	Ins/Ins 11 (29%)	Ins/Ins 5 (24%)	Ins/Ins 5 (31%)
	Ins/del 57 (55%)	Ins/del 27 (50%)	Ins/del 18 (47%)	Ins/del 14 (67%)	Ins/del 8 (50%)
	Del/del 10 (10%)	Del/del 13 (23%)	Del/del 9 (24%)	Del/del 2 (10%)	Del/del 3 (19%)
NFKBIA rs3138053	A/A 61 (58%)	A/A 35 (64%)	A/A 22 (50%)	A/A 14 (6%)	A/A 12 (69%)
	A/G 37 (36%)	A/G 18 (32%)	A/G 15 (50%)	A/G 6 (32%)	A/G 3 (23%)
	G/G 6 (6%)	G/G 2 (4%)	G/G 1	G/G 1 (4%)	G/G 1 (8%)
TIRAP rs8177374	C/C 86 (82%)	C/C 43 (78%)	C/C 30 (79%)	C/C 18 (86%)	C/C 13 (81%)
	C/T 17 (17%)	C/T 11 (20%)	C/T 7 (18%)	C/T 3 (14%)	C/T 3 (19%)
	T/T 1 (1%)	T/T 1 (2%)	T/T 1 (3%)	T/T 0	T/T 0
IRAK1	C/C 61 (59%)	C/C 32 (57%)	C/C 21 (55%)	C/C 12 (57%)	C/C 8 (50%)

SNP Accession number	Allelic frequency in the cohort (n=159)				
	No PI (n=104)	Any PI (n=55)	Fetomaternal pattern (n=38)	Maternal pattern (n=21)	Fetal pattern (n=16)
rs1059703	T/C 14 (13%)	T/C 13 (23%)	T/C 10 (26%)	T/C 3 (14%)	T/C 6 (37%)
	T/T 28 (27%)	T/T 10 (20%)	T/T 7 (18%)	T/T 6 (29%)	T/T 2 (12%)

* P value 0.004 (No PI vs Any PI),

P value 0.012 (No PI vs Maternal paternal of PI. Allelic frequencies of study subjects according to pattern of placental inflammation. Chi-Square or Fisher exact tests were used for comparisons between the groups and for generating P-values