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Maternal human leukocyte antigen-G (HLA-G) genetic variants associate with *in utero* mother-to-child transmission of HIV-1 in Black South Africans

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Abstract

A 14-bp insertion/deletion (indel) within the 3' untranslated region (3'UTR) that affects *HLA-G* expression has been associated with HIV-1 mother-to-child transmission (MTCT). However, other 3'UTR single nucleotide polymorphisms (SNPs) that influence *HLA-G* mRNA stability have been described but not analyzed in the context of MTCT, and little is known about the role of *HLA-G* alleles. We examined *HLA-G* alleles and 3'UTR SNPs, including the 14-bp indel, in 216 mother-infant pairs from Johannesburg, South Africa. Mother-infant pairs were classified as HIV-1 non-transmitting (NT, n=144) or HIV-1 transmitting (TR, n=72) with either intrapartum (IP, n=29) or *in utero* (IU, n=19) infected infants. We found *HLA-G* allele, *G*01:01:02* (in strong linkage disequilibrium with the 14-bp insertion) and +3187G SNP were significantly over-represented in IU-TR mothers compared to NT mothers ($P=0.036$, OR=2.26; $P=0.011$, OR=2.96, respectively). These findings suggest that maternal *HLA-G* alleles and/or SNPs that might alter expression of *HLA-G* potentially influence IU HIV-1 MTCT.

Keywords

Mother-to-child transmission (MTCT); HIV-1; *HLA-G* alleles; *HLA-G* 3'UTR haplotypes

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Conflict of interest

The authors declare that there are no conflicts of interest.

1. Introduction

Prior to current prevention strategies, mother-to-child transmission (MTCT) or vertical transmission of HIV-1 occurred at an estimated rate of more than 30%, and still is the major cause of HIV/AIDS in children (Taha, 2011, da Silva et al., 2013). MTCT can occur during pregnancy (*in utero*, IU), at the time of delivery (intrapartum, IP), or postpartum (PP) through breast feeding (Duri et al., 2010, Kourtis et al., 2001). While the use of antiretroviral therapy (ART) during pregnancy has been shown to reduce the risk of MTCT, in the absence of ART, large proportions of infants remain HIV-1 uninfected and appear to have “natural protection”. Thus, studying the mechanisms of natural protection in HIV-1 exposed but uninfected (EU) infants may help to determine correlates of protection in both infants and adults. Several studies have suggested that host genetic factors, such as human leukocyte antigen (HLA) class I and II alleles (Matt and Roger, 2001, Kuhn et al., 2004, MacDonald et al., 1998, Polycarpou et al., 2002) and killer immunoglobulin-like receptors (KIR) (Hong et al., 2013, Paximadis et al., 2011) contribute to MTCT. Recently, there has been increased interest in the role of *HLA-G* in MTCT, because of its preferential expression at the maternal-foetal interface and its immunosuppressive properties. HLA-G can inhibit differentiation, proliferation, cytolysis, cytokine secretion and immunoglobulin production upon binding to their specific inhibitory receptors: immunoglobulin-like transcript (ILT)-2, ILT-4 and KIR2DL4 expressed by many immune cells, such as B and T lymphocytes as well as natural killer (NK) cells (Amiot et al., 2014).

Unlike the classical class I HLA molecules, the non-classical HLA-G molecule has limited allelic polymorphism in the coding region, with only 50 *HLA-G* alleles having been described to date (IMGT/HLA, version 3.16.0, 2014/04/14) (Robinson et al., 2013). These alleles encode 16 distinct transmembrane proteins (G*01:01, G*01:02, G*01:03, G*01:04, G*01:06, G*01:07, G*01:08, G*01:09, G*01:10, G*01:11, G*01:12, G*01:14, G*01:15, G*01:16, G*01:17, G*01:18) and two truncated proteins (G*01:05N and G*01:13N) (Robinson et al., 2013). Alternative splicing of the *HLA-G* primary transcript can generate seven alternative mRNAs that encode membrane-bound (HLA-G1, -G2, -G3, and -G4) and soluble (HLA-G5, -G6, and -G7) protein isoforms (Donadi et al., 2011). HLA-G1 can also be proteolytically cleaved from the membrane and released as soluble HLA-G1 (sHLA-G) allowing it to have systemic immunoregulatory effects in the absence of localized tissue expression (Solier et al., 2002).

In contrast to the coding region, the 3' untranslated region (UTR) of *HLA-G* (which exhibits several regulatory elements, including AU-rich motifs, a poly-A signal, as well as signals that regulate the spatial and temporal expression of an mRNA) presents a high degree of variation (Lynge Nilsson et al., 2014, Donadi et al., 2011). Amongst these 3' UTR polymorphisms (Figure 1), a 14-bp indel (rs66554220) has been the most studied and has been associated with the magnitude of HLA-G production, with modulation of HLA-G mRNA stability, and with binding for specific microRNAs (Castelli et al., 2014). In general, the presence of a 14-bp insertion (*Ins*) (5'-ATTTGTTTCATGCCT-3') introduces an alternative splicing site that generates a 92-bp deletion in the 3' UTR that influences mRNA stability; and individuals with *Ins/Ins* genotype have been associated with lower mRNA production compared to *Ins/Del* and *Del/Del* genotypes (Hviid et al., 2003, Castelli et al.,

2010, Castelli et al., 2009). Furthermore, other 3'UTR SNPs have also been implicated in the regulation of HLA-G expression. For example, a SNP at position +3142C/G (rs1063320), the presence of a Guanine (G) increases the affinity of this region for microRNAs (miR-148a, miR-148b and miR-152), thereby decreasing HLA-G expression by mRNA degradation and translational suppression (Castelli et al., 2014, Tan et al., 2007, Veit and Chies, 2009). While polymorphisms at positions +3187A/G (rs9380142) and +3196C/G (rs1610696), located near an AU-rich motif in the HLA-G mRNA, have also been associated with mRNA stability and degradation. It was demonstrated that an Adenine (A) at +3187 was associated with decreased HLA-G expression due to the increased number of Adenines in this AU-rich motif (Yie et al., 2008). In addition, polymorphisms within the 3'UTR seem to be arranged in several haplotypes, each of them associated either with a single or a group of coding and/or promoter region polymorphisms (Alvarez et al., 2009, Castelli et al., 2010, Donadi et al., 2011).

In the context MTCT of HIV-1, several reports have associated the 14-bp indel with the risk of MTCT (Segat et al., 2014, Sanches et al., 2013, Segat and Crovella, 2012, Segat et al., 2009, Fabris et al., 2009, Aikhionbare et al., 2006); but only one has associated *HLA-G* alleles with MTCT (Luo et al., 2013). However, some of these reports have been conflicting, for example: in Zambian infants, the *Ins* was associated with protection from IU and IP HIV-1 infection (Segat et al., 2014); yet in Brazilian children, the *Del* allele and *Del/Del* genotype associated with a protective effect from vertical transmission (Fabris et al., 2009). Whilst, in another study, HIV-1 transmitting mothers were found to have almost four times more placental sHLA-G compared to non-transmitting mothers (Moodley and Bobat, 2011). Similarly, while differing in the mode of HIV-1 transmission, higher levels of sHLA-G in the female genital tract were independently associated with both HIV-1 infection and bacterial vaginosis in sex workers from Benin (Thibodeau et al., 2011). Moreover *G*01:03*-alleles, which reportedly translate into lower plasma sHLA-G, were associated with a reduced risk of vertical transmission of HIV-1 (Luo et al., 2013). Whereas, in the context of sexual transmission, *G*01:04:04*, an allele with reportedly higher levels of plasma sHLA-G, was associated with susceptibility to HIV-1 infection (Turk et al., 2013). Of note, amongst all these studies, few have assessed the roles of *HLA-G* alleles and 3'UTR SNPs collectively.

In this study, we investigated the role of *HLA-G* in MTCT of HIV-1 in a Black South African cohort of 216 mother-infant pairs, particularly focusing on the coding region representing extracellular $\alpha 1$, $\alpha 2$ and $\alpha 3$ domains, as well as the 3'UTR, since polymorphisms within these regions might influence peptide binding as well as HLA-G expression, respectively. We found maternal possession of the *G*01:01:02* allele, as well as the G allele at position +3187 of the 3'UTR (indicative of the UTR1 haplotype), independently increased the risk for IU transmission. Overall, these findings suggest that polymorphisms that alter HLA-G expression are likely to influence MTCT of HIV-1 via the IU route.

2. Materials and Methods

2.1. Study population

This study is a nested case-control analysis of data collected prospectively on 216 Black mother-infant pairs recruited as part of four mother-to-infant HIV-1 transmission studies in Johannesburg, South Africa, that took place from 1996 to 2005. The studies aimed to identify immunogenetic correlates of HIV-1 transmission and have been described previously (Kuhn et al., 2007).

All available HIV-1 transmitting mother-infant samples (TR, n=72) from the four transmission studies were selected as “cases” for the nested analysis. As controls, two HIV-1 non-transmitting mother-infant samples (NT, n=144) for each case were randomly selected from each of the four transmission studies giving rise to the cases. HIV-1 infected infants were further characterized according to timing of transmission as determined by an HIV-1 DNA PCR test (Roche Amplicor version 1.5) at birth and at 6 weeks of age. In total 19 were *in utero* (IU) infected (PCR positive at birth and at 6 weeks), 29 were intrapartum (IP) infected (PCR negative at birth but positive at 6 weeks), and the remaining 24 infants were found to be positive at 6 weeks but had no birth sample available (unknown whether IU or IP).

For analyses confined to mother-infant pairs who received maternal sdNVP, in addition to analysing associations by known timing of transmission IU or IP as described above, we also analysed associations for an IU-enriched group called IU2. In IU2 we combined the 19 known IU-infected infants with the 24 infected infants of unknown transmission on the basis that 79% (19/24) of these mothers received sdNVP (Table 1). The rationale for this is that maternal sdNVP administration given only at the onset of labour is known to have little to no effect on reducing IU infection. Thus we can infer that when infection does occur in an sdNVP-exposed infant, there is a greater likelihood that it is due to IU infection than due to IP infection. We confirmed this in our data. Among infected infants with a known timing of infection (Table 1), significantly fewer acquired infection during the IP period when born to mothers that received sdNVP compared to ART-naive mothers (10/29, 34% vs. 19/29, 65%, respectively, $P=0.035$).

Maternal viral load (mVL) determinations were performed on samples collected at the time of delivery using the Roche Amplicor HIV-1 RNA Monitor assay version 1.5 (Roche Diagnostic Systems, Inc., Branchburg, NJ) and the CD4+ T cell counts (cells/ μ L) were quantified using the commercially available FACSCount System from Becton Dickinson (San Jose, CA).

This study was approved by the University of the Witwatersrand Committee for Research on Human Subjects and the Institutional Review Board of Columbia University. Written informed consent was obtained from all women in this study.

2.2. HLA-G genotyping

Genomic DNA was extracted from whole blood using a QIAamp DNA Blood Mini Kit (Qiagen, Dusseldorf, Germany) following manufacturer’s instructions. Exons 2, 3 and 4 of

HLA-G were amplified and sequenced using previously published primers (Turk et al., 2013, Hviid et al., 1997). Briefly, a 994-bp fragment encompassing exons 2 and 3 was amplified using primers HLAG_2/3PCRF (5'-CGGCCCTGCGCGGAGGAGGGAGGGG-3') and HLAG_2/3PCRR (5'-TCAGGACCAGAGGGAGGGCGATATTC-3'); primers HLAG_4PCRF (5'-AGGTGCTG-CTGGAGTGTC-3') and HLAG_4PCRR (5'-TCTGGGAAAGGAGGTGAAG-3') were used to amplify a 463-bp fragment spanning exon 4 (Turk et al., 2013). Thermocycling conditions were: 94°C for 2 min followed by 35 cycles of 94°C for 30 sec, 61.5°C/60°C for 30 sec (for exons 2-3 and exon 4, respectively) and 72°C for 1 min followed by a final extension step of 72°C for 7 min. PCR amplicons were purified using the Invitex MSB Spin PCRapace cleanup kit (Berlin, Germany) and sequenced in both directions by capillary electrophoresis using an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA) with the sequencing primers described by Turk et al. (2013): HLAG_2SEQR (5'-TCGTGATCTGCGCCCTG-3'); HLAG_3SEQF (5'-TGGGCGGGGCTGACCGAGGGGGTGGG-3'); HLAG_3SEQR (5'-TCAGGACCAGAG-GGAGGGCGATATTC-3'); HLAG_4SEQF (5'-GTGCTTGAATT-TTCTGACTCTT-3') and HLAG_4SEQR (5'-TGCTTTCCTAACAGACATGAT-3'). HLAG_2SEQF (5'-CTCCATGAGGTATTTACAG[G]-3') was an in-house primer designed using *HLA-G* intron 1 alignments and synthesised with a lock nucleic acid (LNA) modified 3' end (indicated in square brackets). Sequence analysis and allele assignment were performed using Assign™ SBT version 4.7 software (Conexio Genomics, Fremantle, Western Australia) with the IMGT/HLA-G 2013 reference library compiled and supplied by Conexio Genomics (Fremantle, Western Australia) on personal request.

2.3 *HLA-G* 3'UTR genotyping

Nucleotide sequence variation of the *HLA-G* 3'UTR was evaluated by direct sequencing of a 343-bp fragment encompassing the genomic positions +2885 through to +3228, using PCR primers described by (Sizzano et al., 2012). Briefly, the 3'UTR region was amplified using HLAG_3UTRF (5'-TCACCCCTCACTGTGACTGA-3') and HLAG_3UTRR (5'-CCCATCAA-TCTCTCTTGAAA-3') primers with the following thermocycling conditions: 95°C for 15 min followed by 30 cycles of 93°C for 1 min, 58°C for 1 min, 72°C for 1 min. A final extension step was carried out at 72°C for 10 min. Purified amplicons using the Invitex MSB Spin PCRapace cleanup kit (Berlin, Germany) were sequenced in both directions by capillary electrophoresis using an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA) using the same PCR primers. The chromatograms obtained were analysed using Sequencher version 4.10.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and sequences were aligned with an available *HLA-G* 3'UTR reference sequence (GenBank Accession number NG_029039.1) to identify known polymorphic positions (Castelli et al., 2010) and any other polymorphism that had not been previously described.

2.4 *HLA-G* 3'UTR haplotypes

Arrangement of variations in the *HLA-G* 3'UTR into haplotypes was predicted both by visual examination of the genotypic data as well as using the Bayesian algorithm through the HAPLOTYPER software (Niu et al., 2002). Observed haplotypes were compared to published 3'UTR haplotypes and assigned the published nomenclature when the haplotypes

corresponded in sequence/structure (Sabbagh et al., 2014). The frequencies of haplotypes were calculated by counting the number of alleles harbouring the haplotypes and dividing by the total number of alleles. Counting of the haplotypes was irrespective of the presence of additional SNPs not forming part of the haplotypes in question.

2.5 Classification of mother-infant *HLA-G* concordance

To assess the effect of concordance on MTCT of HIV-1, we directly counted the number of matching mother-infant pairs following the method reported by MacDonald et al. (1998). Since a child will inherit one allele from the mother and one from the father, it will match at least 50% of the mother's *HLA-G* alleles, if the two alleles of the child matched two alleles of the mother, they were considered concordant. If the mother was homozygous at *HLA-G* and matched one of the child's *HLA-G* alleles, she was also considered to be concordant since the child would not recognise any foreign antigens. At the 3'UTR, concordance was considered when both mother and infant had the same polymorphism and/or UTR haplotype.

2.6. Computational and statistical analysis

Allele frequencies at all polymorphic positions were determined by direct counting. Deviations from Hardy-Weinberg Equilibrium (HWE) were assessed using the conventional Monte Carlo exact test (Guo and Thompson, 1992) using the computer program TFPGA (Tools for Population Genetic Analyses version 1.3; 1997: author Mark. P. Miller). Linkage disequilibrium (LD) between *HLA-G* alleles and the polymorphic sites within the 3'UTR were evaluated using the likelihood ratio test as described by (Lewontin, 1964). The statistical significance of the LD between each of the SNP pairs was evaluated by the approximate chi-square described by (Liau et al., 1984). For associations of MTCT risk, comparisons of *HLA-G* allele and genotype frequencies were analyzed between NT and TR maternal groups, as well as EU and HIV-1 infected infants using the online Fisher exact test, VassarStats (<http://www.vassarstats.net/odds2x2.html>) which was also used to estimate the odds ratio (OR) and its 95% confidence interval (95% CI). Reported *P*-values are two-tailed and were considered significant when $P < 0.05$. No adjustments were made for multiple comparisons, as adjustment for multiple comparisons correct for type 1 errors but increase the risk of type 2 errors. Given the complexity and multifactorial nature of maternal-infant HIV-1 transmission, we considered it more important to identify potential factors that may play a role in this route of infection rather than dismissing these leads as due to chance variations brought about by multiple comparisons. Unconditional logistic regression (Pezzullo, 2005) was used to adjust for the effects of the following variables: maternal viral load (mVL), maternal sdNVP, as well as infant possession of *KIR2DS4-v* which we have recently reported to impact on IU MTCT in the same cohort (Hong et al., 2013).

3 Results

3.1 *HLA-G* allele representation in Black South Africans

A total of 216 Black South African mother-infant pairs were genotyped, which included 144 NT/EU pairs and 72 TR/IP, IU or IU2 pairs. The clinical characteristics are shown in Table 1. Sixteen *HLA-G* alleles were identified in the study population (Table 2). These encoded six functional proteins (G*01:01, G*01:03, G*01:04, G*01:06, G*01:08 and G*01:11) and

the truncated protein (G*01:05N). The three most common *HLA-G* alleles were *G*01:01:01*, *G*01:01:02* and *G*01:04:04* with frequencies greater than 15% in both mothers and infants, which corresponded with the most common genotypes *G*01:01:01/G*01:01:01*, *G*01:01:01/G*01:01:02* and *G*01:01:01/G*01:04:04* (Table 3). All *HLA-G* allele frequencies were all in Hardy–Weinberg equilibrium for both mother and infant groups ($P=0.120$ and $P=0.769$, respectively).

3.2 *HLA-G* alleles and mother-to-child transmission of HIV-1

To investigate the influence of *HLA-G* alleles and genotypes on MTCT of HIV-1 we compared HIV-1 non-transmitting mothers (NT) to HIV-1 transmitting mothers (TR) and their respective infants: exposed uninfected (EU), *in utero* infected (IU and IU2) and intrapartum infected (IP). Maternal possession of the *G*01:01:02* allele was associated with increased risk for IU HIV-1 transmission (Table 2). Representation of *G*01:01:02* was higher in IU-TR ($P=0.036$, OR=2.26) and IU2-TR ($P=0.078$, OR=1.68) mothers compared to NT mothers. While *G*01:01:02/G*01:01:02* genotype showed a strong trend ($P=0.072$, OR=4.31) towards increased representation in IU-TR mothers compared to NT mothers (Table 3). In addition, the combination of *G*01:01:02* with *G*01:03:01* (*G*01:01:02/G*01:03:01*) was significantly higher in IU2-TR mothers compared to NT mothers ($P=0.031$, OR=4.61). All of these allele associations were significant post adjustments for mVL but only *G*01:01:02* homozygosity maintained significance post adjustment for maternal sdNVP and infant *KIR2DS4-v* (Table 7). Infant *HLA-G* alleles (Table 2) and *HLA-G* genotypes (Table S1) showed no significant associations with regards to acquisition of HIV-1.

3.3 *HLA-G* 3'UTR allele, genotype and haplotype frequencies in MTCT of HIV-1

Sequencing analysis of *HLA-G* 3'UTR revealed the presence of 11 polymorphic sites in our cohort. Nine of these have been previously described, namely: +2961Ins/Del (rs66554220), +3003T/C (rs1707), +3010C/G (rs1710), +3027C/A (rs17179101), +3032G/C (rs146339774), +3035C/T (rs17179108), +3142G/C (rs1063320), +3187A/G (rs9380142), and +3196C/G (rs1610696). Two new SNPs, +3076C/T and +3114G/A, were found in EU infants, but had frequencies below 1% and were not considered in further analyses. Observed genotype frequencies at all variation sites were in adherence with HWE. Using HAPLOTYPER software and in combination with published 3'UTR haplotypes (Sabbagh et al., 2014), we found eight distinct 3'UTR haplotypes (UTR1-UTR7 and UTR20) within our cohort (Figure 1). UTR1, UTR2 and UTR3 were the most frequent haplotypes, which together accounted for over 65% of the haplotypes in our South African study population, which was most comparable to the Senegalese population (SER, n=239) reported by Sabbagh et al. (2014).

When comparing the nine polymorphic sites within the 3'UTR with risk of MTCT, only one position (+3187A/G) showed an association with increased risk for IU MTCT (Table 4). Maternal frequencies of the *G* allele as well as *G/G* homozygosity at position +3187 were significantly over-represented in IU-TR mothers compared to NT mothers ($P=0.011$, OR=2.96 and $P=0.011$, OR=14.4, respectively). However, only *G/G* homozygosity maintained significance post adjustment for mVL ($P=0.009$, OR=29.6), but the confidence

interval (CI) was considerably large (2.36-371.0), most likely due to the small number (Table 7). Comparison of haplotype associations in MTCT however confirmed the UTR1 haplotype (the only haplotype to have a G at position +3187), as well as the UTR1/UTR1 genotype (Table 5), were both significantly over-represented in IU-TR mothers compared to NT mothers ($P=0.011$, OR=2.96 and $P=0.012$, OR=13.3, respectively). The genotype UTR1/UTR2 also showed a strong trend towards increased representation in IU2-TR mothers compared to NT mothers ($P=0.052$, OR=3.66). Both UTR1/UTR1 and UTR1/UTR2 genotypes maintained significance after adjustment for mVL ($P=0.009$, OR=29.6 and $P=0.019$, OR=5.44, respectively) but not after adjustment for maternal sdNVP or *KIR2DS4-v* (Table 7). Taken together, a Guanine at +3187 and/or the UTR1 haplotype, showed a significant association with increased risk for IU transmission. In contrast, IP-TR mothers had a significantly higher representation of the UTR3/UTR4 genotype compared to NT mothers ($P=0.034$, OR=8.19), but this association was not significant post adjustment for mVL or maternal sdNVP (Table 7). Comparison between genotypes UTR1/UTR1 (associated with IU transmission) and UTR3/UTR4 (associated with IP transmission) showed differences at four positions. The UTR3/UTR4 combination is heterozygous at positions +3003T/C, +3010C/G, +3142G/C, and has an Adenine at position +3187; while UTR1/UTR1 is homozygous at positions +3003T, +3010G, +3142C, and has a Guanine at position +3187.

Comparing the 3'UTR polymorphisms, as well as the haplotypes, in the infant group revealed no significant associations or trends (data not shown); suggesting that in our cohort, polymorphisms within 3'UTR did not play a role in infant acquisition of HIV-1.

3.4 Linkage disequilibrium (LD) between *HLA-G* alleles and the 3'UTR

In order to further investigate the relationships between *HLA-G* alleles and the variants within the 3'UTR, pairwise LD between the *HLA-G* alleles and the 14-bp indel, as well as the other SNPs and haplotypes was calculated. Table 6 shows LD results only for significant pairwise combinations. Seven of the *HLA-G* alleles were in strong LD with the 14-bp indel: *G*01:01:01*, *G*01:04:01* and *G*01:04:04* were significantly linked with the *Del*; while, *G*01:01:02*, *G*01:03:01*, *G*01:05N* and *G*01:01:19* were significantly linked with the *Ins*. As expected, comparison of *G*01:01:01*, *G*01:04:01* and *G*01:04:04* with the 3'UTR haplotypes containing the 14-bp *Del* showed *G*01:01:01* was in strong LD with UTR1, UTR6 and UTR20, while *G*01:04:04* and *G*01:04:04* were in strong LD with UTR3. Alleles associated with the *Ins*, *G*01:01:02*, *G*01:03:01*, *G*01:05N* and *G*01:01:19*, were found to be in significant LD with UTR2, UTR5 and UTR7.

Thus since the *G*01:01:02* allele and +3187G (UTR1) are not in LD ($D'=-0.12$; $X^2=0.09$, $P>0.1$), the effects of these two genotypic variations on IU transmission are independent of each other. Thus the comparison of IU-TR mothers vs. NT mothers having both *G*01:01:02* and +3187G (UTR1) did not show a significant additive association, likely due to the small number of individuals carrying both these variants (Table 7). However, comparison of IU-TR mothers with EU mothers and representation of *G*01:01:02* or +3187G (UTR1), was more significant ($P=0.014$; OR=4.51) than possession of *G*01:01:02* alone, but not more significant than having +3187G (UTR1) alone (Table 7). The significantly greater

representation of having either *G*01:01:02* or +3187G (UTR1) was the only association maintained throughout all adjustments (Table 7).

3.5 Mother-infant concordance and MTCT

HLA concordance amongst the classical class I molecules (HLA-A, -B and -C) (MacDonald et al., 1998), as well as a synonymous SNP in exon 2 of HLA-G (Aikhionbare et al., 2001), have been described as a risk factors for MTCT; thus, we wanted to determine if concordance for *HLA-G* allele and 3'UTR polymorphisms between mother and infant had a similar association. Out of the 216 mother-infant pairs, concordance for *HLA-G* alleles did not significantly differ between NT and TR mother-infant pairs (Table S2). Similarly, mother-infant concordance for the 14-bp *Ins/Ins*, *Ins/Del* or *Del/Del* and other 3' UTR haplotypes showed no significant associations. However, mother-infant concordance at SNP +3187A was found to be significantly lower in IU-TR/IU mother-infant pairs compared to NT/EU pairs (47% vs. 76%, $P=0.014$, OR=0.29).

4. Discussion

The role of HLA-G has become increasingly evident in many infectious diseases, primarily due to its inhibitory effect on the innate and adaptive immune system. By directly binding to the inhibitory receptors KIR2DL4, ILT-2 and ILT-4 that are present on NK and CD8+ T cells, HLA-G can also inhibit differentiation, proliferation, cytolysis, cytokine secretion, and immunoglobulin production (Amiot et al., 2014). Therefore, certain *HLA-G* alleles and/or 3'UTR polymorphisms that alter HLA-G expression may influence the function of NK and CD8+ T cells and ultimately influence the susceptibility to HIV-1 transmission. Several studies have found associations between certain *HLA-G* alleles, as well as a 14-bp indel, with an altered risk for vertical transmission of HIV-1 (Aikhionbare et al., 2006, Lajoie et al., 2009, Luo et al., 2013, Moodley and Bobat, 2011). However, these studies have largely focused on each of these parameters individually and, where studies have been comparable, the results have often not shown consensus. In addition, other SNPs within the 3'UTR have not been extensively analyzed for their clinical relevance in MTCT. In this study, we report on the combined analysis of *HLA-G* alleles as well as the 3'UTR of *HLA-G* in a HIV-1 MTCT cohort of 216 Black South African mother-infant pairs.

We found *G*01:01:01*, *G*01:01:02* and *G*01:04:04* were the most common alleles in our cohort, which corresponded with the most frequent genotypes *G*01:01:01/G*01:01:01*, *G*01:01:01/G*01:01:02*, and *G*01:01:01/G*01:01:04*. With regards to MTCT, maternal possession of *G*01:01:02* allele, a Guanine at the +3187A/G 3'UTR SNP and the UTR1 haplotype, were significantly associated with increased risk for IU transmission (Tables 2, 4 and 5). Furthermore, strong LD between *G*01:01:02* and the 14-bp *Ins*, as well as UTR1 with the 14-bp *Del*, suggested that differences in HLA-G expression altered the risk of MTCT.

Several studies have associated HLA-G with heterosexual transmission of HIV-1. In one study *G*01:01:01* was significantly enriched in HIV-1 resistant sex workers, whereas *G*01:04:04* was significantly associated with susceptibility to HIV-1 infection (Turk et al., 2013). However in Zimbabwean women, *G*01:01:08* was associated with an increased risk

of HIV-1 infection, while *G*01:05N* offered protection from heterosexual HIV-1 infection (Matte et al., 2004). In both these studies, *HLA-G* alleles that had higher HLA-G expression were associated with increased risk of HIV-1 transmission. It was further reported that individuals with *G*01:04* had higher sHLA-G levels than individuals with the more frequent *G*01:01:01*; whereas, *G*01:01:01* individuals had higher sHLA-G levels compared to individuals with *G*01:01:03* and *G*01:05N* (Rebmann et al., 2001). Similarly, the 3'UTR haplotypes have also been associated with differences in sHLA-G expression levels, for example, UTR1 was associated with higher expression of sHLA-G, whereas UTR5 and UTR7 with lower expression and other UTRs (UTR2, 3, 4 and 6) exhibited intermediate levels of sHLA-G (Martelli-Palomino et al., 2013). What is unique about the UTR1 haplotype (14-bp *Del*, +3003T, +3010G, +3027C, +3032G, +3035C, +3142C, +3187G, +3196C) is that it carries the majority of the polymorphisms that associate with increased expression of HLA-G, namely: the 14-bp *Del* that is associated with highly soluble HLA-G expression, Cytosine at +3142 that is less sensitive to specific miRNAs (miR-148a, miR-148b and miR-152) and Guanine at +3187 that increases mRNA stability (Castelli et al., 2014).

Therefore in our study, UTR1 and its strong LD with *G*01:01:01*, may share a similar mechanism to that of heterosexual HIV-1 transmission, through increased expression of HLA-G being a risk factor for IU transmission. It has been postulated that *HLA-G* alleles that have higher HLA-G expression would exert greater NK and CD8+ T cell inhibition, which could result in the decreased ability of these cells to target viral-infected cells and thus increasing the risk for HIV-1 transmission (da Silva et al., 2014, Luo et al., 2013, Matte et al., 2004, Moodley and Bobat, 2011). In agreement with this hypothesis, placental expression of HLA-G was 3.9 times more up-regulated in transmitting mothers compared to non-transmitting mothers (Moodley and Bobat, 2011).

However, while certain alleles and 3'UTR polymorphisms would imply either increased/decreased expression of HLA-G, this is not absolute. Le Discorde et al. (2005) reported that even the null allele, *G*01:05N*, had HLA-G protein expression. *G*01:05N* is characterized by a single cytosine deletion in exon 3 that presents a stop codon in exon 4 blocking the translation of HLA-G1 and HLA-G5 isoforms; it does however, encode both membrane-bound and soluble functional HLA-G proteins that are able to inhibit NK-cell cytolysis (Le Discorde et al., 2005). It is possible that even low expressing *HLA-G* alleles, such as *G*01:01:02* and its strong LD with the 14-bp *Ins*, might have other mechanisms influencing HLA-G expression. Indeed, a fraction of HLA-G mRNA transcripts presenting the 14-bp *Ins* can be further processed (alternatively spliced) by the removal of 92 bases from the mature HLA-G mRNA, which yields smaller HLA-G transcripts, reported to be more stable than the complete mRNA forms (Donadi et al., 2011, Rousseau et al., 2003).

Interestingly in another study, *G*01:01:02*, in the heterozygous and homozygous state, was associated with increased expression of genital sHLA-G in HIV-1-infected sex workers compared with those in both the HIV-1-uninfected sex workers ($P=0.051$) and non-sex workers ($P=0.002$) groups (Thibodeau et al., 2011). Additionally in a Canadian Human Papillomavirus (HPV) study, one of the most common sexually transmitted infections, both *G*01:01:02* and *G*01:03*-alleles (also in high LD with the 14-bp *Ins*) were associated with

increased risk for HPV-16 infection and persistent infections with HPV types from the alpha species (Ferguson et al., 2011). It is possible that polymorphisms within the HLA-G promoter region (5'URR, the upstream regulatory region) may influence HLA-G expression. For example, in a study of recurrent pregnancy loss, the tri-allelic polymorphism $-725C/G/T$ (rs1233334) within the 5'URR was evaluated in relation to plasma sHLA-G concentration, and the CC genotype was found to have significantly lower levels of sHLA-G than the CG and CT genotypes (Jassem et al., 2012). Additionally, it was suggested that the presence of Guanine in position -725 may alter the methylation profile of CpG dinucleotides resulting in a modification of gene expression, influencing the binding of regulatory factors as IRF-1 (Interferon response factor-1) to ISRE (interferon specific regulation element) (Costa et al., 2012). Indeed, $G*01:01:02$ alleles were reported in 5'URR haplotypes having the $-725C$ allele (Costa et al., 2012). It is also important to note that haplotype structures often differ between different ethnic groups and thus analysis of the 5'URR together with HLA-G alleles and the 3'UTR, and the respective haplotypes across this entire region with expression levels of HLA-G would be very informative in trying to determine the role of this molecule in HIV-1 MTCT.

Of note, while these two *HLA-G* variations [$G*01:01:02$ and $+3187G$ (UTR1)] independently associated with increased risk for IU transmission, the effect of having either $G*01:01:02$ or $+3187G$ (UTR1) was significantly over-represented in IU-TR mothers compared to NT mothers and was maintained throughout all adjustments. Interestingly, this association was stronger than the effect of $G*01:01:02$ alone, but weaker than that of UTR1 alone.

With regards to mother-infant concordance, we and others (Luo et al., 2013, Rousseau et al., 2003, Segat and Crovella, 2012) found no association for *HLA-G* alleles and the 14-bp indel with risk of vertical transmission. Only one study reported an association between *HLA-G* discordance at codon 57 in exon 2 in HIV-1 non-transmitting mother-infant pairs, but the sample size was small ($n=34$) and the study did not consider different routes of transmission (Aikhionbare et al., 2001). In keeping with this limitation, we acknowledge that in our study, we too may be biased in our analysis of the IU enriched group, IU2, as IU transmission is assumed based on maternal sdNVP treatment. Nevertheless, "true" IU infected infants were analysed on their own account and in most cases, associations within the IU enriched group (IU2) followed similar trends with the IU group.

In conclusion, we show that certain maternal *HLA-G* alleles ($G*01:01:02$) and 3'UTR polymorphisms ($+3187G$ and UTR1) associated with an increased risk for IU MTCT, and that this is likely through modulation of maternal HLA-G expression at the maternal-foetal interface. However, further investigations in larger cohorts are necessary to confirm these associations and future studies should collectively assess *HLA-G* alleles as well as the 5'URR and the 3'UTR, as polymorphisms in all three regions might impact HLA-G expression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- HLA-G is an immunosuppressive molecule expressed at the maternal-foetal interface
- We assessed both *HLA-G* alleles and the 3'UTR in HIV-1 mother-to child transmission
- Two maternal *HLA-G* variants independently increased *in utero* (IU) HIV-1 transmission
- IU mothers had increased *G*01:01:02* and a SNP within the UTR1 haplotype, +3187G
- Findings suggest that maternal expression of HLA-G can influence HIV-1 transmission

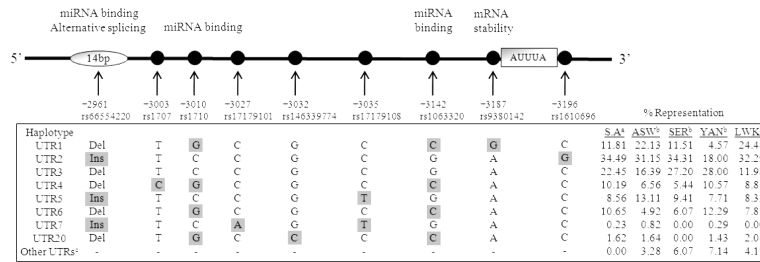


Figure 1. Polymorphisms in the 3' untranslated region (UTR) of the *HLA-G* gene and the 3'UTR haplotypes. Polymorphic positions are relative to the first ATG codon at exon 1. UTR haplotypes were numbered according to (Sabbagh et al., 2014).

Del, Deletion; Ins, insertion.

^a SA is represented by the total group of HIV-1 positive mothers in our MTCT cohort (n=216)

^b Represents 3'UTR frequencies reported by (Sabbagh et al., 2014) which pooled some data from the 1000 genome project. ASW, people of African ancestry from the south western United States (n=61); SER, Serer from Niakhar, Senegal (n=239); YAN, Yansi from Bandundu, Democratic Republic of the Congo (N=175); and LWK, Luhya from Webuye, Kenya (n=96).

^c Represents other 3' UTR haplotypes not identified in SA

Table 1

Clinical characteristics and antiretroviral (ARV) administration in HIV-1 positive mothers who did/did not transmit (TR/NT) HIV-1 to their infants

HIV+ mothers Median (IQR range)	NT (N=144)	TR (N=72)	IP-TR (N=29)	IU-TR (N=19)	Unknown-TR (N=24)
Age (years)	27 (22-30)	29 (24-31)	29 (22-31)	29 (22-31)	28 (25-30)
log ₁₀ VL (copies/ml)	4.0 (3.2-4.6)	4.8 (3.7-5.4)	4.8 (3.9-5.3)	4.9 (4.2-5.5)	4.6 (2.6-5.4)
CD4 (cells/ μ)	449 (319-681)	375 (253-575)	378 (262-508)	350 (182-647)	437 (301-590)
ARV administration N (%)	NT (N=144)	TR (N=72)	IP-TR (N=29)	IU-TR (N=19)	Unknown-TR (N=24)
None	60 (41.7%)	29 (40.3%)	19 (65.5%)	7 (36.8%)	3 (12.5%)
sdNVP	80 (55.5%)	41 (56.9%)	10 (34.5%)	12 (63.2%)	19 (79.2%)
Other ARV	4 (2.8%)	2 (2.8%)	0 (0%)	0 (0%)	2 (0%)

IQR: interquartile range; NT: HIV-1 nontransmitting mother; TR: total group of HIV-1 transmitting mother; IP-TR: intrapartum HIV-1 transmitting mother; IU-TR: *in utero* HIV-1 transmitting mother; Unknown-TR: group of HIV-1 transmitting mothers where the infants' mode of HIV-1 acquisition was unknown.

Table 2

Representation of *HLA-G* allele frequencies in HIV-1 positive mothers and their infants, N (%)

Maternal <i>HLA-G</i> Alleles	NT (2n=288)	IP-TR (2n=58)	IU-TR (2n=38)	IU2- TR (2n=86)	IP-TR vs. NT		IU-TR vs. NT		IU2-TR vs. NT	
					OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
<i>G*01:01:01</i>	85 (29.51)	19 (32.76)	15 (39.47)	25 (29.07)	1.16 (0.63- 2.12)	0.63 9	1.55 (0.77- 3.13)	0.26 1	0.97 (0.57- 1.66)	1.00 0
<i>G*01:01:02</i>	59 (20.49)	11 (18.97)	14 (36.84)	26 (30.23)	0.91 (0.44- 1.86)	0.86 0	2.26 (1.10- 4.64)	0.03 6	1.68 (0.97- 2.89)	0.07 8
<i>G*01:01:08</i>	8 (2.78)	3 (5.17)	1 (2.63)	2 (2.33)	1.91 (0.49- 7.42)	0.40 3	0.94 (0.11- 7.77)	1.00 0	0.83 (1.07- 3.99)	1.00 0
<i>G*01:01:09</i>	11 (3.82)	2 (3.45)	1 (2.63)	1 (1.16)	0.90 (0.19- 4.16)	1.00 0	0.68 (0.08- 5.42)	1.00 0	0.29 (0.04- 2.32)	0.31 0
<i>G*01:01:15</i>	1 (0.35)	0 (0.00)	0 (0.00)	0 (0.00)	-	1.00 0	-	1.00 0	-	1.00 0
<i>G*01:01:17</i>	1 (0.35)	0 (0.00)	0 (0.00)	0 (0.00)	-	1.00 0	-	1.00 0	-	1.00 0
<i>G*01:01:19</i>	8 (2.78)	2 (3.45)	0 (0.00)	4 (4.65)	1.25 (0.25- 6.04)	1.00 0	-	0.60 3	1.70 (0.50- 5.81)	0.48 3
<i>G*01:01:20</i>	1 (0.35)	0 (0.00)	0 (0.00)	0 (0.00)	-	1.00 0	-	1.00 0	-	1.00 0
<i>G*01:03:01</i>	23 (7.99)	3 (5.17)	3 (7.89)	7 (8.14)	0.63 (0.18- 2.16)	0.59 2	0.98 (0.28- 3.45)	1.00 0	1.02 (0.42- 2.46)	1.00 0
<i>G*01:04:01</i>	11 (3.82)	5 (8.62)	0 (0.00)	1 (1.16)	2.37 (0.79- 7.11)	0.16 0	-	0.37 5	0.29 (0.04- 2.32)	0.31 0
<i>G*01:04:04</i>	53 (18.40)	10 (17.24)	3 (7.89)	15 (17.44)	0.92 (0.44- 1.94)	1.00 0	0.38 (0.11- 1.28)	0.11 6	0.93 (0.50- 1.76)	0.87 5
<i>G*01:05N</i>	27 (9.38)	2 (3.45)	0 (0.00)	4 (4.65)	0.34 (0.08- 1.49)	0.19 4	-	0.05 6	0.47 (0.16- 1.38)	0.18 8
<i>G*01:06</i>	0 (0.00)	1 (1.72)	0 (0.00)	0 (0.00)	-	0.16 8	-	1.00 0	-	1.00 0
<i>G*01:11</i>	0 (0.00)	1 (1.72)	1 (2.63)	1 (1.16)	-	1.00 0	-	0.11 7	-	0.23 0
Infant <i>HLA-G</i> Alleles	EU (2n=288)	IP (2n=58)	IU (2n=38)	IU2 (2n=86)	IP vs. EU		IU vs. EU		IU2 vs. EU	
<i>G*01:01:01</i>	96 (33.33)	22 (37.93)	13 (34.21)	21 (24.42)	1.22 (0.68- 2.19)	0.54 5	1.04 (0.51- 2.12)	1.00 0	0.64 (0.37- 1.12)	0.14 5

<i>G*01:01:02</i>	59 (20.49)	6 (10.34)	9 (23.68)	20 (23.26)	0.45 (0.18- 1.09)	0.09 6	1.20 (0.54- 2.68)	0.67 2	1.17 (0.66- 2.09)	0.65 2
<i>G*01:01:08</i>	10 (3.47)	4 (6.90)	3 (7.89)	4 (4.65)	2.05 (0.62- 6.81)	0.26 5	2.38 (0.62- 9.07)	0.37 7	1.35 (0.41- 4.43)	0.74 6
<i>G*01:01:09</i>	7 (2.43)	4 (6.90)	1 (2.63)	1 (1.16)	2.97 (0.84- 10.5)	0.09 4	1.08 (0.12- 9.06)	1.00 0	0.47 (0.05- 3.89)	0.68 8
<i>G*01:01:19</i>	11 (3.82)	2 (3.45)	0 (0.00)	5 (5.81)	0.90 (0.19- 4.17)	1.00 0	-	0.37 5	1.55 (0.52- 4.60)	0.54 2
<i>G*01:03:01</i>	29 (10.07)	4 (6.90)	4 (10.53)	8 (9.30)	0.66 (0.22- 1.95)	0.48 5	1.05 (0.35- 3.17)	1.00 0	0.92 (0.40- 2.08)	1.00 0
<i>G*01:04:01</i>	11 (3.82)	1 (1.72)	1 (2.63)	3 (3.49)	0.44 (0.06- 3.49)	0.49 7	0.68 (0.08- 5.42)	1.00 0	0.91 (0.25- 3.33)	1.00 0
<i>G*01:04:04</i>	41 (14.24)	11 (18.97)	6 (15.79)	19 (22.09)	1.41 (0.67- 2.94)	0.41 9	1.12 (0.44- 2.87)	0.80 6	1.71 (0.93- 3.13)	0.09 4
<i>G*01:04:05</i>	1 (0.35)	0 (0.00)	0 (0.00)	0 (0.00)	-	1.00 0	-	1.00 0	-	1.00 0
<i>G*01:05N</i>	21 (7.29)	3 (5.17)	1 (2.63)	5 (5.81)	0.69 (0.20- 2.41)	0.60 0	0.34 (0.05- 2.63)	0.34 3	0.78 (0.29- 2.15)	0.81 0
<i>G*01:06</i>	0 (0.00)	1 (1.72)	0 (0.00)	0 (0.00)	-	0.16 8	-	1.00 0	-	1.00 0
<i>G*01:08</i>	1 (0.35)	0 (0.00)	0 (0.00)	0 (0.00)	-	1.00 0	-	1.00 0	-	1.00 0
<i>G*01:11</i>	1 (0.35)	0 (0.00)	0 (0.00)	0 (0.00)	-	1.00 0	-	1.00 0	-	1.00 0

NT: HIV-1 nontransmitting mother; IP-TR: intrapartum HIV-1 transmitting mother; IU-TR: *in utero* HIV-1 transmitting mother; IU2-TR: enriched group of presumed *in utero* transmitting mothers; EU: HIV-1 exposed uninfected infant; INF; IP: intrapartum infected infant; IU: *in utero* infected infant; IU2: enriched group of presumed *in utero* infected infants. Highlighted and bold *P* values indicate significant differences ($P < 0.05$) and bold *P* values indicate trends ($P < 0.090$).

Table 3

Representation of maternal HLA-G genotypes, N (%)

HLA-G Genotypes*	NT (n=14)	IP-TR (n=2)	IU-TR (n=19)	IU-TR (n=4)	IP-TR vs. NT OR (95% CI)	P	IU-TR vs. NT OR (95% CI)	P	IU2-TR vs. NT OR (95% CI)	P
G*01:01:01/G*01:01:01	17 (11.81)	3 (10.3)	5 (26.32)	5 (11.6)	0.86 (0.23-3.16)	1.00	2.66 (0.85-8.34)	0.14	0.98 (0.34-2.84)	1.00
G*01:01:01/G*01:01:02	15 (11.81)	4 (13.7)	3 (15.7)	4 (9.30)	1.37 (0.42-4.49)	0.74	1.61 (0.42-6.18)	0.69	0.88 (0.27-2.81)	1.00
G*01:01:01/G*01:01:09	4 (2.78)	0 (0.00)	1 (5.26)	1 (2.33)	-	0.60	1.94 (0.21-18.3)	1.00	0.83 (0.09-7.65)	1.00
G*01:01:01/G*01:03:01	4 (2.78)	0 (0.00)	1 (5.26)	2 (4.65)	-	0.60	1.94 (0.21-18.3)	1.00	1.71 (0.30-9.66)	0.62
G*01:01:01/G*01:04:01	3 (2.08)	2 (6.90)	0 (0.00)	0 (0.00)	3.48 (0.56-21.8)	0.19	-	1.00	-	0.58
G*01:01:01/G*01:04:04	17 (11.81)	4 (13.7)	0 (0.00)	6 (13.9)	1.19 (0.37-3.85)	0.75	-	0.22	1.21 (0.44-3.29)	0.79
G*01:01:01/G*01:05N	7 (4.86)	1 (3.45)	0 (0.00)	0 (0.00)	0.70 (0.08-5.91)	1.00	-	0.60	-	0.20
G*01:01:02/G*01:01:02	6 (4.17)	2 (6.90)	3 (15.79)	4 (9.30)	1.70 (0.33-8.89)	0.62	4.31 (0.98-18.9)	0.07	2.35 (0.63-8.77)	0.24
G*01:01:02/G*01:01:08	3 (2.08)	1 (3.45)	0 (0.00)	1 (2.33)	1.67 (0.17-16.7)	1.00	-	1.00	1.11 (0.11-11.0)	1.00
G*01:01:02/G*01:01:19	4 (2.78)	0 (0.00)	0 (0.00)	0 (0.00)	-	0.60	-	1.00	-	0.57
G*01:01:02/G*01:03:01	4 (2.78)	0 (0.00)	2 (10.53)	5 (11.6)	-	0.60	4.11 (0.70-24.1)	0.14	4.61 (1.17-17.9)	0.03
G*01:01:02/G*01:04:01	3 (2.08)	0 (0.00)	0 (0.00)	1 (2.33)	-	1.00	-	1.00	1.11 (0.11-11.0)	1.00

HLA-G Genotypes*	NT (n=14)	IP-TR (n=29)	IU-TR (n=19)	IU-TR (n=43)	IP-TR vs. NT		IU-TR vs. NT		IU2-TR vs. NT	
					OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
<i>G*01:01:02/G*01:04:04</i>	10 (6.94)	2 (6.90)	2 (10.53)	4 (9.30)	1.00 (0.21-4.78)	1.00	1.57 (0.32-7.81)	0.63	1.37 (0.41-4.62)	0.74
<i>G*01:01:02/G*01:05N</i>	6 (4.17)	0 (0.00)	0 (0.00)	2 (4.65)	-	0.38	-	0.61	1.12 (0.22-5.77)	1.00
<i>G*01:01:08/G*01:04:04</i>	3 (2.08)	0 (0.00)	1 (5.26)	1 (2.33)	-	1.00	2.61 (0.26-26.5)	0.39	1.11 (0.11-11.0)	1.00
<i>G*01:01:09/G*01:05N</i>	3 (2.08)	0 (0.00)	0 (0.00)	0 (0.00)	-	1.00	-	1.00	-	0.58
<i>G*01:03:01/G*01:04:04</i>	3 (2.08)	1 (3.45)	0 (0.00)	0 (0.00)	1.67 (0.17-16.7)	1.00	-	1.00	-	0.58
<i>G*01:03:01/G*01:05N</i>	4 (2.78)	0 (0.00)	0 (0.00)	0 (0.00)	-	0.60	-	1.00	-	0.57
<i>G*01:04:04/G*01:04:04</i>	6 (4.17)	0 (0.00)	0 (0.00)	0 (0.00)	-	0.38	-	0.61	-	0.33
<i>G*01:04:04/G*01:05N</i>	6 (4.17)	1 (3.45)	0 (0.00)	2 (4.65)	0.82 (0.09-7.09)	1.00	-	0.61	1.12 (0.22-5.77)	1.00

* indicates HLA-G genotype frequencies > 2% in the NT group. NT: HIV-1 nontransmitting mother; IP-TR: intrapartum HIV-1 transmitting mother; IU-TR: *in utero* HIV-1 transmitting mother; IU2-TR: enriched group of presumed *in utero* transmitting mothers. Highlighted and bold *P* values indicate significant differences (*P*<0.05) and bold *P* values indicate trends (*P*<0.090).

Table 4

Representation 3'UTR polymorphisms of *HLA-G* in HIV-1 positive mothers, N (%)

Polymorphism	NT (n=144)	IP-TR (n=29)	IU-TR (n=19)	IU2-TR (n=43)	IP-TR vs. NT		IU-TR vs. NT		IU2-TR vs. NT	
					OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
D	166 (57.64)	36 (62.07)	19 (50.00)	43 (50.00)	1.20 (0.67- 2.14)	0.562	0.73 (0.37- 1.45)	0.388	0.73 (0.45- 1.19)	0.218
I	122 (42.36)	22 (37.93)	19 (50.00)	43 (50.00)	1	1	1	1	1	1
+2961 (Indel)										
DD	53 (36.81)	11 (37.93)	6 (31.58)	12 (27.91)	1.61 (0.47- 5.48)	0.564	0.58 (0.17- 1.97)	0.527	0.58 (0.23- 1.45)	0.344
ID	60 (41.67)	14 (48.28)	7 (36.84)	19 (44.19)	1.81 (0.55- 5.96)	0.414	0.52 (0.16- 1.69)	0.348	0.81 (0.35- 1.90)	0.668
II	3 (21.53)	4 (13.79)	6 (31.58)	12 (27.91)	1	1	1	1	1	1
C	31 (10.76)	9 (15.52)	1 (2.63)	4 (4.65)	1.52 (0.68- 3.40)	0.366	0.22 (0.03- 1.69)	0.149	0.40 (0.14- 1.18)	0.095
T	257 (89.24)	49 (84.48)	37 (97.37)	82 (95.35)	1	1	1	1	1	1
+3003.T/C										
CC	4 (2.78)	1 (3.45)	0 (0.00)	0 (0.00)	1.39 (0.15- 13.1)	1.000	-	1.000	-	0.573
TC	23 (15.97)	7 (24.14)	1 (5.26)	4 (9.30)	1.69 (0.36- 4.45)	0.418	0.28 (0.03- 2.22)	0.311	0.52 (0.17- 1.60)	0.329
TT	117 (81.25)	21 (72.41)	18 (94.74)	39 (90.70)	1	1	1	1	1	1
G	100 (34.72)	22 (37.93)	15 (39.47)	26 (30.23)	1.14 (0.64- 2.06)	0.653	1.23 (0.61- 2.45)	0.590	0.81 (0.48- 1.37)	0.516
C	188 (65.28)	36 (62.07)	23 (60.53)	60 (69.77)	1	1	1	1	1	1
+3010.C/G										
GG	23 (15.97)	4 (13.79)	5 (26.32)	5 (11.63)	1.06 (0.31- 3.66)	1.000	1.61 (0.49- 5.32)	0.518	0.66 (0.22- 1.95)	0.609
CG	54 (37.50)	14 (48.28)	5 (26.32)	16 (37.21)	1.58 (0.66- 3.76)	0.379	0.69 (0.22- 2.18)	0.582	0.90 (0.43- 1.89)	0.853
CC	67 (46.53)	11 (37.93)	9 (47.37)	22 (51.16)	1	1	1	1	1	1
+3027.C/A										
A	1 (0.35)	0 (0.00)	0 (0.00)	0 (0.00)	-	1.000	-	1.000	-	1.000

Polymorphism	NT (n=144)	IP-TR (n=29)	IU-TR (n=19)	IU2-TR (n=43)	IP-TR vs. NT		IU-TR vs. NT		IU2-TR vs. NT		
					OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	
C	287 (99.65)	58 (100.00)	38 (100.00)	86 (100.00)	1	1	1	1	1	1	
	AA	0 (0.00)	0 (0.00)	0 (0.00)	-	1.000	-	1.000	-	1.000	
	CA	1 (0.69)	0 (0.00)	0 (0.00)	0 (0.00)	-	1.000	-	1.000	-	
	CC	143 (99.31)	29 (100.00)	19 (100.00)	43 (100.00)	1	1	1	1	1	
+3032.G/C	C	4 (1.39)	1 (1.72)	1 (2.63)	2 (2.33)	1.00 (0.11- 8.66)	1.000	1.52 (0.17- 13.5)	1.000	1.34 (0.26- 7.07)	1.000
	G	284 (98.61)	57 (98.28)	37 (97.37)	84 (97.67)	1	1	1	1	1	
	CC	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	-	1.000	-	1.000	-	
	GC	4 (2.78)	1 (3.45)	1 (5.26)	2 (4.65)	1.67 (0.16- 16.6)	1.000	2.59 (0.26- 26.4)	0.396	2.28 (0.37- 14.1)	0.591
GG	140 (97.22)	28 (96.55)	18 (94.74)	41 (95.35)	1	1	1	1	1	1	
	C	263 (91.32)	53 (91.38)	34 (89.47)	78 (90.70)	1.01 (0.37- 2.75)	1.000	0.81 (0.26- 2.46)	0.760	0.93 (0.40- 2.14)	1.000
		T	25 (8.68)	5 (8.62)	4 (10.53)	8 (9.30)	1	1	1	1	1
		CC	121 (84.03)	24 (82.76)	15 (78.95)	35 (81.40)	-	1.000	-	1.000	-
CT		21 (14.58)	5 (17.24)	4 (21.05)	8 (18.60)	-	1.000	-	1.000	-	
TT	2 (1.39)	0 (0.00)	0 (0.00)	0 (0.00)	1	1	1	1	1	1	
	C	100 (34.72)	22 (37.93)	15 (39.47)	26 (30.23)	1.14 (0.64- 2.06)	0.653	1.23 (0.61- 2.45)	0.590	0.81 (0.48- 1.37)	0.516
		G	188 (65.28)	36 (62.07)	23 (60.53)	60 (69.77)	1	1	1	1	1
		CC	23 (15.97)	4 (13.79)	5 (26.32)	5 (11.63)	1.06 (0.31- 3.65)	1.000	1.61 (0.49- 5.33)	0.518	0.66 (0.22- 1.95)
GC		54 (37.50)	14 (48.28)	5 (26.32)	16 (37.21)	1.58 (0.66- 3.76)	0.379	0.68 (0.21- 2.18)	0.582	0.90 (0.43- 1.89)	0.853
GG	67 (46.53)	11 (37.93)	9 (47.37)	22 (51.16)	1	1	1	1	1	1	

Polymorphism	NT (n=144)	IP-TR (n=29)	IU-TR (n=19)	IU2-TR (n=43)	IP-TR vs. NT		IU-TR vs. NT		IU2-TR vs. NT	
					OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
G	31 (10.76)	6 (10.34)	10 (26.32)	14 (16.28)	0.96 (0.37- 2.41)	1.000	2.96 (1.31- 6.67)	0.011	1.61 (0.81- 3.19)	0.187
A	257 (89.24)	52 (89.66)	28 (73.68)	72 (83.72)	1	1	1	1	1	1
+3187A/G GG	2 (1.39)	0 (0.00)	3 (15.79)	3 (6.98)	-	1.000	14.4 (2.18- 94.7)	0.011	5.39 (0.86- 33.7)	0.080
AG	27 (18.75)	6 (20.69)	4 (21.05)	8 (18.60)	1.11 (0.41- 2.99)	1.000	1.42 (0.42- 4.74)	0.740	1.06 (0.44- 2.57)	1.000
AA	115 (79.86)	23 (79.31)	12 (63.16)	32 (74.42)	1	1	1	1	1	1
G	97 (33.68)	17 (29.31)	15 (39.47)	35 (40.70)	0.82 (0.44- 1.51)	0.545	1.28 (0.64- 2.57)	0.586	1.35 (0.82- 2.21)	0.249
C	191 (66.32)	41 (70.69)	23 (60.53)	51 (59.30)	1	1	1	1	1	1
+3196C/G GG	18 (12.50)	2 (6.90)	4 (21.05)	7 (16.28)	0.52 (0.11- 2.48)	0.514	1.81 (0.48- 6.68)	0.464	1.68 (0.60- 4.76)	0.399
CG	61 (42.36)	13 (44.83)	7 (36.84)	21 (48.84)	0.98 (0.43- 2.27)	1.000	0.93 (0.32- 2.73)	1.000	1.49 (0.71- 3.16)	0.347
CC	65 (45.14)	14 (48.28)	8 (42.11)	15 (34.88)	1	1	1	1	1	1

NT: HIV-1 nontransmitting mother; IP-TR: intrapartum HIV-1 transmitting mother; IU-TR: *in utero* HIV-1 transmitting mother; IU2-TR: enriched group of presumed *in utero* transmitting mothers. Highlighted and bold *P* values indicate significant differences ($P < 0.05$) and bold *P* values indicate trends ($P < 0.090$).

Table 5

Representation 3'UTR haplotype and genotype frequencies of *HLA-G* in HIV-1 positive mothers, N (%)

3' UTR	NT (n=144)		IP-TR (n=29)		IU-TR (n=19)		IU2-TR (n=43)		IP-TR vs. NT		IU-TR vs. NT		IU2-TR vs. NT	
									OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
<i>UTR1</i>	31 (10.76)	6 (10.34)	10 (26.32)	14 (16.28)	0.96 (0.38-2.41)	1.00 0	2.96 (1.31-6.67)	0.01 1	1.61 (0.81-3.19)	0.18 7				
<i>UTR2</i>	97 (33.68)	17 (29.31)	15 (39.47)	35 (40.70)	0.82 (0.44-1.51)	0.54 5	1.28 (0.64-2.57)	0.58 6	1.44 (0.87-2.37)	0.15 5				
<i>UTR3</i>	66 (22.92)	14 (24.14)	4 (10.53)	17 (19.77)	1.07 (0.55-2.07)	0.86 5	0.39 (0.13-1.16)	0.09 4	0.82 (0.46-1.51)	0.55 9				
<i>UTR4</i>	31 (10.76)	9 (15.52)	1 (2.63)	4 (4.65)	1.52 (0.68-3.39)	0.36 6	0.22 (0.03-1.69)	0.14 9	0.40 (0.14-1.18)	0.09 5				
<i>UTR5</i>	24 (8.33)	5 (8.62)	4 (10.53)	8 (9.30)	1.03 (0.39-2.84)	1.00 0	1.29 (0.42-3.96)	0.75 6	1.13 (0.49-2.61)	0.82 6				
<i>UTR6</i>	34 (11.81)	6 (10.34)	3 (7.89)	6 (6.98)	0.86 (0.34-2.16)	0.82 7	0.64 (0.18-2.19)	0.59 6	0.56 (0.23-1.38)	0.23 8				
<i>UTR7</i>	1 (0.35)	0 (0.00)	0 (0.00)	0 (0.00)	-	1.00 0	-	1.00 0	-	1.00 0				
<i>UTR20</i>	4 (1.39)	1 (1.72)	1 (2.63)	2 (2.33)	1.24 (0.14-11.3)	1.00 0	1.91 (0.21-17.6)	0.46 4	1.69 (0.30-9.39)	0.62 4				
<i>UTR1/UTR1</i>	2 (1.39)	0 (0.00)	3 (15.79)	3 (6.98)	-	1.00 0	13.3 (2.07-85.7)	0.01 2	5.32 (0.86-32.9)	0.08 1				
<i>UTR1/UTR2</i>	5 (3.47)	3 (10.34)	2 (10.53)	5 (11.63)	3.21 (0.72-14.3)	0.13 2	3.27 (0.59-18.2)	0.19 0	3.66 (1.01-13.3)	0.05 2				
<i>UTR1/UTR3</i>	12 (8.33)	1 (3.45)	0 (0.00)	0 (0.00)	0.39 (0.05-3.14)	0.47 5	-	0.36 3	-	0.07 1				
<i>UTR1/UTR4</i>	5 (3.47)	1 (3.45)	0 (0.00)	0 (0.00)	0.99 (0.11-8.83)	1.00 0	-	1.00 0	-	0.34 7				
<i>UTR1/UTR5</i>	2 (1.39)	0 (0.00)	1 (5.26)	2 (4.65)	-	1.00 0	3.94 (0.34-45.7)	0.31 2	3.46 (0.47-25.4)	0.22 7				
<i>UTR1/UTR6</i>	3 (2.08)	0 (0.00)	1 (5.26)	1 (2.33)	-	1.00 0	2.61 (0.26-26.5)	0.39 4	1.12 (0.11-11.0)	1.00 0				

3' UTR	NT (n=144)	IP- TR (n=29)	IU- TR (n=19)	IU2- TR (n=43)	IP-TR vs. NT			IU-TR vs. NT			IU2-TR vs. NT		
					OR (95% CI)	P		OR (95% CI)	P		OR (95% CI)	P	
UTR1/UTR 20	0 (0.00)	1 (3.45)	0 (0.00)	0 (0.00)	-	0.16	8	-	1.00	0	-	1.00	0
UTR2/UTR 2	18 (12.50)	2 (6.90)	4 (21.05)	7 (16.28)	0.52 (0.11- 2.37)	0.53	4	1.87 (0.56- 6.25)	0.47	4	1.36 (0.53- 3.51)	0.61	0
UTR2/UTR 3	23 (15.97)	4 (13.79)	3 (15.79)	10 (23.26)	0.84 (0.27- 2.64)	1.00	0	0.99 (0.26- 3.66)	1.00	0	1.59 (0.69- 3.68)	0.36	1
UTR2/UTR 4	12 (8.33)	3 (10.34)	0 (0.00)	1 (2.33)	1.27 (0.33- 4.81)	0.71	9	-	0.36	3	0.26 (0.03- 2.07)	0.30	4
UTR2/UTR 5	10 (6.94)	2 (6.90)	2 (10.53)	5 (11.63)	0.99 (0.21- 4.78)	1.00	0	1.58 (0.32- 7.81)	0.63	4	1.76 (0.57- 5.47)	0.34	2
UTR2/UTR 6	9 (6.25)	1 (3.45)	0 (0.00)	0 (0.00)	0.54 (0.06- 4.40)	0.69	9	-	0.39	0	-	0.12	1
UTR2/UTR 7	1 (0.69)	0 (0.00)	0 (0.00)	0 (0.00)	-	1.00	0	-	1.00	0	-	1.00	0
UTR2/UTR 20	1 (0.69)	0 (0.00)	0 (0.00)	0 (0.00)	-	1.00	0	-	1.00	0	-	1.00	0
UTR3/UTR 3	8 (5.56)	0 (0.00)	0 (0.00)	0 (0.00)	-	0.35	5	-	0.59	8	-	0.20	1
UTR3/UTR 4	2 (1.39)	3 (10.34)	0 (0.00)	2 (4.65)	8.19 (1.30- 51.5)	0.03	4	-	1.00	0	3.46 (0.47- 25.4)	0.22	7
UTR3/UTR 5	5 (3.47)	3 (10.34)	0 (0.00)	0 (0.00)	3.21 (0.72- 14.3)	0.13	2	-	1.00	0	-	0.34	7
UTR3/UTR 6	7 (4.86)	3 (10.34)	1 (5.26)	4 (9.30)	2.25 (0.55- 9.31)	0.37	4	1.09 (0.12- 9.35)	1.00	0	2.01 (0.56- 7.21)	0.46	1
UTR3/UTR 20	1 (0.69)	0 (0.00)	0 (0.00)	1 (2.33)	-	1.00	0	-	1.00	0	-	1.00	0
UTR4/UTR 4	4 (2.78)	1 (3.45)	0 (0.00)	0 (0.00)	1.25 (0.13- 11.6)	1.00	0	-	1.00	0	-	0.57	5
UTR4/UTR 5	1 (0.69)	0 (0.00)	1 (5.26)	1 (2.33)	-	1.00	0	7.94 (0.48- 132.6)	0.22	0	3.40 (0.21- 55.6)	0.40	8
UTR4/UTR 6	2 (1.39)	0 (0.00)	0 (0.00)	0 (0.00)	-	1.00	0	-	1.00	0	-	1.00	0

3' UTR	NT (n=144)	IP-TR (n=29)		IU-TR (n=19)		IU2-TR (n=43)		IP-TR vs. NT		IU-TR vs. NT		IU2-TR vs. NT	
								OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
UTR4/UTR 20	1 (0.69)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	-	1.00	-	1.00	3.40 (0.21- 55.6)	0.40 8
UTR5/UTR 5	2 (1.39)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	-	1.00	-	1.00	-	1.00 0
UTR5/UTR 6	2 (1.39)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	-	1.00	-	1.00	-	1.00 0
UTR6/UTR 6	5 (3.47)	1 (3.45)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0.99 (0.11- 8.83)	1.00	-	1.00	-	0.34 7
UTR6/UTR 20	1 (0.69)	0 (0.00)	1 (5.26)	1 (2.33)	1 (2.33)	1 (2.33)	1 (2.33)	-	1.00	7.94 (0.48- 132.6)	0.22	3.40 (0.21- 55.6)	0.40 8

NT: HIV-1 nontransmitting mother; IP-TR: intrapartum HIV-1 transmitting mother; IU-TR: *in utero* HIV-1 transmitting mother; IU2-TR: enriched group of presumed *in utero* transmitting mothers. Highlighted and bold *P* values indicate significant differences ($P < 0.05$) and bold *P* values indicate trends ($P < 0.090$).

Table 6

Significant linkage disequilibrium (LD) between *HLA-G* alleles, the 14-bp indel, 3'UTR SNPs and haplotypes in HIV-1 positive mothers (n=216)

LD comparisons	N	(%)	D'	X ²	P-value	
<i>G*01:01:01</i>	Del	126	29.17	0.95	45.19	<0.001
	UTR1	44	10.19	0.80	37.70	<0.001
	UTR4	33	7.64	0.64	19.97	<0.001
	UTR6	45	10.42	0.94	47.47	<0.001
	UTR20	6	1.39	1.00	5.93	<0.05
<i>G*01:01:02</i>	Ins	96	22.22	1.00	66.88	<0.001
	UTR2	96	22.22	1.00	98.49	<0.001
<i>G*01:01:19</i>	Ins	14	3.24	1.00	6.98	<0.01
	UTR2	14	3.24	1.00	10.27	<0.01
<i>G*01:03:01</i>	Ins	33	7.64	1.00	17.64	<0.001
	UTR5	33	7.64	1.00	189.72	<0.001
<i>G*01:04:01</i>	Del	17	3.94	1.00	4.16	<0.05
	UTR3	17	3.94	1.00	27.24	<0.001
<i>G*01:04:04</i>	Del	78	18.06	1.00	24.37	<0.001
	UTR3	78	18.06	1.00	159.40	<0.001
<i>G*01:05N</i>	Ins	33	7.64	1.00	17.64	<0.001
	UTR2	33	7.64	1.00	25.98	<0.001
	UTR7	1	0.23	1.00	5.83	<0.05
<i>Del</i>	+3003 C	44	10.19	1.00	11.94	<0.01
	+3010 G	148	34.26	1.00	65.44	<0.001
	+3035 C	245	56.71	1.00	7.30	<0.05
	+3142 C	148	34.26	1.00	65.44	<0.001
	+3187 G	51	11.81	1.00	14.23	<0.001
	+3196 C	245	56.71	1.00	126.48	<0.001
<i>Ins</i>	+3003 T	187	43.29	1.00	4.78	<0.05
	+3010 C	187	43.29	1.00	60.58	<0.001
	+3035 T	38	8.80	1.00	20.71	<0.001
	+3142 G	187	43.29	1.00	60.58	<0.001
	+3187 A	187	43.29	1.00	6.44	<0.01
	+3196 G	149	34.49	1.00	136.23	<0.001

N: the number of individuals; D': measure of linkage disequilibrium; X²: Chi-Square value from which the P-value was determined significant at P<0.05.

Table 7

Logistic regression analysis, adjustments made for maternal factors that influence MTCT of HIV-1

Allele/genotype	Association	Unadjusted		Adjusted mVL		Adjusted mNVP		Adjusted Infant <i>KIR2DS4-v</i>	
		OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
<i>G*01:01:02</i>	IU-TR vs. NT	2.26 (1.10-4.64)	0.036	3.22 (1.03-10.1)	0.043	2.02 (0.74-5.52)	0.168	2.13 (0.79-5.78)	0.134
<i>G*01:01:02</i>	IU2-TR vs. NT	1.68 (0.97-2.89)	0.078	2.39 (1.10-5.20)	0.027	1.54 (0.73-3.22)	0.252	1.79 (0.88-3.65)	0.105
<i>G*01:01:02/G*01:01:02</i>	IU-TR vs. NT	4.31 (0.98-18.9)	0.072	8.79 (1.46-52.8)	0.017	4.81 (1.04-22.3)	0.044	5.08 (1.12-22.9)	0.035
<i>G*01:01:02/G*01:03:01</i>	IU2-TR vs. NT	4.61 (1.17-17.9)	0.031	10.0 (2.15-46.8)	0.003	3.14 (0.73-13.5)	0.122	3.81 (0.89-16.3)	0.071
<i>3187G</i>	IU-TR vs. NT	2.96 (1.31-6.67)	0.011	2.17 (0.67-6.99)	0.192	2.56 (0.90-7.29)	0.077	2.42 (0.86-6.83)	0.094
<i>3187GG</i>	IU-TR vs. NT	14.4 (2.18-94.7)	0.011	29.6 (2.36-371.0)	0.009	-	-	-	-
<i>3187GG</i>	IU2-TR vs. NT	5.39 (0.86-33.7)	0.080	6.69 (0.91-49.4)	0.062	10.9 (1.06-113.6)	0.044	6.51 (1.04-40.7)	0.045
<i>UTR1</i>	IU-TR vs. NT	2.96 (1.31-6.67)	0.011	2.17 (0.67-6.99)	0.192	2.56 (0.90-7.29)	0.077	2.42 (0.86-6.83)	0.094
<i>UTR1/UTR1</i>	IU-TR vs. NT	13.3 (2.07-85.7)	0.012	29.6 (2.36-371.0)	0.009	-	-	-	-
<i>UTR1/UTR2</i>	IU2-TR vs. NT	3.66 (1.01-13.3)	0.052	5.44 (1.32-22.4)	0.019	3.43 (0.91-12.8)	0.067	3.57 (0.95-13.3)	0.058
<i>UTR3/UTR4</i>	IP-TR vs. NT	8.19 (1.30-51.5)	0.034	6.17 (0.91-41.4)	0.061	4.24 (0.63-28.5)	0.137	N/A	N/A
Combined effect									
<i>G*01:01:02 & 3187G</i>	IU-TR vs. NT	4.12 (0.70-24.2)	0.145	6.62 (0.82-52.9)	0.074	3.81 (0.64-22.5)	0.140	4.19 (0.70-24.9)	0.116
<i>G*01:01:02 or 3187G</i>	IU-TR vs. NT	4.51 (1.25-16.1)	0.014	4.93 (1.24-19.5)	0.023	4.14 (1.14-15.1)	0.031	4.12 (1.13-14.9)	0.031

mVL: maternal viral load; mNVP: maternal single-dose Nevirapine; *KIR2DS4-v*: non-functional *KIR2DS4*; N/A: not an applicable adjustment, since infant possession of *KIR2DS4-v* has been associated with IU HIV-1 transmission and not IP HIV-1 transmission. Highlighted and bold *P* values indicate significant differences ($P < 0.05$).