Sanches et al., 2:2
http://dx.doi.org/10.4172/scientificreports.652

Open Access Scientific Reports

Research Article Open Access

Human Leukocyte Antigen-G 14 Base Pairs Polymorphism in the Human Immunodeficiency Virus -1 Perinatal Transmission

Roberta Seron Sanches[1], Juliana Doblas Massaro[2], Erick da Cruz Castelli[3], Celso Teixeira Mendes-Junior[4], Francielly Maiara da Penna Matos[1], Juliana Martinez[1], Simone Kashima Haddad[2], Silvana Maria Quintana[5], Maria Célia Cervi[6], Luciana Castelar Tsuda[1], Eduardo Antônio Donadi[2] and Ana Paula Morais Fernandes[1]*

[1]*Department of General and Specialized Nursing, Nursing School of RibeirãoPreto, University of São Paulo, 3900 Av Bandeirantes, CEP14 040 - 902, RibeirãoPreto, SP, Brazil*
[2]*Department of Medical Clinical, Faculty of Medicine of RibeirãoPreto, University of São Paulo, 3900 Av Bandeirantes, CEP14 049 - 900, RibeirãoPreto, SP, Brazil*
[3]*Department of Biology, Biological Sciences Institute, Federal University of Goias, Goiânia – GO, Brazil*
[4]*Department of Chemistry, Faculty of Philosophy, Sciences and Literature of RibeirãoPreto, University of São Paulo, 3900 Av Bandeirantes, CEP14 040 - 901, RibeirãoPreto, SP, Brazil*
[5]*Department of Gynecology, Faculty of Medicine of RibeirãoPreto, University of São Paulo, 3900 Av Bandeirantes, CEP14 049 - 900, RibeirãoPreto, SP, Brazil*
[6]*Department of Pediatric, Faculty of Medicine of RibeirãoPreto, University of São Paulo, 3900 Av Bandeirantes, CEP14 049 - 900, RibeirãoPreto, SP, Brazil*

Abstract

Background: *HLA-G* is expressed at maternal-fetal interface and is strongly involved on maternal-fetal tolerance. The 3' untranslated region of the *HLA-G* gene presents an insertion (INS) or deletion (DEL) of a 14-base pair (bp) fragment, which is associated with stability and expression levels of *HLA-G* mRNA. In this study we verified the influence of the 14-bp INS/DEL polymorphism on the HIV vertical transmission.

Methods: Blood samples were obtained from 49 mother-child pairs (26 pairs with and 23 without vertical transmission). All children were born from HIV-positive mothers who did not receive antiretroviral therapy during pregnancy. The 14-bp polymorphism was detected by PCR-amplified DNA using specific primers.

Results: The presence of the DEL/DEL genotype was more frequent among mothers with HIV-infected child (P=0.05). In addition, the INS/DEL genotype similarity among mother and child pairs was more frequent on vertical transmission (69%) than in the absence of vertical transmission.

Conclusion: The presence of the DEL/DEL genotype, associated with high production of *HLA-G*, and the INS/DEL genotype similarities between mother and child may favor HIV mother to child transmission.

Keywords: *HLA-G*; HIV; Mother-to-child; Vertical; Transmission

Introduction

HLA-G is highly expressed in trophoblast and it is considered to be an important mediator of maternal–fetal tolerance due to its ability to inhibit maternal cytotoxic cells [1]. Genetic polymorphisms observed at the *HLA-G* 3′ untranslated region (UTR) have been associated with post-transcriptional control of *HLA-G* mRNA expression. One of these polymorphisms is the presence (insertion-INS) or absence (deletion-DEL) of a 14-bp fragment (5′-ATTTGTTCATGCCT-3′, in which the DEL/DEL genotype has been associated with high expression of *HLA-G* mRNA [2-5].

Some viruses have developed the ability to increase *HLA-G* expression to evade host immune response [6], and an altered *HLA-G* expression induced by viruses may influence mother's immune response and, theoretically, the vertical virus transmission. Studies associating polymorphic sites at coding and 3'UTR*HLA-G* regions in vertical transmission [7-9] or only in children presenting or not vertical HIV transmission have been reported (10,11).

Considering that the 14-bp INS/DEL polymorphism has a well-recognized role on the *HLA-G* mRNA expression, we evaluated the frequency of the 14-bp INS/DEL polymorphism and the INS/DEL genotype similarity in Southeastern Brazilian mother-child pairs perinatally exposed to HIV-1 infection.

The study was conducted on 49 mother-child pairs, stratified into two groups: i) 26 HIV-1-positive mother/HIV-1-positive child pairs and 23 HIV-1-positive mother/HIV-1-negative child pairs. All mother-child pairs were not treated with antiretroviral drugs and did not receive breastfeed. Patients were selected from the Acquired Immunodeficiency Outpatient Clinic at the University Hospital of the Faculty of Medicine of RibeirãoPreto, University of São Paulo, Brazil. HIV-1-positive mother/HIV-1-positive child pair samples were obtained from 1993 – 1996, when antiretroviral therapy was not freely dispensed by the Brazilian Ministry of Health. The local Ethics Committee of the University Hospital of Faculty of Medicine of RibeirãoPreto approved the study protocol, and informed consent was obtained from all individuals (HCFMRP-USP # 9060/2006).

Genomic DNA was obtained from peripheral blood mononuclear cells using a salting out procedure. The *HLA-G* 14-bp INS/DEL polymorphism was evaluated as previously described [12]. Allelic and genotypic frequencies were computed by the direct counting method. The frequency of each allele or genotype was compared between patients and controls by the two-sided Fisher exact test, with the aid of the GraphPadInStat3.06 software, which was also used to estimate the

***Corresponding author:** Ana Paula M Fernandes, Department of General and Specialized Nursing, School of Nursing of RibeirãoPreto, University of São Paulo, 3900 Av. Bandeirantes, Monte Alegre, 14 049-902, Ribeirão Preto, SP, Brazil, Tel: +55 16 3602 3414; Fax: +55 16 3633 3271; E-mail: anapaula@eerp.usp.br

Received March 02, 2013; **Published** March 27, 2013

Citation: Sanches RS, Massaro JD, da Cruz Castelli E , Mendes-Junior CT, da Penna Matos FM, et al. (2013) Human Leukocyte Antigen-G 14 Base Pairs Polymorphism in the Human Immunodeficiency Virus -1 Perinatal Transmission. 2: 652 doi:10.4172/scientificreports.652

odds ratio (OR) and its 95% confidence interval (95%CI). GENEPOP 3.4 software was used to observe the adherences of genotypic proportions to expectations under Hardy-Weinberg equilibrium (HWE) by the complete enumeration method [13].

The 14-bp INS/DEL allele and genotype frequencies were compared between the two groups, i.e., HIV-1 positive transmission and HIV-1 negative transmission, and only an increased frequency of the DEL/DEL genotype among mothers with HIV-infected child (P=0.05) (Table 1) was detected. In addition, the 14-bpgenotype similarity among mother and child pairs was more frequent on vertical transmission (69%) than in the absence of vertical transmission (P=0.09).

The role of the *HLA-G* polymorphisms in HIV-1 infection has not been elucidated. Considering the HIV horizontal transmission, the *HLA-G**01:05N allele, which codes truncated non-functional *HLA-G* molecules, was associated with protection against the HIV-1 infection, while the *HLA-G**01:01:08 allele, which codes functional soluble and membrane-bound molecules, was associated with an increased risk of HIV-1 infection in Zimbabwean women [4,5]. Since *HLA-G* inhibits NK cell-mediated lysis [14-16], the absence or reduced expression of *HLA-G* molecules may allow NK cells to destroy HIV-infected cells, protecting against HIV infection.

Regarding the role of *HLA-G* gene on HIV vertical transmission, contradictory results have been reported. The first published paper on this issue reported that mother-child pair discordance at *HLA-G* codon 57 was associated with reduced risk of HIV-1 transmission in North-American Caucasians [7], a finding that was not confirmed in the Zimbabwean population [9]. Since the polymorphism at codon 57 does not change the *HLA-G* amino acid composition and presumably *HLA-G* function, it is difficult to understand how this silent mutation could have a direct influence on HIV transmission. Such finding could reflect the effect of other polymorphic sites at the *HLA-G* gene in linkage disequilibrium with the codon 57 polymorphism. Then, other gene regions have been studied, and a likely candidate region is *HLA-G*3' UTR, which has several post-transcriptional control elements, and the most widely studied is the 14 bp INS/DEL polymorphism.

Two independent studies reported an association between *HLA-G* nucleotide sequences located at 3′UTR, including the C variant at position +3777C/G (also designated as +3010C/G) and 14-bp DEL polymorphism, with decreased risk of HIV-1 vertical transmission [8,10]. Moreover, two other independent studies have shown that the *HLA-G*+3777 and 14-bp polymorphisms are in linkage disequilibrium, especially the association between the 14-bp DEL and the +3777C allele [11,17]. The *HLA-G* +3777C variant alone has no effect on vertical transmission of HIV-1 but, when linked with the 14-bp DEL allele, it exerts a protective role [11]. In two studies performed in Brazilian children from Recife (North East Brazil), evaluating the transmission of *HLA-G* 3' UTR alleles only in children infected or not with HIV and born from HIV-infected mothers, reported that the DEL/INS and +3777G > C polymorphisms were in linkage disequilibrium and the presence of the DEL-C/DEL-C combined genotype conferred protection against HIV mother-to-child transmission [10,11,18].

We analyzed Brazilian mother-child pairs from São Paulo (South East Brazil) and observed an increased frequency of the DEL/DEL homozygote genotype was observed among mothers with HIV-infected child, and the 14-bp INS/DEL genotype similarities were more frequent on vertical transmission than on its absence. Aikhionbare et al. [8] showed similarities at the *HLA-G* 3' UTR polymorphic sites (+3742, +3743 and +3777) in mother-child HIV-infected pairs. Since the +3742 and +3743 polymorphic sites are associated with 14-bpDEL and are in linkage disequilibrium with +3777, our results are similar to the mentioned study. Considering that the population backgrounds of both studies are different, concordant associations reinforces the power of such finding.

HLA-G alleles presenting the 14-bp DEL have been associated with increased production of *HLA-G*, but with lower stability of mRNA. Considering that *HLA-G* is highly expressed in trophoblast at the maternal-fetal interface, exhibiting an important role on fetal immunological tolerance [19,20], the role of *HLA-G* in the HIV-1 mother-to-child transmission shall be important. To corroborate this idea, a recent study reported high expression placental *HLA-G*1 expression among HIV-1-infected mothers with infected babies when compared to uninfected babies [21].

Taken together, the expression of *HLA-G* is a complex process modulated by many factors, such as *HLA-G* polymorphisms, which may contribute to an immunological environment affecting the outcome of HIV-1 mother-to-child transmission. In conclusion, the presence of the DEL/DEL genotype in mothers, associated with high production of *HLA-G*, and 14-bp genotype similarities between mother and child may favor HIV mother to child transmission.

***HLA-G* 14-bp alleles and genotypes**	**HIV-1 positive transmission group (*n*=26)**	**HIV-1 negative transmission group (n=23)**	***P value*[1]**	**OR (95%C.I.)**
Mothers				
INS/INS	4 (0.15)	6 (0.26)	0.48	-
DEL/DEL	7 (0.27)	1 (0.04)	0.05	8.11 (0.91-71.98)
INS/DEL	15 (0.58)	16 (0.70)	0.55	-
INS	23 (0.44)	28 (0.61)	0.11	-
DEL	29 (0.56)	18 (0.39)	0.11	-
Children				
INS/INS	6 (0.23)	3 (0.13)	0.47	-
DEL/DEL	9 (0.35)	5 (0.22)	0.36	-
INS/DEL	11 (0.42)	15 (0.65)	0.15	-
INS	23 (0.44)	21 (0.46)	1.00	-
DEL	29 (0.56)	25 (0.54)	1.00	-
Mother/Child pairs				
HLA-G 14-bp similarity	18 (0.69)	10 (0.43)	0.09	2.93 (0.91-9.45)

[1]P value estimated by the Fisher exact test

Table 1: Allelic and genotypic frequencies of the 14-bp INS/DEL polymorphisms in HIV-positive mothers and in their children. Samples were distributed in two groups, according to perinatal HIV transmission. Mother/child pairs were also organized according to genotype similarity.

Acknowledgements

This work received financial support from FAPESP (Fernandes APM #2011/08177-1) and CNPq.

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