Role of β-Defensin-1 Polymorphisms in Mother-to-Child Transmission of HIV-1

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Background and Objectives: Mother-to-child transmission (MTCT) of HIV-1, the main source of pediatric AIDS, is multifactorial. Defensins provide microbial barriers and function as effectors of innate immunity. This study investigated the relationship between genetic variants of β-defensin-1 gene and MTCT of HIV-1.

Patients and Methods: Three hundred children, 118 HIV-1 infected and 182 HIV-1 uninfected, born to HIV-1–infected mothers who had not undergone antiretroviral therapy during pregnancy, and 84 HIV-1–infected mothers were analyzed. The single nucleotide polymorphisms -44C/G (rs1800972) and -52G/A (rs1799946) were genotyped by TaqMan allelic discrimination assay and sequencing. Statistical analyses were performed using SNPStats and Bonferroni correction for multiple tests.

Results: In children, the -52GG genotype and the -44G/-52G haplotype had a protective role against HIV-1 infection [odds ratio (OR) = 0.52, 95% confidence interval (CI) 0.31 to 0.86, $P = 0.03$ and OR = 0.50, 95% CI 0.31 to 0.83, $P = 0.014$, respectively]. In mothers, the -52GG genotype and the -44G/-52G haplotype were associated with low levels of HIV-1 plasma viremia (<1000 copies/mL) and a lower risk of maternal HIV-1 transmission (OR = 0.14, 95% CI 0.03 to 0.67, $P = 0.009$ and OR = 0.23, 95% CI 0.08 to 0.66, $P = 0.012$, respectively).

Conclusions: These results demonstrate a significant relationship between genetic variants of β-defensin-1 gene, viral load, and MTCT of HIV-1, thus supporting a critical role of innate immunity in pediatric HIV-1 infection.

Key Words: HIV-1, mother-to-child transmission, natural immunity, β-defensins

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INTRODUCTION

Approximately 2 million infants live with HIV-1 infection and more than 500,000 were born HIV-1 infected in 2007 worldwide. Mother-to-child transmission (MTCT) is the main source of pediatric HIV-1 infection, and it primarily occurs during the intrapartum period. Without antiretroviral therapy (ART), MTCT of HIV-1 ranges from 15% to 40% and it is multifactorial: high maternal viral load, low CD4 T-cell number, and mode of delivery are important maternal factors.[1,2] Characteristics specific to the newborn are also relevant; in neonates, the immune system is under development, and factors conferring "innate resistance" and/or "innate immunity" might be critical. Chemokines and other circulating factors, and deletion in the CCR5 viral coreceptor, have been described as host factors that confer resistance to infection by HIV-1.[2,3]

Defensins provide microbial barriers and are important effectors of innate immunity.[4] They are small cationic peptides mainly produced by leukocytes and epithelial cells and classified into α, β, and θ groups, according to size and binding patterns of disulfide bonds within mature peptides.[4] Human β-defensins, mainly expressed by epithelial cells, serve as an antimicrobial host defense system at critical interfaces between host and environment.[5,6] The antiviral activity of β-defensins involves several mechanisms, including direct interaction with viral envelopes and target cells. The β-defensins 2 and 3 inhibit HIV-1 replication through HIV-1 inactivation and downregulation of CXCR4 coreceptor.[7] Antiviral activity of β-defensins may also be related to their ability to attract cells of the immune system to a target tissue.[8] A recent study demonstrated that vaginal and endocervical mucosa of exposed seronegative individuals had higher levels of β-defensins 1, 2, and 3 than healthy controls; in addition, vaginal mucosa of exposed seronegative subjects had higher level of β-defensin-1 than seropositive individuals.[9] Recent studies suggest that single nucleotide polymorphisms (SNPs) in the 5′-untranslated region of the β-defensin-1 (*DEFB1*) gene (MIM#602056) influence susceptibility/resistance to infection by microbial[10,11] and viral[12,13] agents. Two SNPs, -44C/G (rs1800972) and -52G/A (rs1799946), have been proposed to be involved in MTCT of HIV-1 but their role has not yet been clearly defined. Both SNPs are located in the 5′-untranslated region of the *DEFB1* gene; they do not induce amino acid change, but the -44C/G transversion creates a putative binding site for the nuclear factor-kB,[10] and both SNPs affect gene expression.[14,15] A role for the -52G/A SNP was suggested in a study performed in Brazilian HIV-1–infected and exposed uninfected children,[13] whereas a study performed in Italian

HIV-1–infected and unexposed uninfected children did not disclose this association but rather indicated a relationship between the -44C/G SNP and risk of MTCT.[12] However, this latter study did not investigate SNPs in exposed uninfected children. Furthermore, no data are available concerning the relationship between SNPs in mothers, viral load at delivery, and MTCT.

We investigated the role of the -44C/G and -52G/A SNPs in perinatal infection of HIV-1 by analyzing a large cohort of HIV-1–infected and HIV-1–exposed uninfected infants, all born to HIV-1–infected mothers. In addition, in a subset of mothers with available samples at delivery, the relationship between these 2 SNPs, viral load, and risk of HIV-1 transmission was defined.

SUBJECTS AND METHODS

Subjects

This study involved children born to HIV-1–seropositive mothers between 1984 and 1996, whose virological analyses for diagnosis of HIV-1 infection were conducted at the AIDS Reference Center of Padova University. Inclusion criteria were the known HIV-1–seropositive status of the mother at delivery, the absence of ART prophylaxis during gestation and/or at delivery, and ethnicity. The study population included 300 white children; 282 were born by vaginal delivery and 18 by cesarean section, and none was breastfeed. Most of the children attended the Pediatric Department of Padova University, and 239 were followed since birth. Peripheral blood samples were obtained every 30 days in the first 3 months of life and every 2–3 months thereafter. Diagnosis of HIV-1 infection was performed by virus culture and polymerase chain reaction (PCR), as detailed elsewhere.[16,17] For all children included in this study, 118 HIV-1–infected and 182 HIV-1–uninfected children, the infection was confirmed by disease onset and/or persistence of HIV-1 antibody after 18 months of age, whereas the lack of infection was confirmed by the loss of HIV-1 antibodies. For a subset of children (n = 84; 20 HIV-1–infected and 64 HIV-1–uninfected children), maternal samples at delivery were available for SNP analysis. The study was approved by the local Ethical Committee.

SNP Analysis

Genomic DNA was extracted from peripheral blood mononuclear cells with the QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Polymorphic sites in genomic DNA were analyzed by the TaqMan allelic discrimination assay. Primers and probes to specifically determine the SNP sites -44C/G (rs1800972) and -52G/A (rs1799946) were designed with the Primer Express software (version 1.5, Applied Biosystems, Foster City, CA) on the genomic DNA sequence of the *DEFB1* gene (GenBank accession number U50930). The primers were forward 5′-gAggTTgTgCAATCCACCAgTCT-3′ and reverse 5′-gTTCTCATggCgA CTggCA-3′. The probes were as follows (allele-specific nucleotides are underlined): FAM-5′-AGCCAGCGTCTCCCCAGTTCC-3′-TAMRA (for -44G), VIC-5′-AGCCAGCCTCTCCCCA GTTCC-3′-TAMRA (for -44C), FAM-5′-GCTCAGCCTCCAAAGAAGCC-3′-TAMRA (for -52A), and VIC-5′-GCTCAGCCTCCAAAGGAGCC-3′-TAMRA (for -52G). PCR was performed in a thermal cycler (ABI PRISM 7700, Applied Biosystems) in a reaction volume of 25 μL containing 600 nM of each primer, 100 nM of each probe, 12.5 μL of 2× TaqMan Universal PCR Master Mix (Applied Biosystems), and 1 ng of sample DNA. Thermal cycling conditions were 2 minutes at 50°C, 10 minutes at 95°C, and 45 cycles each of 95°C for 15 seconds and 60°C for 1 minute. The genotypes were assigned using the Sequence Detection System software (version 1.9, Applied Biosystems), analyzing the threshold cycle of amplification curves, and using 3 positive controls, 1 for each of the possible genotypes for both polymorphisms. Accuracy of genotyping was confirmed by direct sequencing of randomly selected samples. PCR amplification was performed using the same primers employed for the TaqMan assay. Reaction mixtures contained 5 μL of 10× PCR Buffer II (Applied Biosystems), 700 nM of each primer, 200 μM of deoxyribonucleotide tri phosphates, 1.6 mM of $MgCl_2$, 1.5 U of AmpliTaq DNA polymerase (Applied Biosystems), 100 ng of DNA template, and water up to a final volume of 45 μL. Amplification was carried out in a thermal cycler (2720, Applied Biosystems) for 40 cycles, each of 30 seconds at 94°C, 1 minute at 68°C, and 1 minute at 72°C. PCR products were purified using a commercial assay (ExoSAP-IT, Amersham Biosciences, Piscataway, NJ) and directly sequenced using the automatic sequencer ABI PRISM 3130*xl* genetic analyzer (Applied Biosystems) and the Big Dye Terminator v1.1 cycle sequencing ready reaction kit (Applied Biosystems), according to the manufacturer's instructions. Sequences were analyzed with Sequencing Analysis software (version 5.2, Applied Biosystems). Results obtained with different methods were fully correlated, which demonstrated the reliability of the TaqMan system used for genotyping SNPs.

Quantification of HIV-1 RNA in Plasma

Plasma HIV-1 RNA levels were determined by reverse transcriptase–PCR (Roche Amplicor Monitor System, Branchburg, NJ) according to the manufacturer's instructions. The lower limit of detection of this assay was 50 HIV-1 RNA copies per milliliter using the ultrasensitive protocol.

Statistical Analysis

Genotype, allele frequencies, and Hardy–Weinberg equilibrium tests of the *DEFB1* -44C/G (rs1800972) and -52G/A (rs1799946) SNPs were performed separately among HIV-1–uninfected and HIV-1–infected children using SNPStats.[18] Estimation of the power of the study to detect an association between SNPs and HIV-1 infection was evaluated using the genetic power calculators available at web sites http://pngu.mgh.harvard.edu/~purcell/gpc/ and http://www.sph.umich.edu/csg/abecasis/CaTS/. With this sample size and considering allele frequencies in this population, our study had 80% power to detect significant ($P < 0.05$) protective effects with odds ratios (ORs) ≤0.58 for the G allele of -44C/G SNP and ≤0.62 for the G allele of -52G/A SNP. Logistic regression analysis was performed with SNPStats, a user-friendly interface that uses the free software environment *R* for statistical computing implemented by the algorithm of the package "genetics," to investigate the association

between SNPs and infection status in children, maternal viral load, and transmission of HIV-1. To increase statistical power, 3 genetic models (codominant, dominant, and recessive) were considered.[19] The ORs and 95% confidence intervals (CIs) were calculated for each group compared with the class with the highest risk of infection (set as having risk = 1). The *P* values were derived from a likelihood ratio test, and the Akaike information criterion (AIC) was used to select the genetic model that best fits the data (ie, the model with the lowest AIC score was the best fitting). Linkage disequilibrium between the SNPs -44C/G and -52G/A was tested using SNPStats and Haploview software (version 3.32). Haplotype analysis was performed with the SNPStats program, which offers the possibility of estimating haplotype frequencies from genotype frequencies using the Expectation-Maximization (EM) algorithm coded into the haplo.stats package. Association of haplotypes to HIV-1 infection was analyzed similarly to that of genotypes, and results are given as OR and 95% CI. The haplotype with the highest risk of HIV-1 infection was selected as the reference category. Univariate comparisons between viral load and genotypes were tested with the nonparametric Kruskall–Wallis test for continuous variables and with the SNPStats program when viral load was reported as a categorical variable. The effects of mode of delivery (vaginal or cesarean) and viral load on SNPs association with HIV-1 infection and transmission were tested with multivariate regression analysis implemented in the SNPStats program. To account for multiple testing, appropriate Bonferroni multiple test correction of *P* values were applied. The *P* values were corrected for 3 and 2 comparisons for genotype and haplotype analyses, respectively.

RESULTS

Relationship Between SNPs in the *DEFB1* Gene and Risk of HIV-1 Infection in Infants

To analyze the impact of -44C/G and -52G/A SNPs in perinatal infection of HIV-1, a cohort of HIV-1–infected (n = 118) and exposed HIV-1–uninfected (n = 182) children were analyzed with different genetic models (Table 1). Genotype and allelic frequencies for the 2 SNPs were in Hardy–Weinberg equilibrium in both groups and were in agreement with white population data reported in the National Center for Biotechnology Information database (data not shown). -44CC and -52AG were the most frequent genotypes in both HIV-1–uninfected and HIV-1–infected children, whereas -44GG and -52GG genotypes were of a higher prevalence in HIV-1–uninfected infants. The -44GG genotype was slightly associated with lower risk of HIV-1 infection in all considered genetic models, but these associations were not confirmed after stringent Bonferroni multiple test correction, whereas the -52GG genotype remained significantly associated with lower risk of HIV-1 infection in the recessive genetic model, even after Bonferroni multiple test correction (OR = 0.52, 95% CI 0.31 to 0.86, $P = 0.03$; AIC 399.5) (Table 1). As expected, linkage disequilibrium analysis using both SNPStats and Haploview software confirmed a strong disequilibrium level with $D' = 0.9989$ and $r^2 = 0.14$ between the 2 SNPs. To investigate their combined effect, given their close proximity, haplotype frequencies were estimated in both groups of children. The most frequent haplotype among HIV-1–infected children was the -44C/-52A, whereas in HIV-1–uninfected children, it was the -44C/-52G haplotype (Table 2). As expected, the -44G/-52G haplotype had a low frequency and was more prevalent in HIV-1—uninfected infants than HIV-1–infected infants. Moreover, no carriers of a -44G/-52A haplotype were predicted or observed. Using the most common haplotype (-44C/-52A) as a reference, the -44G/-52G haplotype was associated with a significantly lower risk of HIV-1 infection (OR = 0.50, 95% CI 0.31 to 0.83, $P = 0.014$) (Table 2), thus supporting a combined effect of the 2 SNPs.

Most children (94%) were born by vaginal delivery. Multivariate regression analysis disclosed that after adjustment for maternal delivery, the -52GG genotype and -44G/-52G haplotype remained significantly associated with a lower risk

TABLE 1. Genotypes of DEFB1 in Infants and Risk of HIV-1 Infection According to 3 Genetic Models

SNPs	Genetic Model		Uninfected Children, No. (%)	Infected Children, No. (%)	OR	95% CI LCL	95% CI UCL	*P*	Corrected *P**	AIC
-44 (rs1800972)	Codominant	C/C	119 (65.4)	90 (76.3)	1	—	—	0.033	0.10	401.3
		C/G	54 (29.7)	27 (22.9)	0.66	0.39	1.13			
		G/G	9 (5.0)	1 (0.8)	0.15	0.02	1.18			
	Dominant	C/C	119 (65.4)	90 (76.3)	1	—	—	0.043	0.13	402.0
		C/G + G/G	63 (34.6)	28 (23.7)	0.59	0.35	0.99			
	Recessive	C/C + C/G	173 (95.0)	117 (99.2)	1	—	—	0.034	0.10	401.6
		G/G	9 (5.0)	1 (0.8)	0.16	0.02	1.31			
-52 (rs1799946)	Codominant	A/A	29 (15.9)	26 (22.0)	1	—	—	0.032	0.10	401.3
		A/G	79 (43.4)	61 (51.7)	0.86	0.46	1.61			
		G/G	74 (40.7)	31 (26.3)	0.46	0.23	0.92			
	Dominant	A/A	29 (15.9)	26 (22.0)	1	—	—	0.190	0.57	404.4
		G/G + A/G	153 (84.1)	92 (78.0)	0.67	0.37	1.2			
	Recessive	A/G + A/A	108 (59.3)	87 (73.7)	1	—	—	0.010	0.03	399.5
		G/G	74 (40.7)	31 (26.3)	0.52	0.31	0.86			

LCL, lower confidence limit; UCL, upper confidence limit.
*Bonferroni multiple test correction.

TABLE 2. DEFB1 Haplotype Frequency and Risk of HIV-1 Infection

-44 (rs1800972)	-52 (rs1799946)	Uninfected Children, %	Infected Children, %	OR	95% CI LCL	95% CI UCL	*P*	Corrected *P**
C	A	37.6	47.9	1	—	—	—	—
C	G	42.6	39.8	0.75	0.52	1.07	0.110	0.220
G	G	19.8	12.3	0.50	0.31	0.83	0.007	0.014

LCL, lower confidence limit; UCL, upper confidence limit.
*Bonferroni multiple test correction.

of HIV-1 infection (OR = 0.52, 95% CI 0.31 to 0.86, $P = 0.033$ and OR = 0.51, 95% CI 0.31 to 0.84, $P = 0.017$, respectively).

Data on maternal viral load at delivery were available in a subgroup of 109 infants. Analysis of allele and genotype frequencies and the Hardy–Weinberg equilibrium in HIV-1–infected and HIV-1–uninfected children in this subgroup were in agreement with analysis on the entire cohort (data not shown). In this group of children, the -52GG genotype and -44G/-52G haplotype tended to be associated with a lower risk of HIV-1 infection in the univariate (OR = 0.39, 95% CI 0.14 to 1.07, $P = 0.054$ and OR = 0.49, 95% CI 0.20 to 1.12, $P = 0.083$, respectively) and multivariate analysis after adjustment for maternal viral load (OR = 0.38, 95% CI 0.14 to 1.05, $P = 0.051$ and OR = 0.48, 95% CI 0.19 to 1.10, $P = 0.082$, respectively).

Relationship Between SNPs in *DEFB1* Gene and Risk of HIV-1 Transmission From Mother-to-Child

The -44C/G and -52G/A SNPs were analyzed in 84 mothers. The genotype distribution for both polymorphisms was in Hardy–Weinberg equilibrium in this subgroup of mothers. The -44CC genotype was more frequent in transmitting mothers, whereas the -44CG genotype was more prevalent in nontransmitting mothers, but these associations were not significant, probably due to the small numbers of -44GG carriers; notably, the 2 women with the -44GG genotype did not transmit infection (Table 3). The -52GG genotype was more prevalent among nontransmitting than transmitting mothers and was significantly associated with a lower risk of HIV-1 transmission, in particular, in the recessive genetic model (OR = 0.14, 95% CI 0.03 to 0.67, $P = 0.009$; AIC 87.4) (Table 3). By comparing mother/child genotypes, it was possible to establish the maternal origin of the alleles in 62 children; the G allele remained significantly associated with a protective effect (OR = 0.28, 95% CI 0.08 to 0.96, $P = 0.04$) even when considering only children who inherited from their mothers the G allele (41 cases) compared with those who inherited the A allele (21 cases). Consistent with what was observed in infants, the most common haplotype in nontransmitting mothers was -44C/-52G, whereas -44C/-52A was more frequent in transmitting mothers, and the -44G/-52G haplotype was associated with a lower risk of HIV-1 transmission (OR = 0.23, CI 0.08 to 0.66, $P = 0.012$) (Table 4). Multivariate regression analysis disclosed that the -52GG genotype and -44G/-52G haplotype remained significantly associated with a lower risk of HIV-1 transmission also after adjustment for maternal viral load (OR = 0.08, 95% CI 0.014 to 0.45, $P < 0.005$ and OR = 0.16, 95% CI 0.08 to 0.31, $P < 0.005$, respectively).

TABLE 3. Genotypes of DEFB1 in Mothers and Risk of HIV-1 Transmission According to 3 Genetic Models

SNPs	Genetic Model		Nontransmitting Mothers, No. (%)	Transmitting Mothers, No. (%)	OR	95% CI LCL	95% CI UCL	*P*	Corrected *P**	AIC
-44 (rs1800972)	Codominant	C/C	41 (64.1)	17 (85.0)	1	—	—	0.14	0.42	94.3
		C/G	21 (32.8)	3 (15.0)	0.34	0.09	1.31			
		G/G	2 (3.1)	0 (0)	0	—	—			
	Dominant	C/C	41 (64.1)	17 (85.0)	1	—	—	0.063	0.189	92.8
		C/G + G/G	23 (35.9)	3 (15.0)	0.31	0.08	1.19			
	Recessive	C/C + C/G	62 (96.9)	20 (100)	1	—	—	0.29	0.87	95.1
		G/G	2 (3.1)	0 (0)	0	—	—			
-52 (rs1799946)	Codominant	A/A	9 (14.1)	5 (25.0)	1	—	—	0.012	0.036	89.4
		A/G	27 (42.2)	13 (65.0)	0.87	0.24	3.11			
		G/G	28 (43.8)	2 (10.0)	0.13	0.02	0.78			
	Dominant	A/A	9 (14.1)	5 (25.0)	1	—	—	0.27	0.81	95.0
		G/G + A/G	55 (85.9)	15 (75.0)	0.49	0.14	1.69			
	Recessive	A/G + A/A	36 (56.2)	18 (90.0)	1	—	—	0.003	0.009	87.4
		G/G	28 (43.8)	2 (10.0)	0.14	0.03	0.67			

LCL, lower confidence limit; UCL, upper confidence limit.
*Bonferroni multiple test correction.

TABLE 4. DEFB1 Haplotype Frequency and Risk of HIV-1 Transmission

-44 (rs1800972)	-52 (rs1799946)	Nontransmitting Mothers, %	Transmitting Mothers, %	OR	95% CI		P	Corrected P*
					LCL	UCL		
C	A	35.2	57.5	1	—	—	—	—
C	G	45.3	35.0	0.47	0.24	0.92	0.027	0.054
G	G	19.5	7.5	0.23	0.08	0.66	0.006	0.012

LCL, lower confidence limit; UCL, upper confidence limit.
*Bonferroni multiple test correction.

Nonetheless, these SNPs may influence expression of β-defensin-1[14,15] and this in turn may affect viral load.[4,20] Mothers with the -44CG genotype had a lower median (range) level of plasma HIV-1 RNA than mothers with the -44CC genotype, that is, 2.65 (1.60–5.00) versus 3.94 (2.30–6.06) $\log_{10}$ copies per milliliter ($P = 0.011$); the 2 mothers with the -44GG genotype had a high viral load (4.42 and 5.07 $\log_{10}$ copies/mL). The median (range) levels of plasma HIV-1 RNA were 3.73 (2.34–5.00), 4.01 (1.60–6.06), and 3.27 (2.30–5.07) $\log_{10}$ copies per milliliter for the -52AA, -52AG, and -52GG genotypes, respectively; mothers with the -52GG had significantly lower levels of HIV-1 RNA than mothers with the -52AA and -52AG genotypes ($P = 0.028$). Notably, for prevention of MTCT of HIV-1, ART was recently recommended for all women with HIV-1 RNA levels of ≥ 3 $\log_{10}$ copies per milliliter.[21] The distribution of genotypes for both SNPs was then studied categorizing mothers according to low (<3 $\log_{10}$ HIV-1 RNA copies/mL) and high (≥ 3 $\log_{10}$ HIV-1 RNA copies/mL) viral load. The -44CG genotype was significantly associated with low viral load in the codominant and dominant genetic models (Table 5). The OR was not estimated in the recessive model due to the low frequency of the -44GG genotype. The -52GG genotype was significantly associated with low viral load, in particular in the recessive genetic model (OR = 0.23, 95% CI 0.08 to 0.63, $P = 0.011$) (Table 5). The most frequent haplotype among mothers with high viral load was the -44C/-52A, whereas the -44C/-52G haplotype was the most frequent in mothers with low viral load. No carrier of the -44G/-52A haplotype was predicted (Table 6). The -44G/-52G haplotype was strongly associated with low viral load (OR = 0.22, 95% CI 0.08 to 0.55, $P = 0.024$) (Table 6).

DISCUSSION

MTCT of HIV-1 is a multifactorial event; even in the absence of prophylaxis, about 70%–80% of infants of HIV-1–infected mothers are born uninfected. Virus threshold levels required to establish infection in the host depend on virus–host interactions. In newborns, in whom the acquired immune response is not fully developed, the innate immune system might play a critical role in conferring resistance to infections.[22] β-defensins are key proteins of innate immunity as they exhibit a broad spectrum of activity against bacteria and viruses and attract cells of the acquired immune system.[8,23] β-defensin-1 is widely expressed, and recent data suggest that polymorphisms in *DEFB1*, the β-defensin-1-encoding gene, may influence susceptibility/resistance to infection. In particular, the promoter region of *DEFB1* displays elevated nucleotide diversity and carries potential

TABLE 5. Genotypes of DEFB1 and Their Association With Viral Load in Mothers According to 3 Genetic Models

SNPs	Genetic Model		Low Viral Load, No. (%)	High Viral Load, No. (%)	OR	95% CI		P	Corrected P*	AIC
						LCL	UCL			
-44 (rs1800972)	Codominant	C/C	10 (43.5)	48 (78.7)	1	—	—	0.0023	0.007	92.4
		C/G	13 (56.5)	11 (18)	0.18	0.06	0.51			
		G/G	0 (0)	2 (3.3)	0	—	—			
	Dominant	C/C	10 (43.5)	48 (78.7)	1	—	—	0.0024	0.007	93.4
		C/G + G/G	13 (56.5)	13 (21.3)	0.21	0.07	0.58			
	Recessive	C/C + C/G	23 (100)	59 (96.7)	1	—	—	0.25	0.75	101.3
		G/G	0 (0)	2 (3.3)	0	—	—			
-52 (rs1799946)	Codominant	A/A	2 (8.7)	12 (19.7)	1	—	—	0.014	0.042	96
		A/G	7 (30.4)	33 (54.1)	0.78	0.14	4.32			
		G/G	14 (60.9)	16 (26.2)	0.19	0.04	1			
	Dominant	A/A	2 (8.7)	12 (19.7)	1	—	—	0.2	0.60	101
		G/G + A/G	21 (91.3)	49 (80.3)	0.39	0.08	1.89			
	Recessive	A/G + A/A	9 (39.1)	45 (73.8)	1	—	—	0.0035	0.011	94.1
		G/G	14 (60.9)	16 (26.2)	0.23	0.08	0.63			

LCL, lower confidence limit; UCL, upper confidence limit.
*Bonferroni multiple test correction.

TABLE 6. DEFB1 Haplotype Frequency and Viral Load in Mothers

-44 (rs1800972)	-52 (rs1799946)	Low Viral Load, %	High Viral Load, %	OR	95% CI		P	Corrected P*
					LCL	UCL		
C	A	23.9	46.7	1	—	—	—	—
C	G	47.8	41.0	0.44	0.22	0.89	0.022	0.044
G	G	28.3	12.3	0.22	0.08	0.55	0.012	0.024

LCL, lower confidence limit; UCL, upper confidence limit.
*Bonferroni multiple test correction.

functional variants.[24] Although the biological function of the *DEFB1* genetic variants are largely unknown, -44 C/G and -52 A/G SNPs have been reported to affect levels of protein expression.[14,15]

This study analyzed the role of these SNPs of the *DEFB1* gene on risk of MTCT of HIV-1. Availability of samples from a large cohort of children born to HIV-1–infected mothers and from a representative subset of mothers allowed us to analyze for the first time the impact of these SNPs on both infant HIV-1 acquisition and maternal HIV-1 transmission. The -52GG genotype was highly protective against perinatal HIV-1 infection; children with these genetic variants were at significantly lower risk for infection. The protective function of the -52GG genotype was confirmed by evidence that mothers with this genotype were at lower risk of transmission. This protective effect against infection and transmission was stronger and statistically significant even after multiple test correction in the recessive genetic model. In contrast, the association of the -44GG genotype with a lower risk of infection did not remain statistically significant after multiple test correction in any genetic model. However, this may have been due to its low frequency; indeed, its tendency to protect against MTCT was supported by the finding that no mothers with this genotype transmitted the virus despite high viral load and by evidence that the haplotype -44G/-52G played a strongly protective role against both infant HIV-1 acquisition and maternal HIV-1 transmission.

These results partially agree and expand previous data concerning the interplay between the 2 *DEFB1* polymorphisms and risk of MTCT of HIV-1. A study performed in Italian HIV-1–infected children and unexposed HIV-uninfected children indicated that the -44CC genotype significantly increased susceptibility to HIV-1 infection,[12] whereas the -52G/A SNP was not found to be significantly associated with risk of MTCT. In contrast, a study performed in Brazilian HIV-1–infected children and exposed HIV-uninfected children did not confirm the role of -44C/G SNP and indicated an association of the -52G/A SNP with risk of MTCT.[13] Our study supports a strongly significant role of -52G/A SNP and for the first time, indicated a role of the -44G/-52G haplotype and risk of MTCT in the white population.

MTCT of HIV-1 mainly occurs during delivery, and maternal viral load is an important risk factor for infants born by vaginal delivery.[1] Polymorphisms may modulate defensin expression, which in turn may influence viral load.[4,20] The -52GG genotype in mothers was associated with low viral load; this is consistent with the finding that the -52A allele reduces β-defensin-1.[14,15] The -44CC was the most frequent genotype in mothers with high viral load; this is consistent with previous reports indicating that this genotype is associated with increased risk of HIV-1 infection[12,25] and with in vitro findings that -44C decreased β-defensin-1 expression.[14] Notably, both the maternal -52GG genotype and -44G/-52G haplotype were associated with lower level of plasma viremia and a lower risk of HIV-1 transmission. The main mechanism by which these SNPs influence MTCT may be by controlling virus levels. Unfortunately, we had only 84 mother/child pairs, and viral load values at delivery were available only in a subgroup of children. However, it should be pointed out that mothers with the -44GG genotype did not transmit infection despite high plasma viral load, and the -52GG genotype and -44G/-52G haplotype were associated with lower risk of MTCT of HIV-1, even after adjustment for maternal viral load.

β-defensin-1 is mainly produced by epithelial cells.[4] It has been recently demonstrated that vaginal mucosa of exposed seronegative subjects had higher level of β-defensin-1 than seropositive individuals.[9] These SNPs might increase protective activity of β-defensin-1 by increasing protein levels in vaginal and other epithelial tissues to a greater extent than in other compartments. It is conceivable that β-defensin-1 might protect against HIV-1 infection through multiple pathways; it may be crucial in killing the virus directly and in protecting skin and mucosa of newborns during delivery. Functional studies would deepen our knowledge of the mechanisms by which the SNPs mediate the protective effects of β-defensin-1.

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