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A combination of single nucleotide polymorphisms in the 3′ untranslated region of HLA-G is associated with preeclampsia

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Abstract

Reduced expression of human leukocyte antigen-G (HLA-G) has been linked to onset of preeclampsia. Associations have also been reported between preeclampsia and single nucleotide polymorphisms (SNP) in the 3′-untranslated region (UTR) of the HLA-G gene. However, there are conflicting results between studies. This studied examined whether a SNP, by itself or in combination with other SNPs, in the 3′UTR of the HLA-G gene is associated with an increased risk of preeclampsia. Placenta samples were obtained from 47 preeclamptic and 68 control cases. DNA was extracted, and the 3′UTR was sequenced and analyzed for nine polymorphisms using different genetic models of inheritance. Four of these polymorphisms have never been analyzed for an association with preeclampsia. Disputing existing reports, preeclamptic cases were suggestively associated with a G/G-genotype at SNP +3187 ($p < 0.05$). Several SNP combinations were more prevalent in preeclampsia cases. Following corrections for multiple testing, one SNP combination (+3027C/C and +3187G/G) was significantly more prevalent in preeclampsia cases using co-dominant, additive, and dominant models ($p < 0.001$). Taken together with the current literature, the data suggests that HLA-G 3′UTR SNP-pair associations, and not individual SNPs, could be useful in a predictive test for the susceptibility to preeclampsia.

Keywords

HLA-G; Preeclampsia; SNP; 3′UTR; +3187A/G

1. Introduction

Preeclampsia is a disease of pregnancy that is most common in first pregnancies with a sexual partner, and is one of the leading causes of morbidity and mortality for both the mother and her fetus [1,2]. This disease affects ~6–8% of all pregnancies and is characterized by varying degrees of hypertension and proteinuria [3]. Maternal endothelial

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dysfunction appears to be an important underlying factor leading to the clinical features of this condition. Although it is a topic of extensive study, the underlying cause of preeclampsia remains an enigma. One hypothesis proposes that preeclampsia occurs due to a maladaptation in the mechanism that is in place to prevent the maternal immune system from rejecting the semi-allogenic fetus [2,4].

At the maternal–fetal interface, trophoblast cells of the placenta do not express most classical human leukocyte antigens (HLA) class Ia and II. However, a low amount of HLA-C is expressed. Compared to classical HLAs, invasive cytotrophoblast cells, which come in direct contact with the mother, express a unique combination of HLA's, namely HLA-C, -E and -G. The non-classical class Ib molecule, HLA-G, is highly expressed in cytotrophoblast cells [5,6]. While classical HLA molecules (HLA-A, -B, and -C) are important for self-antigen recognition, non-classical HLA molecules are thought to play a very different role [6]. There is much evidence that HLA-G acts as a cloaking molecule, allowing placental cytotrophoblast cell invasion into the maternal spiral arteries, without hindrance from the maternal immune system [7–9].

HLA-G protein is expressed not only on cytotrophoblast cells of the placenta, but also on oocytes, early embryos, thymus, and activated monocytes to name a few [5,10–12]. We, and others, have observed lower placental expression and/or secretion of HLA-G in association with preeclampsia [8,13–16]. We have also previously shown that high levels of HLA-G stimulate a pregnancy protective Th2 response, whereas low levels of HLA-G paradoxically stimulate a non-protective Th1 response in an *in vitro* model [17]. Given this evidence, it suggests that reduced expression of HLA-G *in vivo* may paradoxically elicit an adverse maternal immune system response. This could result in abnormally shallow trophoblast invasion of the maternal spiral arteries, leading to diminished remodeling of the maternal spiral arteries and thus, reduced arterial blood flow.

Although initially considered to be non-polymorphic, it is now widely accepted that variations of the HLA-G DNA sequence exists, specifically in the 3′-untranslated region (UTR). The HLA-G 3′-UTR contains several regulatory elements, including AU-rich elements, polyadenylation signals, and signals that regulate the spatial and temporal expression of mRNA [18,19]. Various research groups have studied individual polymorphisms in the 3′UTR of HLA-G as a potential explanation for the observed reduction in HLA-G mRNA and protein levels in association with preeclampsia [20,21]. The gene sequence of the 3′UTR is quite polymorphic, containing various single nucleotide polymorphisms (SNPs) and a 14bp insertion/deletion polymorphism. Some of these variants may lead to functional consequences at the mRNA and protein levels [22–24]. Initially believed to be functionally insignificant, current evidence supports the hypothesis that 3′UTR variants can affect the intrinsic properties and function of proteins [25,26]. In addition, it has been shown that multiple SNP–SNP interactions could have functional effects on genes, potentially contributing to disease [27]. Although the effect of an individual SNP is generally small, we hypothesize that combinations of functionally relevant SNPs in the HLA-G 3′UTR may additively or synergistically contribute to changes in HLA-G expression.

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Although there have been several published studies on the association of these polymorphisms to preeclampsia, there are conflicting results and no consensus to date (Table 1). Most studies have focussed on the 14bp insertion/deletion polymorphism, reporting that the presence of the 14bp insertion is either associated or not associated with preeclampsia [21,22,28–31]. Unlike the 14bp polymorphism, other SNPs found in the 3′UTR have not been extensively evaluated for their association to preeclampsia. Thus, the goal of this study was to analyze whether there is a SNP by itself, or a combination of SNPs, in the 3′UTR of the HLA-G gene in placental tissue that is predictive of preeclampsia.

2. Materials and methods

2.0.1. Samples

We obtained 115 de-identified frozen placental samples from the Research Centre for Women's and Infants' Health BioBank at Mount Sinai Hospital under REB approval (10-0067E). All patients were given an informed consent before participation in the study. Placental samples were carefully collected from four quadrants of the fetal side only, and were washed thoroughly with 1×PBS to reduce maternal contamination. Of these samples, 47 came from preeclampsia cases and 68 from non-preeclamptic controls. All the preeclampsia cases had a measured systolic blood pressure of >150 mmHg and a diastolic blood pressure of 90 mmHg and pro-teinuria of 1–4+ on dipstick testing. A summary of the clinical characteristics of the preeclampsia versus the control groups can be found in Table 2.

2.0.2. DNA extraction

DNA was extracted from frozen placental tissue using the GE Illustra Triple Prep Kit™ according to the manufactures instructions. A piece of placental tissue, weighing 100 mg, was placed in a Eppendorf™ safety lock tube with 350 μL of 90% Lysis buffer type 15 (GE kit), 1% β-mercapto-ethanol and 1 scoop full of 0.5 mm stainless steel beads (Next Advance Inc.). The tube was placed in the bullet blender blue (Next Advance Inc.) for 4 min at maximum speed to homogenize the sample. The homogenate was ran through a QIA Shredder column (QIAGEN™) and centrifuged for 2 min at maximum speed. Following centrifugation, the flow-through was used for the GE Illustra Triple Prep Kit™ protocol. The resulting DNA was stored in a −20 °C freezer until analyzed.

2.0.3. Sequencing and SNP analysis

DNA samples were sequenced for the 3′UTR region of the HLA-G gene using the 5′-CAGGAAACAGCTATGACCAAAGTTCATGGTGGCCTGAG-3′ primer. The sequence was amplified by PCR using the following forward: 5′-TGTAAAACGACGGCCAGTTCACCCCTCACTGTGACTGA-3′ and reverse: 5′-CAGGAAACAGCTATGACCAAAGTTCATGGTGGCCTGAG-3′ primers yielding a 534bp PCR length. The sequences were analyzed for the allele and genotype frequencies of the eight SNPs and the 14bp insertion/deletion (ins/del) polymorphism that exists in the 3′UTR of HLA-G. The analyzed polymorphisms can be seen on the schematic (Fig. 1).

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2.0.4. Statistical analysis

We sought evidence for an association between each of the nine SNPs and preeclampsia in a multi-step analysis. Allele and genotype frequencies were compared between preeclampsia cases and the event of the controls using chi-square tests or Fisher's exact tests in the cases of low expected cell counts. Hardy–Weinberg equilibrium was evaluated using a Pearson's chi-squared test. Linkage disequilibrium (LD) was calculated using a chi squared test. LD frequencies were represented by the correlation coefficient, r^2

In order to assess the association between a polymorphism and preeclampsia, a logistic regression model for preeclampsia status with a term in the model for the SNP effect was used. Five different inheritance models were used for coding the SNP effect: dominant, recessive, co-dominant, over dominant and additive [32]. In the dominant model, both the heterozygous variant and rare homozygous variant were combined. In the recessive model, the variant was defined as only the rare homozygous genotype. In the co-dominant model, both rare homozygous and heterozygous variants were evaluated for an association with preeclampsia. In the over-dominant model, the heterozygous variant was compared to a pool of both homozygous genotypes. In the additive model, the homozygous variant genotypes have double the risk than the heterozygous genotype. Each copy of the variant allele alters the risk in an additive form. In all analyses, the common homozygote genotype in the control population was defined as the reference category. The magnitude of the association was summarized by the odds ratio (OR) from the logistic regression model, using a confidence interval of 95%. The statistical significance and *p*-value were adjusted according to Bonferonni's correction for multiple testing.

We also examined SNP–SNP interactions using a log-likelihood ratio test with additive, dominant, or co-dominant SNP effects. SNP-by-SNP interactions were shown in Table 6, Supplementary Tables S1 and S2, and Fig. 2. Due to the large number of SNP–SNP pairs being tested, the Bonferonni's method was used to adjust for multiple testing [33].

3. Results

3.0.1. Clinical characteristics of preeclampsia and control groups

The distribution of selected clinical and epidemiologic factors in cases and controls are shown in Table 2. Maternity age and gravidity were similar between the preeclampsia and control groups, while the proportion of primiparas, gestational age, and birth weight were different between the two groups, as expected. 64% of the preeclampsia group were classified as primiparas compared to 51% in the control group. No patient in the control group had a previous history of preeclampsia, or had a diagnosis of hypertension. The gestational age of preeclampsia patients ranged from 26.6 to 39.3 weeks with a mean of 33.0 weeks. Only patients who delivered at term (above 37 weeks of gestation) were selected as controls for this study. The gestational age of the control group was significantly greater than the preeclampsia group with a mean of 39.3 weeks (range 37.3–41.1 weeks; $p < 0.001$). Given that the gestational age was greater in the control group, the average birth weight was expectedly higher in the control group (3410 g) compared to the preeclampsia group (1706 g) ($p < 0.001$).

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3.0.2. Allelic frequencies between placentas of preeclampsia cases and controls

Among all samples, nine variation sites were detected in the 3′UTR region. Among these nine polymorphisms, the 14bp insertion/deletion polymorphism (NCBI dbSNP: accession number rs1704) and the SNPs +3142G/C (rs1063320) and +3187A/G (rs9380142), are thought to affect HLA-G expression [23,24,34–36]. In addition, six other SNPs were detected in the positions +3003T/C (rs1707), +3010G/C (rs1710), +3027A/C (rs17179101), +3035C/T (rs17179108), +3196C/G (rs1610696), and +3227C/T (rs1233331). Among the nine polymorphisms studied, only +3187A/G had a different allele frequency ($p = 0.03$) between cases and controls based on the crude p-value of 0.05 (Table 3). There was a higher proportion of the G-allele in the preeclampsia group. Similarly, allele frequency was statistically different when pre-eclampsia patients were divided into mild, moderate and severe cases and compared to controls (data not shown). However, after adjusting for multiple testing, there was now only a suggestive association between SNP +3187A/G and preeclampsia. None of the SNP distributions showed deviation from Hardy–Weinberg equilibrium (Supplementary Table S1), indicating that allelic frequencies have remained constant from generation to generation. As expected, no two SNPs were in perfect linkage disequilibrium (Supplementary Table S2).

3.0.3. Genotypic frequencies between placentas of preeclampsia cases and controls

Placental DNA was analyzed for genotype frequencies of the eight SNPs and the 14bp insertion/deletion between preeclampsia and control groups (Table 4). There were no statistical differences between preeclampsia and control cases in the genotype frequencies of all nine polymorphisms. In addition to using Fisher's exact tests to probe for genotype differences between groups, the variant allele of each SNP was analyzed for its association with preeclampsia using five different models of inheritance. The +3187A/G variation site was the only SNP to show a significant ($p < 0.05$) association with preeclampsia (Table 5). This significant association was observed in a recessive model of inheritance, which states that the homozygous dominant and heterozygous genotypes (A/A and A/G, respectively) have the same effect and that both copies of the variant allele (G) are required to confer risk to preeclampsia ($p = 0.009$). In the recessive model of inheritance individuals with a G/G genotype have a 9.80 OR of preeclampsia. Similar to the recessive model, in the co-dominant model of inheritance we observe a 10.85 OR of preeclampsia in individuals who have the G/G genotype at +3187A/G ($p = 0.03$). The log-additive model of inheritance predicted that every copy of a recessive allele (G) at +3187A/G conferred an increased risk to preeclampsia ($p = 0.03$). After using Bonferonni's correction for multiple testing, SNP +3187A/G was associated with preeclampsia, but only when utilizing a recessive model of inheritance.

3.0.4. Pair wise combinations of polymorphisms

Results of various two-way interaction analyses are shown in Table 6 and Supplementary Tables S3–S5. Since the genetic risk models depend on the type of inheritance, SNPs were analyzed using an additive (Supplementary Table S3), co-dominant (Table 6), dominant (Supplementary Table S4), over-dominant (Supplementary Table S5), and recessive (data not shown) models. From the combinations analyzed, there were nine SNP interactions that

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showed significant differences between preeclamptic cases and controls. While we did not find any significant SNP interactions in the recessive model of inheritance (data not shown), there were several SNP interactions in other models. In an additive model, +3003C/T–+3027A/C ($p = 0.02341$), +3027A/C–+3142C/G ($p = 0.02508$), and +3027A/C–+3187A/G ($p = 0.00141$) had a significant interaction with preeclampsia (Table S3). In the co-dominant model, +3003C/T-rs17179108 ($p = 0.04593$), +3027A/C–+3142C/G ($p = 0.01607$), and +3027A/C–+3187A/G ($p = 0.00021$) had a significant interaction with preeclampsia (Table 6). In a dominant model, +3003C/T–+3027A/C ($p = 0.02556$), +3027A/C–+3142C/G ($p = 0.02109$), +3027A/C–+3187A/G ($p = 0.00075$), and +3187A/G–+3196C/G ($p = 0.0499$) were significant (Table S4). In an over-dominant model, 14bp ins/del-rs1710 ($p = 0.03332$), +3003C/T–+3035C/T ($p = 0.04150$), 14bp ins/del-+3142C/G ($p = 0.03382$), and +3027A/C–+3187A/G ($p = 0.03550$) were significant (Table S5). After adjustment for false positives using a conservative method, Bonferroni's correction, only the SNP pair of C/C at +3027 and G/G at +3187 was statistically significant at a 95% confidence level in the additive (OR: 5.65), co-dominant (OR: 9.13), and dominant (OR: 10.85) models of inheritance. It is worth nothing that unlike preeclampsia cases, there were no control patients with the genotype combination of A/C at +3027 and A/G or G/G at +3187.

4. Discussion

In this study, we examined the contribution of nine polymorphisms, including the 14bp insertion/deletion polymorphism and eight SNPs in the 3′UTR of HLA-G from placental tissue. There was only a suggestive association between +3187A/G SNP and pre-eclampsia cases. Interestingly, gene frequencies of this SNP have also been linked to other illnesses and disorders, including septic shock and systemic lupus erythematosus [37,38]. In our study, the frequency of the G allele in the preeclampsia group (28.7%) was almost twice the amount as controls (16.2%). Furthermore, the G/G genotype was associated with preeclampsia cases in co-dominant, additive, and recessive models of inheritance. Similar to our findings, a previous study reported that there was a higher frequency of an A allele at SNP +3187A/G in the normal control group compared to the preeclampsia group, depending on the haplotype combination [29]. Interestingly, SNP +3187A/G is located 4-bp upstream of an adenylate/uridylate (AU)-rich motif that mediates mRNA degradation. This SNP is not only adjacent to the AUUUA pentameter, but a mutation in this SNP decreases mRNA stability *in vitro* [23]. Decreased RNA stability of the 3′UTR was associated with the presence of the +3187A allele, and the association of a A/A genotype was increased in severe preeclampsia cases compared to mild preeclampsia [23]. This allele effect on HLA-G expression was corroborated by Martelli-Palomino et al., who reported that healthy individuals with an A/A genotype have lower levels of soluble HLA-G compared to their A/G and G/G counterparts; although, this did not reach significance [39]. In contrast, our current study found an association of the G/G genotype with preeclampsia cases, rather than the A/A genotype. To determine whether this relationship was related to disease severity, we subdivided our preeclampsia cases into three groups; mild, moderate and severe. However, all three preeclampsia subgroups still had higher levels of G/G genotypes compared to controls. In keeping with this finding and highlighting the importance of SNP interactions, another group reported that their control group had a significantly higher proportion of the

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A/G or A/A genotype, but only when in combination with homozygosity or heterozygosity for the 14bp deletion [29]. The association of an A-allele with normal individuals is not novel. A population analysis of 155 healthy individuals, who were donating bone marrow, revealed a significantly higher frequency of an A allele and A/A genotype, followed by A/G and then G/G [36].

There are multiple possible reasons for the differences in allele and genotype frequencies seen between studies. For example, the findings of our current investigation contradict our previous study [23]. In the previous investigation, the 3′UTR was amplified with low stringent conditions (ex. Annealing temperature of 51 °C), increasing the likelihood for non-specific binding and an increased base pair error rate. In addition, several studies do not specify the details of placental collection, opening the possibility of maternal contamination. Samples must be carefully collected from the fetal side and washed thoroughly. Because of the more stringent methodology used in this study for tissue collection, it is also possible that maternal contamination was a factor in the differences we observed in the current study, compared to our previous study. Differences in SNP rates observed between studies may also be a result of small population bias or multiple comparisons of the statistical test, giving rise to the possibility that any associations observed are random. Although this was accounted for using Bonferonni's correction in our study, there is still a possibility of spurious results. Lastly, preeclampsia is a multi-factorial disease, and recent investigations have identified subpopulations of preeclamptic individuals based on large cohort microarrays [40,49]. If there are subgroups within the preeclamptic population, then there may be mechanisms that lead to or are indicative of a disease state, other than through HLA-G.

Aside from +3187A/G, other SNP genotype frequencies, like the 14bp insertion/deletion, were not different between controls and cases. An association between the 14bp sequence and preeclampsia has been inconsistent and thus, inconclusive [20–22,28–31,41]. The polymorphic nature of the 14bp sequence is primarily seen in HLA-G sequences compared to other HLA class I genes, suggesting that there may be a biological reason for this variability in the 3′UTR of HLA-G [42]. Therefore, most HLA-G studies that examined genetic mutations in the 3′UTR of exon 8 have focused on the 14bp polymorphism. Given that the deleted 14bp sequence appears in such a high frequency in the general population, there may be a selection pressure on the deleted sequence. The 14bp insertion/deletion site is in close proximity to an AU-rich element (AUUUG), and is predicted to affect HLA-G mRNA splicing, leading to more stable transcripts and expression levels [24,34,43,44]. Decreased HLA-G expression could be mediated by the presence of miRNAs that bind to the 14bp insertion sequence [45], leading to reduced birth weight and placenta weight [46]. Aside from the 14bp polymorphism, the presence of a guanine at +3142C/G influences the expression of HLA-G in asthmatic individuals [35]. A guanine at this SNP increases the affinity for miR148a, miR148b, and miR-152 to bind HLA-G mRNA, thereby decreasing mRNA levels and increasing mRNA degradation and translation suppression [35]. However, our study did not find an association between +3142C/G and preeclampsia.

Several of the SNPs analyzed in this report have already been investigated by other groups [29]. However, four of these sites (+3003C/T, +3027A/C, +3035C/T, and +3227G/A) have

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not been previously examined for an association with preeclampsia. These four SNPs encompass a region of only 32 nucleotides, and mutations in these SNPs could influence the binding of several miRNAs. When analyzing this expanded repertoire of SNPs, we were able to demonstrate that the frequency of a G/G genotype at +3187A/G in conjunction with a C/C genotype at +3027A/C was significantly higher in placental tissue samples from patients with preeclampsia than our control group (Table 7). Conversely, it could be argued that given the moderate sample size in this study, a SNP-by-SNP interaction model may not be reliable, as there may not be enough counts within each genotype and interactions may be a result of chance findings. Regardless, the finding that a combination of SNPs may further increase the susceptibility to preeclampsia is not surprising, as there are numerous examples of SNP combinations that have been reported to be associated with complex diseases [47,48]. Interestingly, SNP +3027A/C is not located adjacent to the AU-motif. Thus, this SNP may be affecting HLA-G expression by a different mechanism than through the AU-motif, or it may interact somehow with the +3187A/G SNP site.

Other potential SNP combinations include the 14bp insertion sequence with other polymorphisms (Table 1). In some cases, it is noteworthy that the 14bp insertion was often accompanied by the +3142G and +3187A SNPs in preeclampsia cases [36]. Larsen et al. found that the control group was more often represented by a homozygous 14bp deletion with an A-allele at +3187A/G [29]. In contrast to the control group, the preeclampsia group was overrepresented by a genotype combination of a homozygous 14bp deletion with a +3187 genotype of G/G. However, the association between −14bp/−14bp and G/G at +3187 did not reach statistical significance. Although we did not find a correlation between the 14bp insertion and other polymorphisms, this does not negate the possibility that the 14bp polymorphism may be interacting with other SNPs to affect HLA-G mRNA levels.

SNPs and SNP–SNP interactions have the potential to act as a target site for miRNAs, which are small non-coding RNAs, approximately 22 nucleotides long. miRNAs negatively regulate gene expression by inducing cleavage of mRNA or cause translation repression with resultant effects on post-transcriptional gene expression. Notably, it has been previously shown that there are a number of miRNA that bind +3187A/G, +3003T/C, +3142G/C allele with both high and low affinity [35,45]. Therefore, it is possible that binding of miRNA species to sequences that contain these SNPs results in the reduction of placental HLA-G mRNA and protein levels that is observed with this disease. We are currently investigating this possibility.

In summary, the HLA-G 3′UTR contains an AU-rich motif, poly-A signal, and several polymorphic sites that may potentially influence HLA-G transcription, translation, or both, by several different mechanisms. Among them, we found that the G/G genotype at +3187A/G is more prevalent in preeclampsia cases. The SNP combination of +3027 (C/C) and +3187 (G/G) was highly correlated with preeclampsia, as compared to controls. This combination is a promising biomarker to investigate further.

Supplementary Material

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Abbreviations

HLA-G human leukocyte antigen-G

SNP single nucleotide polymorphism

UTR untranslated region

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.humimm.2014.10.009.

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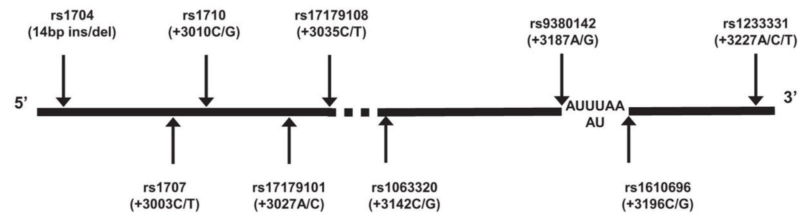

Fig. 1.
Schematic of the gene regulatory elements and polymorphisms that were analyzed in the 3′UTR of HLA-G. The 3′UTR of HLA-G consists of 8 SNPs and one 14bp insertion/deletion polymorphism.

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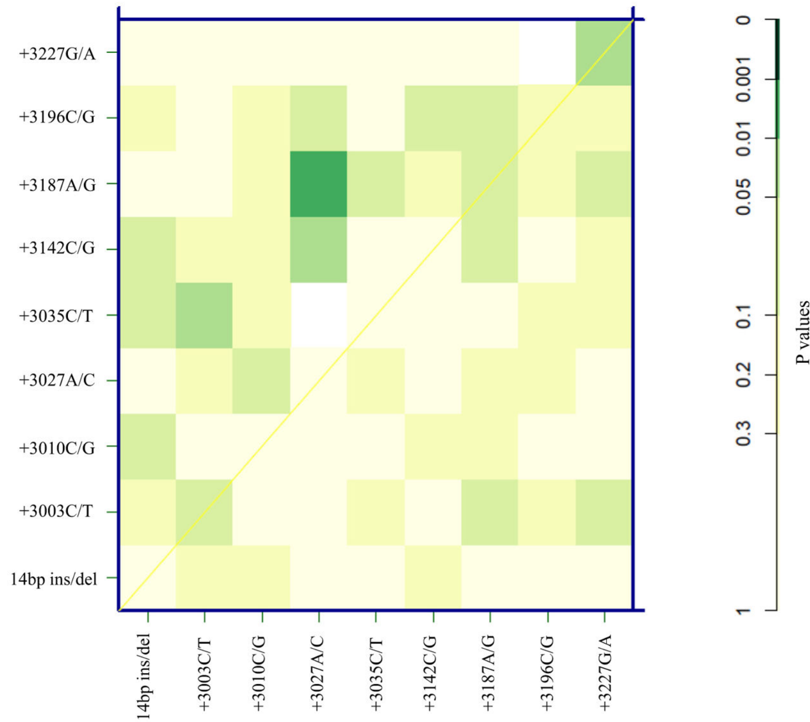

Fig. 2.

A plot of the SNP-by-SNP interactions showing the *p*-values using a likelihood ratio test for a co-dominant model. Different colored cubes are indicative of different statistical significant levels. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Table 1

Literature summary of SNPs found in the 3′UTR of HLA-G in preeclampsia cases.

SNP	Bases	Citation	Outcome
rs1704 (+2959)	14bp	[30]	Comparison of the 14-bp allele between PE and controls were not significantly different
		[28]	The 14-bp insertion allele and homozygous genotype were found at a statistically higher frequency in preeclampsia cases and the respective fathers. There was a trend towards paternal transmission of the +14-bp allele from heterozygote parents to preeclampsia children, while the opposite was true for controls
		[50]	The presence of the 14-bp sequence was prominent in PE compared to controls, and homozygotes with deletion were not detected in the pathology
		[21]	PE cases were neither associated with the HLA-G 14-bp genotype, nor with altered decidual HLA-G 14-bp gene expression
		[29]	Overrepresentation of the +14/+14-bp HLA-G genotype in the PE primipara cases
		[22]	Over representation of the +14/+14-bp HLA-G genotype in PE cases
rs1710 (+3010)	C/G	[29]	There was no statistically significant differences in genotype distributions between PE cases and controls
rs1063320 (+3142)	C/G	[29]	Overrepresentation of G/G homozygous genotypes was found in the PE group compared to controls
rs9380142 (+3187)	A/G	[29]	There were no differences in genotype distribution between PE cases and controls In primiparas, there was a statistically significant difference between two haplotypes DelGCAC and DelGCGC, where only rs9380142 differs and the other polymorphisms are fixed. The G allele was found at a higher frequency (35.5%) compared haplotypes with the A allele (6.3%) in preeclampsia cases. The A allele was more prominent in the control group (26.4%) Genotype combinations of the 14bp sequence and rs9380142 were significantly different between PE and controls. Whether the A or G allele in rs9380142 was most prominent in cases or in controls depended on the 14bp polymorphism –14bp/–14bp (del/del) and the rs9380142 genotype A/A or A/G was overrepresented in the control group –14bp/–14bp and the genotype G/G was overrepresented the PE group +14bp/–14bp (ins/del) combined with an A/A genotype was associated with the control group +14bp/+14bp combined with A/A was associated with PE cases
		[23]	The frequency of the A allele was found to be significantly higher in the PE group compared to controls Although the frequency of the A allele in the severe PE group was 0.86 (12/14), and 0.63 (9.5/15) in the mild PE group, but there was no statistical significance was found between these two groups The homozygous A/A genotype was found in 12/14 of severe PE placentas, while only 4/15 were found among those with mild disease
rs1610696 (+3196)	C/G	[29]	No statistically significant differences in genotype distributions between PE cases and controls
rs1707 (+3003)	C/T		N/A
rs17179101 (+3027)	A/C		N/A
rs17179108 (+3035)	C/T		N/A
rs1233331 (+3227)	G/A		N/A

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Table 2

Clinical characteristics of control cases and women diagnosed with preeclampsia.

Characteristics	Preeclampsia cases ($n = 47$)	Control cases ($n = 68$)
Age (years)	32.4 ± 6.6	32.8 ± 3.9
Gravidity	2.0 ± 1.2	1.8 ± 0.9
Primiparas	30/47 (64%)	35/68 (51%)
Mean gestational age (weeks)***	33.0 ± 3.4 (range 26.9–39.3)	39.3 ± 0.9 (range 37.3–41.1)
Mean birth weight (grams)***	1706 ± 395 (range 580–3460)	3410 ± 463 (range 2760–4760)
Disease severity¥	Mild – 25.5% Medium – 53.2% Severe – 21.3%	n/a

*** $p < 0.001$.

¥ Mild = systolic BP between 150 and 160 mmHg and a diastolic BP between 100 and 110 mmHg and proteinuria from +1 to +4; Medium = systolic BP of >160 mmHg and diastolic BP > 90 mmHg and proteinuria from +1 to +4; Severe = systolic BP of >160 mmHg and diastolic BP > 110 mmHg and proteinuria of +4.

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Table 3

Allele frequencies of polymorphisms.

	Control		Preeclampsia		
	n	%	*n*	%	
14bp ins/del					$p = 0.08$
del	60	44.12	53	56.38	
ins	76	55.88	41	43.62	
+3003C/T					$p = 0.39$
C	17	12.50	8	8.51	
T	119	87.50	86	91.49	
+3010C/G					$p = 0.33$
C	90	66.18	56	59.57	
G	46	33.82	38	40.43	
+3027A/C					$p = 1.00$
A	12	8.82	9	9.57	
C	124	91.18	85	90.43	
+3035C/T					$p = 0.37$
C	110	80.88	81	86.17	
T	26	19.12	13	13.83	
+3142C/G					$p = 0.49$
C	89	65.44	57	60.64	
G	47	34.56	37	39.36	
+3187A/G*					$p = 0.03$
A	114	83.82	67	71.28	
G	22	16.18	27	28.72	
+3196C/G					$p = 0.26$
C	51	37.50	28	29.79	
G	85	62.50	66	70.21	
+3227G/A					$p = 0.08$
C	131	96.32	94	100.00	
T	5	3.68	0	0.00	

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* $p < 0.05$.

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Table 4

Genotype frequencies of polymorphisms.

		Control		Preeclampsia		
		n	%	*n*	%	
14bp ins/del	del/del	15	22.06	15	31.91	$p = 0.19$
	del/ins	30	44.12	23	48.94	
	ins/ins	23	33.82	9	19.15	
+3003C/T	C/C	1	1.47	0	0.0	$p = 0.78$
	C/T	15	22.06	8	17.02	
	T/T	52	76.47	39	82.98	
+3010C/G	C/C	31	45.59	18	38.3	$p = 0.60$
	C/G	28	41.18	20	42.55	
	G/G	9	13.24	9	19.15	
+3027A/C	A/A	0	0.0	0	0.0	$p = 1.00$
	A/C	12	17.65	9	19.15	
	C/C	56	82.35	38	80.85	
+3035C/T	C/C	43	63.24	34	72.34	$p = 0.59$
	C/T	24	35.29	13	27.66	
	T/T	1	1.47	0	0.0	
+3142C/G	C/C	30	44.12	18	38.30	$p = 0.73$
	C/G	29	42.65	21	44.68	
	G/G	9	13.24	8	17.02	
+3187A/G	A/A	47	69.12	26	55.32	$p = 0.14$
	A/G	20	29.41	15	31.91	
	G/G	1	1.47	6	12.77	
+3196C/G	C/C	13	19.12	4	8.51	$p = 0.31$
	C/G	25	36.76	20	42.55	
	G/G	30	44.12	23	48.94	
+3227G/A	C/C	63	92.65	47	100.0	$p = 0.08$
	C/T	5	7.35	0	0.0	
	T/T	0	0.0	0	0.0	

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Table 5

Association of SNP +3187A/G with preeclampsia.

Model	C	Percent	*P*	Percent	OR	*p* value	AIC
Co-dominant*							
A/A	47	69.10	26	55.30	1.00	0.03	154.60
A/G	20	29.40	15	31.90	1.36		
G/G	1	1.50	6	12.80	10.85		
Dominant							
A/A	47	69.10	26	55.30	1.00	0.13	157.30
A/G-G/G	21	30.90	21	44.70	1.81		
Recessive**							
A/A-A/G	67	98.50	41	87.20	1.00	0.009	153.10
G/G	1	1.50	6	12.80	9.80		
Overdominant							
A/A-G/G	48	70.60	32	68.10	1.00	0.77	159.50
A/G	20	29.40	15	31.90	1.12		
Log-additive*							
0, 1, 2	68	59.10	47	50.00	1.99	0.03	154.80

* $p < 0.05$.

** $p < 0.01$.

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Table 6

p-value for SNP-by-SNP interaction based on an co-dominant model.

Pair	*p* value
+3010C/G–+3035C/T	0.04593 *
+3027A/C–+3142C/G	0.01607 *
+3027A/C–+3187A/G	0.00021 ***

* $p < 0.05$.

*** $p < 0.001$.

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Table 7

Estimated odds ratio (OR) for +3187A/G with respect to the non-variant (+3027A/C) genotype using a co-dominant model.

	C/C, control	C/C, PE	OR	A/C, control	A/C, PE	OR	A/A, control	A/A, PE
A/A	35	23	1	12	3	0.38	0	0
A/G	20	9	0.68	0	6	0	0	0
G/G	1	6	9.13	0	0	0	0	0