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DEFB1 polymorphisms and HIV-1 Mother-To-Child Transmission in Zambian population

Running title: ***DEFB1* SNPs and MTCT**

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Abstract

Introduction. Human Beta Defensin-1 (hBD-1) is a component of the innate immune system, the first line of defence against pathogens, already reported as involved in the susceptibility to HIV-1 infection and HIV-1 mother-to-child transmission (MTCT) in different populations.

We investigated the role of *DEFB1*gene (encoding for hBD-1) functional polymorphisms in the susceptibility to HIV-1 MTCT in a population from Zambia.

Methods. Four selected polymorphisms within *DEFB1* gene, three at the 5' untranslated region (UTR), namely –52G>A (rs1799946), –44C>G (rs1800972) and –20G>A (rs11362) and one in the 3'UTR, c.*87A>G (rs1800972), were genotyped in 101 HIV-1 positive mothers (26 transmitters – 27% and 75 not transmitters – 73%) and 331 infants born to HIV-1 infected mothers (85 HIV-1 positive - 26% and 246 exposed but not infected – 74%).

Results. *DEFB1* c.*87 A allele was more frequent among HIV- children respect to HIV+ (with intra-uterine MTCT). Concerning *DEFB1*haplotypes, GCGA haplotype resulted more represented in HIV- than HIV+ infants and *DEFB1*ACGG haplotype presented increased frequency in HIV- children respect to HIV+ (with intra-partum MTCT) (p=0.02, p=0.002 and p= 0.006 respectively).

Conclusion. *DEFB1* polymorphisms were significantly associated with decreased risk of HIV-1 infection acquisition in the studied Zambian population suggesting that they may play a role in HIV-1 MTCT.

Introduction

Human immunodeficiency type-1 (HIV-1) mother-to-child transmission (MTCT) has been dramatically reduced with the introduction of HIV test in all pregnant women and consequent antiretroviral drugs administration in those positive for virus infection; also alternative to breastfeeding and caesarean partum contributed to prevent MTCT [1]. Nevertheless, data from past years in which antiretroviral drugs were not yet available for prevention provide a unique model to understand the role of host genetic factors in the modulation of HIV-1 infection susceptibility, a multifactorial trait [2].

So far several genes and genetic polymorphisms (e.g. single nucleotide polymorphisms, SNPs) have been described as conferring risk or protection towards HIV-1 infection [3]. Since innate immunity is known to play a crucial role in the immune system of the foetus, we focused our attention on functional genetic variations distributed in primary natural defence genes. Therefore, we studied *DEFB1* (8p23.1) gene encoding for the human beta defensins 1 (hBD-1), an antimicrobial peptide [4], known for its antimicrobial properties against bacteria, fungi but also viruses [5] and already investigated in the context of HIV-1 MTCT [6, 7, 8, 9]. *DEFB1* expression has been detected in the placenta, and a role in the protection against HIV-1 mother-to-foetus transmission has been hypothesized [9].

DEFB1 gene presents different functional polymorphisms, the –52G>A (rs1799946), –44C>G (rs1800972) and –20G>A (rs11362) at the 5' untranslated region (5'UTR) and c.*87A>G (rs1800971) at the 3'UTR possibly modulating *DEFB1* gene expression in different cellular models [10, 11, 12, 13].

In this study, taking into account the previously reported role of *DEFB1* genetic variations in the context of HIV-1 infection, we analysed the four above-mentioned

DEFB1 functional polymorphisms in 101 HIV-1 positive mothers and 331 infants born to HIV-1-positive mothers from Zambia with the aim of investigating their potential impact in the susceptibility to HIV-1 perinatal infection.

Material and Methods

Study population

The Zambia Exclusive Breastfeeding Study (ZEBS, Lusaka Zambia, ClinicalTrials.gov Identifier: NCT00310726) population recruited for this study was previously analysed in another work by Segat et al. [14]: briefly the ZEBS was a randomized clinical trial that investigated if exclusive breastfeeding up to 4 months could reduce the risk of HIV-1 transmission respect to longer breastfeeding through a median of 16 months. Nine hundred and fifty-eight HIV-1 positive women were enrolled during pregnancy at two prenatal care clinics (May 2001 to September 2004), and they were followed to delivery and 24 months post partum with their infants who were tested regularly for HIV. All women were counseled to breastfeed to at least 4 months, then, half of the women were randomized to stop all breastfeeding and the other half to continue breastfeeding for as long as they usually would. Detailed information is provided in table supplementary 1.

For the current genetic analysis 331 infants were selected: 85 were HIV-1 infected (designed as HIV+): 22 (6.9%) had intrauterine MTCT (IU - defined as a positive polymerase chain reaction (PCR) result within 2 days of birth), 25 (7.5%) had intrapartum MTCT (IP - defined as a positive PCR result within 42 days of birth with an earlier negative result) and 38 (14.4%) had postnatal (breastfeeding) MTCT (PP - defined as a positive PCR results older than 42 days with a negative earlier result

in a breastfed child). The remainder 246 were HIV-1-exposed uninfected children (designed as HIV-). The samples of 101 available HIV-1 positive mothers (mean age 26 years, range 18-45) of these children were selected and included in the genotyping analysis: 26 of these transmitted the HIV-1 infection to their newborns (designed as TR): 26.9% (7/26) were IU trnsmitted MTCT, 30.8% (8/26) were IP transmitted, and 42.3% (11/26) were PP transmitted via breastfeeding; 75 mothers did not transmit HIV-1 to their infants (designed as NTR). The numbers of enrolled children and mother is different since a subset of 331 out of 632 infants and 101 mothers out of 958 recruited in the trial had available dried blood spot specimen useful for DNA extraction and subsequent genotyping.

All women provided written informed consent for participating in the study. All the study experiments and procedures have been performed in accordance with ethical standards of the 1975 Declaration of Helsinki (7th revision, 2013) and the ethical committee of IRCCS Burlo Garofolo approved the study (protocol L-1106, 1 May 2010).

DEFB1 genotyping

DNA extraction was performed from dried blood spots as described in Segat et al. [14]. The four polymorphisms at *DEFB1* gene were detected using TaqMan SNPs genotyping assays and TaqMan® GTXpress™ Master Mix on the ABI7900HT Real Time PCR platform (Applied Biosystems - Life Technologies, Carlsbad, California, U.S.A.), following manufacturer instructions: *DEFB1* 5'UTR -52G>A (rs1799946), -44C>G (rs1800972), -20G>A (rs11362) and 3'UTR c.*87A>G (rs1800971) polymorphisms using respectively C__11636795_20, C__11636794_10, C__11636793_20 and C___8845559_10 assays.

Statistical analysis

*DEFB1*allele and genotype frequencies were calculated by direct counting, while haplotype frequencies and linkage disequilibrium were computed using the Arlequin software version 3.5.1.2 [15].

Nonparametric Wilcoxon rank sum test with continuity correction were used to compare continuous variables. Fisher's exact test was used for pairwise comparison of allele, genotype and haplotype frequencies. Logistic regression and Wald's test were conducted to examine the association between polymorphisms genotypes and the risk of HIV-1 MTCT. The statistical tests were performed with the free software R version 3.1.3 [16]. P-value for linkage disequilibrium analysis was calculated using the permutation test with the EM algorithm, on Arlequin [15], whereas D' and r^2 measures were computed with SNPstat [17]. Post-hoc power calculations were performed with G*Power software version 3.1.9.2 using post-hoc calculation using Fisher's exact test [18].

Results

HIV-1 MTCT status significantly correlated with maternal CD4 cells count and plasma viral load (Wilcoxon rank sum test with continuity correction p=3.544e-08 and p=2.35e-13 respectively) (table supplementary 1).

DEFB1 polymorphisms at position -52G>A, -44C>G and -20G>A were in Hardy Weinberg equilibrium (HWE) in all groups (both of mothers and children) with

the exception of the -44C>G variation in HIV- children group (table 1, 2 and supplementary table 2). The polymorphisms were in linkage disequilibrium in the children but not in the mothers (D'>0.51, r^2>0.01, p<0.04 and D'>0.645, r^2>0.02, p<0.07 respectively) and combined to form three major haplotypes, namely ACGA, GCAA, ACGG and other minor haplotypes (with frequency <0.05).

Analysing *DEFB1* polymorphisms' allele and genotype frequency distribution no statistical significant differences were observed between HIV+ and HIV- children, also when stratifying for HIV-1 routes of MTCT (table 1, table 2). An exception was the c.*87 G allele, more frequent among IU HIV+ and associated with increased risk of IU HIV-1 MTCT (p=0.02, OR=2.09, CI=1.05-4.10, power=0.57; table 2).

When *DEFB1* haplotypes were considered, the GCGA haplotype was significantly more frequent among HIV- children than ACGA more represented in HIV+ and associated with protection towards HIV-1 infection (p=0.002; OR=0.18; CI=0.03-0.61; power: 0.93; table 1). When children were stratified according to HIV-1 route of MTCT the ACGG haplotype was more represented among HIV- respect to the ACGA haplotype, more frequent among IP HIV+, and associated with a protection towards IP HIV-1 MTCT (p=0.006 OR= 0.23; CI=0.06-0.70; power: 0.80 table 2).

No statistical significant association was detected between maternal *DEFB1* polymorphisms and the risk of HIV-1 MTCT, comparing NTR and TR mothers (table supplementary 1).

Finally no association was found between the maternal *DEFB1*polymorphisms' genotypes and maternal plasma viral load (data not shown).

The mothers and children data were also stratified based on maternal CD4 cells count and plasma viral load (CD4+cells count: cut off=350 cells/mm^3; mothers group (n=101): low=17-347 cells/mm^3, high=350-925 cells/mm^3; children's mothers

(n=331): low=17-349 cells/mm^3, high=350-1207 cells/mm^3; plasma viral load cut off=50000 copies/ml; mothers group (n=101): low=399-48180 copies/ml, high=50800-75001 copies/ml; children's mothers (n=331): low=399-49748 copies/ml, high=50291-75001 copies/ml. *DEFB1*polymorphisms frequencies were not significantly different comparing HIV+ and HIV- children and also between TR and NTR mothers (table supplementary 3 and 4 respectively).

Discussion

In our study we tested the possible association between functional variations at *DEFB1* gene and susceptibility to HIV-1 vertical transmission in mothers and children from Zambia: specifically we analysed the frequency distributions of four polymorphisms within *DEFB1* gene comparing TR and NTR mothers and HIV-1 positive and negative children.

DEFB1 c.*87 A allele associated with decreased susceptibility towards IU HIV-1 MTCT; additionally considering *DEFB1* haplotypes, the GCGA associated with protection towards acquiring HIV-1 MTCT, moreover when the children were stratified according to the route of virus MTCT, the ACGG haplotypes were correlated with decreased IP HIV-1 MTCT susceptibility.

Other previous works showed associations between *DEFB1* polymorphisms and HIV-1 infection in other ethnic groups. In Italians (European Caucasian) the increased HIV-1 infection susceptibility was associated with -44 C/C genotype [19, 20], with -52 A allele [6] and with -52A/-44C haplotype [6] among children. Moreover, the maternal -52G/G genotype and 52G/-44G haplotype were correlated with protection against HIV-1 MTCT [6]. Instead, in Brazilians children the HIV-1 infection susceptibility was associated with -52 A/A and -20 G/G genotype [10], although

another work by Segat et al. did not highlight any statistically significant associations [21].

Another study investigated the role of the 5'UTR *DEFB1* polymorphisms in seropositive mother and their infants comparing them with healthy women and their newborns in a population from a Colombia, but no statistically significant different polymorphism frequencies were observed between the two groups [9].

The differences between our findings respect to the previous studies cited above could be explained based upon the different ethnic origin of the population analysed, and considering the fact that in some studies the comparison has been conducted between healthy subjects and HIV-1 infected individuals and not between exposed un-infected and infected subjects; furthermore only our study considered the virus route of transmission, while the others just reported general susceptibility to HIV-1 MTCT. Instead, for the 3'UTR c.*87A>G polymorphism this was the first study that considered this genetic variant in the context of HIV-1 infection and reported its association with the risk of MTCT.

Beta defensins are important mediators of innate mucosal defence against microbial infection and are also known for their antiviral activities [22]. There are controversial reports regarding *DEFB1* expression in various fluid tissues or cell lines [11, 12, 23, 24, 25], nevertheless hBD-1 is constitutively expressed at the mucosa surface [22] and, very important for HIV-1 MTCT, in the placenta [9]. On the other hand, recent evidences suggested that hBD-1 could be also induced by virus infection *in vitro* and *in vivo*[26], and also in conventional monocytes from HIV-1 infected patients in the acute phase but not in the chronic stage [27]

Our study indicated that the four *DEFB1* polymorphisms could be involved in the HIV-1 infection susceptibility, suggesting also an additive and cooperative effect: it is possible to speculate that the *DEFB1* c.*87 G allele genotype and *DEFB1* ACGA haplotype, more frequent among HIV+ children might decrease mRNA *DEFB1*

expression, consequently diminishing hBD-1 levels, thus leading to an increased risk of acquiring HIV-1 infection. Indeed, a possible explanation of the 5'UTR polymorphisms haplotypes effect as a post-transcriptional regulation was suggested: haplotypes could impact on RNA folding and so on its expression as suggested by previous works [23, 28]. Moreover, our results were supported by our previous research [24], where the A/G genotype and *DEFB1* ACGA haplotype were correlated with low concentration of hBD-1 in saliva, although the samples were Italian healthy controls.

We are aware of a limitation of our study since the lack of biological samples other than dried blood spots used for DNA extraction, didn't allow us to quantify hBD-1 protein to validate our hypothesis.

In our study *DEFB1* polymorphisms were not correlated with maternal viral load or CD4 cells count, however former works evidenced that -52 G/G genotype was correlated with low levels of HIV-1 RNA in breast milk of Mozambican women [29]. Moreover, -52 G/G genotype and -44C/G genotype were associated with low plasma HIV-1 RNA in Italian women [6]. The difference could be due to different states of disease progression not specified in these studies.

Despite the importance of subject's genetic background, other major factors are supposed to be involved in the susceptibility to the HIV-1 infection such as maternal viral load, virus subtypes and advancement of immune deficiency status [30].

In agreement with previous research, in our study HIV-1 MTCT was associated with low maternal CD4 cells count and high plasma viral load. Garcia et al. found high HIV-1 RNA plasma levels associated with a significant risk of HIV-1 MTCT [31], similarly in population maternal viral loads were significantly higher in transmitters than in non-transmitters.Moreover, low maternal CD4 cells count correlated with an increased risk of HIV-1 MTCT and this finding was in agreement with previous studies [32].

In spite of our positive findings, taken into account some deviations from HWE, probably due to the low number of subjects present in some groups, and the medium value of the power analysis, further studies should be necessary to clarify the role of *DEFB1* gene polymorphisms in the multifactorial trait HIV-1 MTCT, since we just analysed the genome component of our samples not being available any biological material other than dried blood spot, for the functional validation (i.e. ELISA quantification of hBD1) of the associations observed, although as mentioned above, our previous results [24] supported our current data.

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Conflict of interest

The authors declared no conflict of interest

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Table **1**: analysis of HIV-1 MTCT according to infant *DEFB1*polymorphismsallele, genotype and haplotype frequencies comparing HIV-1 infected (HIV+) and HIV-1 exposed but not infected (HIV-) children.

	HIV+[a]	HIV-[b]	HIV+[a] vs. HIV-[b]
	n=85	n=246	**p-value, O.R.[c], 95% C.I.[d]**
	0.61 (103)	0.60 (293)	reference
	0.39 (67)	0.40 (199)	p=0.86; OR[c]=0.96; CI[d]=0.66-1.39
	0.36 (31)	0.36 (89)	reference
	0.48 (41)	0.47 (115)	p=1.00; OR[c]=1.02; CI[d]=0.57-1.83
	0.15 (13)	0.17 (42)	p=0.85; OR[c]=0.89; CI[d]=0.38-1.97
	χ^2=0.01; p=0.93	χ^2=0.22; p=0.64	
	0.95 (161)	0.94 (462)	reference
	0.05 (9)	0.06 (30)	p=0.85; OR[c]=0.86 CI[d]=0.35-1.91
	0.89 (76)	0.89 (219)	reference
	0.11 (9)	0.10 (24)	p=0.83; OR[c]=1.08; CI[d]=0.42-2.54
	0.00 (0)	0.01 (3)	Not calculable
	χ^2=0.27; p=0.61	χ^2=5.39; p=0.02	
	0.69 (117)	0.71 (348)	reference
	0.31 (53)	0.29 (144)	p=0.63; OR[c]=1.09; CI[d]=0.73-1.62
	0.48 (41)	0.51 (125)	reference
	0.41 (35)	0.40 (98)	p=.079; OR[c]=1.09 CI[d]=0.62-1.90

	0.11 (9)	0.09 (23)	p=0.66; OR[c]=1.19; CI[d]=0.45-2.94
	χ^2=0.14; p=0.71	χ^2=0.35; p=0.55	
c.*87A>G			
	0.70 (119)	0.73 (361)	reference
	0.30 (51)	0.27 (131)	p=0.43; OR[c]=1.18; CI[d]=0.79-1.76
	0.47 (40)	0.53 (131)	reference
	0.46 (39)	0.40 (99)	p=0.36; OR[c]=1.29; CI[d]=0.75-2.22
	0.07 (6)	0.06 (16)	p=0.79; OR[c]=1.23; CI[d]=0.37-3.58
	χ^2=0.73; p=0.39	χ^2=0.22; p=0.64	
	0.33 (56)	0.30 (147)	reference
	0.25 (43)	0.25 (121)	p=0.81; OR[c]=0.93; CI[d]=0.57-1.52
	0.22 (38)	0.24 (117)	p=0.55; OR[c]=0.85 CI[d]=0.51-1.41
	0.02 (3)	0.09 (43)	p=0.002; OR[c]=0.18; CI[d]=0.03-0.61
	0.18 (30)	0.13 (64)	p=0.49; OR[c]=1.23; CI[d]=0.69-2.16

a HIV+ = HIV-1 infected children

b HIV- = HIV-1 exposed but not infected children

c OR= odds ratio

d CI= confidence interval

e HWE = Hardy Weinberg equilibrium

Table **2**: *DEFB1* polymorphisms allele, genotype frequencies (and counts) in HIV-1 exposed but not infected children (HIV-) and HIV-1 infected children stratifying for

timing of HIV-1 MTCT in intrauterine (IU), intrapartum (IP) and postpartum (PP) groups.

CHILDREN	IU[a]	IP[b]	PP[c]	HIV-[d]	IU[a] vs HIV-[d]	IP[b] vs HIV-[d]	PP[c] vs HIV-[d]
	n=22	n=25	n=38	n=246	**p-value, O.R.[e], 95% C.I.[f]**	**p-value, O.R.[e], 95% C.I.[f]**	**p-value, O.R.[e], 95% C.I.[f]**
DEFB1							
-52A>G rs1799946							
A	0.57 (25)	0.56 (28)	0.66 (50)	0.60 (293)	reference	reference	reference
G	0.43 (19)	0.44 (22)	0.34 (26)	0.40 (199)	p=0.75; OR[e]=1.12; CI[f]=0.57-2.18	p=0.65; OR[e]=1.16; CI[f]=0.61-2.16	p=0.38; OR[e]=0.77; CI[f]=0.44-1.30
A/A	0.41 (9)	0.28 (7)	0.39 (15)	0.36 (89)	reference	reference	reference
G/A	0.32 (7)	0.56 (14)	0.53 (20)	0.47 (115)	p=0.43; OR[e]=0.60; CI[f]=0.18-1.90	p=0.49; OR[e]=1.54; CI[f]=0.55-4.72	p=1.00; OR[e]=1.03; CI[f]=0.47-2.30
G/G	0.27 (6)	0.16 (4)	0.08 (3)	0.17 (42)	p=0.57; OR[e]=1.41; CI[f]=0.39-4.77	p=0.75; OR[e]=1.21; CI[f]=0.25-5.07	p=0.27; OR[e]=0.43; CI[f]=0.07-1.62
HWE[g]	χ^2=2.72 p=0.10	χ^2=0.46 p=0.50	χ^2=1.09 p=0.30	χ^2=0.22 p=0.64			
-44C>G rs1800972							
C	0.93 (41)	0.92 (46)	0.97 (74)	0.94 (462)	reference	reference	reference
G	0.07 (3)	0.08 (4)	0.03 (2)	0.06 (30)	p=0.75; OR[e]=1.13; CI[f]=0.21-3.87	p=0.54; OR[e]=1.34; CI[f]=0.33-4.04	p=0.29; OR[e]=0.42; CI[f]=0.05-1.70
C/C	0.86 (19)	0.84 (21)	0.95 (36)	0.89 (219)	reference	reference	reference
C/G	0.14 (3)	0.16 (4)	0.05 (2)	0.10 (24)	p=0.48; OR[e]=1.44; CI[f]=0.25-5.44	p=0.31; OR[e]=1.73; CI[f]=0.40-5.77	p=0.55; OR[e]=0.51; CI[f]=0.06-2.20
G/G	0.00 (0)	0.00 (0)	0.00 (0)	0.01 (3)	p=1.00; OR[e]=0.00; CI[f]=0.00-29.21	p=1.00; OR[e]=0.00; CI[f]=0.00-26.33	p=1.00; OR[e]=0.00; CI[f]=0.00-15.13
HWE[g]	χ^2=0.12 p=0.73	χ^2=0.19 p=0.66	χ^2=0.03 p=0.87	χ^2=5.39 p=0.02			

-20A>G rs11362							
G	0.70 (31)	0.70 (35)	0.67 (51)	0.71 (348)	reference	reference	reference
A	0.30 (13)	0.30 (15)	0.33 (25)	0.29 (144)	p=1.00; OR[e]=1.01; CI[f]=0.47-2.06	p=1.00; OR[e]=1.04; CI[f]=0.51-2.02	p=0.50; OR[e]=1.18; CI[f]=0.68-2.03
G/G	0.59 (13)	0.48 (12)	0.42 (16)	0.51 (125)	reference	reference	reference
G/A	0.23 (5)	0.44 (11)	0.50 (19)	0.40 (98)	p=0.22; OR[e]=0.49; CI[f]=0.13-1.53	p=0.82; OR[e]=1.17; CI[f]=0.45-3.03	p=0.27; OR[e]=1.51; CI[f]=0.69-3.32
A/A	0.18 (4)	0.08 (2)	0.08 (3)	0.09 (23)	p=0.48; OR[e]=1.67; CI[f]=0.36-6.05	p=1.00; OR[e]=0.91; CI[f]=0.09-4.50	p=1.00; OR[e]=1.02; CI[f]=0.18-4.00
HWE[g]	χ^2=4.54 p=0.03	χ^2=0.06 p=0.81	χ^2=0.67 p=0.41	χ^2=0.35 p=0.55			
c.*87A>G rs1800971							
A	0.57 (25)	0.82 (41)	0.70 (53)	0.73 (361)	reference	reference	reference
G	0.43 (19)	0.18 (9)	0.30 (23)	0.27 (131)	p=0.02; OR[e]=2.09; CI[f]=1.05-4.10	p=0.23; OR[e]=0.60; CI[f]=0.25-1.31	p=0.49; OR[e]=1.20; CI[f]=0.67-2.08
A/A	0.27 (6)	0.64 (16)	0.47 (18)	0.53 (131)	reference	reference	reference
G/A	0.59 (13)	0.36 (9)	0.45 (17)	0.40 (99)	p=0.05; OR[e]=2.85; CI[f]=0.97-9.49	p=0.53; OR[e]=0.74; CI[f]=0.28-1.88	p=0.59; OR[e]=1.25; CI[f]=0.57-2.71
G/G	0.14 (3)	0.00 (0)	0.08 (3)	0.06 (16)	p=0.08; OR[e]=4.04; CI[f]=0.60-21.25	p=0.37; OR[e]=0.00; CI[f]=0.00-2.39	p=0.81; OR[e]=1.36; CI[f]=0.23-5.48
HWE[g]	χ^2=0.92 p=0.34	χ^2=1.20 p=0.27	χ^2=0.14 p=0.71	χ^2=0.22 p=0.64			
Haplotypes							
ACGA	0.23 (10)	0.44 (22)	0.32 (24)	0.30 (147)	reference	reference	reference
GCAA	0.25 (11)	0.26 (13)	0.25 (19)	0.25 (121)	p=0.65; OR[e]=1.34; CI[f]=0.50-3.64	p=0.47; OR[e]=0.72; CI[f]=0.32-1.56	p=1.00; OR[e]=0.96; CI[f]=0.47-1.93
ACGG							

	0.32 (14)	0.08 (4)	0.26 (20)	0.24 (117)	p=0.20; OR[e]=1.76; CI[f]=0.70-4.59	p=0.006; OR[e]=0.23; CI[f]=0.06-0.70	p=1.00; OR[e]=1.05; CI[f]=0.52-2.09
GCGA	0.00 (0)	0.02 (1)	0.03 (2)	0.09 (43)	p=0.12; OR[e]=0.00; CI[f]=0.00-1.60	p=0.05; OR[e]=0.16; CI[f]=0.00-1.02	p=0.11; OR[e]=0.29; CI[f]=0.03-1.23
others	0.20 (9)	0.20 (10)	0.14 (11)	0.13 (64)	p=0.13; OR[e]=2.06; CI[f]=0.70-5.95	p=1.00; OR[e]=1.04; CI[f]=0.42-2.46	p=1.00; OR[e]=1.05; CI[f]=0.44-2.39

a IU = intrauterine HIV-1 mother to child transmission

b IP = intrapartum HIV-1 mother to child transmission

c PP = postpartum HIV-1 mother to child transmission

d HIV- = HIV-1 exposed but not infected children

e OR= odds ratio

f CI= confidence interval

g HWE = Hardy Weinberg equilibrium