

Role of Beta-Defensin-1 in Mother-to-Child HIV-1 Transmission

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ABSTRACT

Background: In mother-to-child transmission (MTCT) of HIV-1, the main source of paediatric AIDS, host-HIV-1 interaction occurs when the host's immune system is still under development. Defensins are small cationic antimicrobial peptides that play an important role in host defense, as part of the innate immune system. The aim of this study was to investigate the influence of two nucleotide polymorphisms (SNPs) in the 5'-untranslated region (UTR) of the beta-defensin-1 (DEFB1) gene on the MTCT of HIV-1.

Methods: 300 children, 118 HIV-1 infected and 182 HIV-1 uninfected, were analyzed. All children were born to HIV-1 positive mothers who had not undergone any antiretroviral therapy during pregnancy to prevent vertical transmission. Genomic DNA, extracted from peripheral blood mononuclear cells, was evaluated for -44C/G and -52G/A polymorphisms by the TaqMan allelic discrimination assay. Genotypes were confirmed in randomly selected samples by sequence analysis. Hardy-Weinberg equilibrium tests, linkage disequilibrium estimation, haplotype frequencies and association of genotype with HIV-infection status were evaluated using SNPStats and Haploview programs.

Results: Genotype distributions of the two polymorphisms were significantly different between the HIV-1 infected and uninfected children ($p \leq 0.05$). The children with -52GG genotype were at lower risk of HIV-1 infection than children with -52AA genotype (odds ratio (OR) = 0.47, 95% confidence interval (CI) 0.24 - 0.92, $p = 0.03$). The analysis of the -44C/G polymorphism indicated that the -44GG genotype tended to be associated with a lower risk of HIV-1 infection compared to the -44CC genotype (OR = 0.15, 95% CI 0.02 - 1.18, $p = 0.07$). Moreover, the haplotype -44G/-52G showed a significant protective role against HIV-1 infection compared to the haplotype -44C/-52A (OR = 0.49, 95% CI 0.30 - 0.79, $p = 0.007$).

Conclusions: Our results demonstrate a significant correlation between the two SNPs located in the 5' UTR of the DEFB1 gene and risk of HIV-1-infection in a paediatric population confirming the importance of innate immunity in HIV-1 infection.

INTRODUCTION

Approximately two million infants live with HIV-1 infection and more than 500,000 were born HIV-1-infected in 2007. Mother-to-child transmission (MTCT) is the main source of paediatric HIV-1 infection. Without antiretroviral therapy (ART), MTCT of HIV-1 ranges from 15% to 30%. MTCT of HIV-1 is multifactorial; maternal plasma viral load and mode of delivery are important maternal factors (1). Factors related to the newborn may also be important; as in neonates the immune system is under development, factors conferring "innate resistance" and/or "innate immunity" may play an important role (2). Defensins are small cationic antimicrobial peptides that function as effectors of innate immunity (3). Human β -defensins are variably expressed and it has been proposed that susceptibility/resistance to infection might depend on expression of the DEFB1 genes that encode β -defensins. Recent studies suggested that single nucleotide polymorphisms (SNPs) in the 5' untranslated region (UTR) of the β -defensin-1 gene (DEFB1) may influence susceptibility/resistance to infection by micobacterial (4, 5) and viral (6, 7) agents.

Two SNPs, -44C/G (rs1800972) and -52G/A (rs1799946), both located in the 5'-UTR region of the DEFB1 gene have been involved in the MTCT of HIV-1. The frequency of the -44CC genotype was significantly higher in Italian HIV-1-infected infants than in unexposed-uninfected children (6). The role of the -52G/A SNP emerged in a study performed in Brazilian HIV-1-infected and exposed-uninfected children (7).

In this study, we investigated the role of these two SNPs, -44C/G (rs1800972) and -52G/A (rs1799946), in determining susceptibility to HIV-1 infection in exposed HIV-1-infected and uninfected Italian children, all born to HIV-1-infected mothers.

PATIENTS AND METHODS

Patients. The study population included 300 children born to HIV-1-seropositive mothers between 1984 and 1996. 118 children were diagnosed as infected by virus isolation and polymerase chain reaction (PCR). All of these children were born to HIV-1-infected mothers who had not undergone any ART during gestation and/or to prevent vertical transmission. Most children (94%) were born by vaginal delivery. This study was approved by the local Ethical Committee.

SNP analysis. Genomic DNA was extracted from peripheral blood mononuclear cells (PBMC). Polymorphic sites in genomic DNA were analyzed by the TaqMan allelic discrimination assay. Primers and probes to specifically determine SNP sites -44C/G (rs1800972), and -52G/A (rs1799946) were designed with the Primer Express software (version 1.5, Applied Biosystems) on the basis of the genomic DNA sequence of the DEFB1 gene (GenBank accession number U50930). The primers were: forward 5'-GAGGTTGTGCAATCCACCACTCT-3' and reverse 5'-GTTCTCATGGCGACTGGCA-3'. The probes were (allele-specific nucleotides are underlined): FAM-5'-AGCCAGCGTCTCCCAAGTTCC-3'-TAMRA (for -44G), VIC-5'-AGCCAGCGTCTCCCAAGTTCC-3'-TAMRA (for -44C), FAM-5'-GCTCAGCCTCCAAAGAGCC-3'-TAMRA (for -52A), and VIC-5'-GCTCAGCCTCCAAAGAGCC-3'-TAMRA (for -52G). The PCR was performed in a thermal cycler (ABI PRISM 7700, Applied Biosystems) in a reaction volume of 25 μ l containing 600 nM of each primer, 100 nM of each probe, 12.5 μ l of 2X TaqMan Universal PCR Master Mix (Applied Biosystems) and 1 ng of sample DNA. The thermal cycling conditions were 2 min at 50°C, 10 min at 95°C, and 45 cycles each of 95°C for 15 s and 60°C for 1 min. The genotypes were assigned using the Sequence Detection System software (version 1.9, Applied Biosystems), analyzing the threshold cycle of amplification curves. The accuracy of genotyping was confirmed by known DNA samples of each genotype and by direct sequencing of randomly selected samples.

Statistical analysis. Comparisons of DEFB1 SNPs -44C/G (rs1800972) and -52G/A (rs1799946) frequencies and infection status were tested for significance with the SNPStats, a free-based tool designed for genetic epidemiology purposes (8). Logistic conditional regression models, with HIV-1 infection status as the dependent variable, was employed to obtain odds ratios (OR) and 95% Confidence Intervals (CI), both univariate and adjusted for potential confounders. To increase statistical power, three inheritance genetic models (codominant, dominant and recessive) were considered; p -values were derived from a likelihood ratio test. Using genotype frequencies of the SNPs, the Haploview program estimated the haplotype frequencies that characterized a group of individuals. Association analysis of the haplotypes to HIV-1 infection was translated into 95% CI ORs. To account for multiple testing, the Bonferroni's correction method was applied.

RESULTS

118 children were born HIV-1-infected and 182 were born HIV-1-uninfected. The genotype frequencies of the two DEFB1 SNPs are shown in Figure 1. -44CC and -52GA were the most frequent genotypes in both uninfected and infected children. Genotype and allelic frequencies for the two SNPs were in Hardy-Weinberg equilibrium in both groups and were also in agreement with Caucasian population data reported in the NCBI database (data not shown).

Figure 1. Frequencies of DEFB1 genotypes in HIV-1-uninfected and HIV-1-infected children born to HIV-1-seropositive mothers.

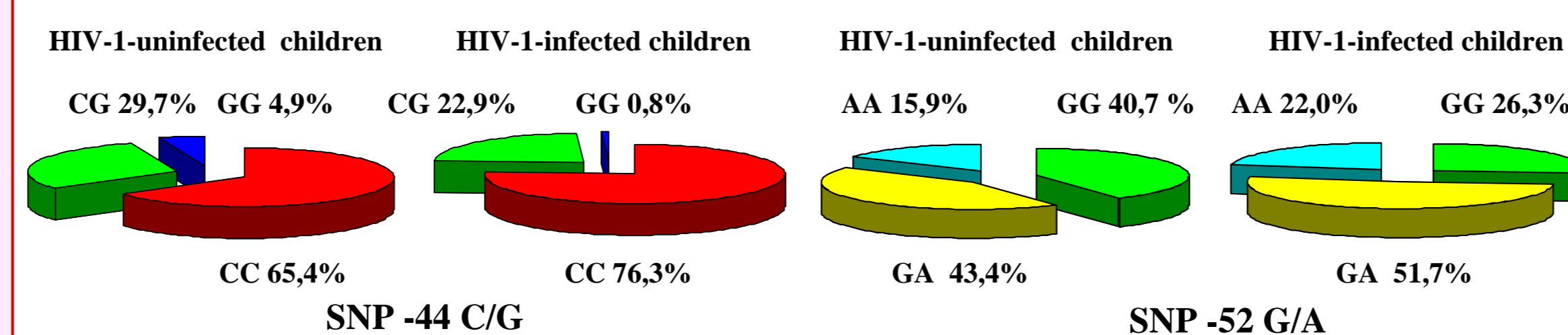


Table 1. Genotypes of DEFB1 and risk of HIV-1 infection.

SNP	Genotype	OR	95% CI		p
			LCL	UCL	
-44 (rs1800972)	GG vs CC	0.15	0.02	1.18	0.07
	GG vs CG	0.22	0.03	1.84	0.16
	CG vs CC	0.66	0.39	1.13	0.13
	G vs C	0.57	0.36	0.91	0.018
-52 (rs1799946)	GG vs AA	0.47	0.24	0.92	0.03
	GG vs GA	0.54	0.32	0.93	0.02
	GA vs AA	0.86	0.46	1.61	0.64
	G vs A	0.66	0.47	0.92	0.013

OR, odds ratio; CI, confidence intervals; LCL, lower confidence limit; UCL, upper confidence limit; p , p -value.

To improve statistical power, the association between SNPs and HIV-1 infection was evaluated considering three inheritance genetic models (codominant, dominant and recessive). The -44GG genotype was slightly significant associated with lower risk of HIV-1 infection in all considered genetic models, but these associations were not confirmed after Bonferroni multiple testing correction. Conversely, for the -52 SNP, the -52GG genotype was significantly associated with a lower risk of HIV-1 infection; when analyzed in the recessive genetic model, this significance was confirmed even after Bonferroni's multiple test correction (Table 2).

Table 2. Genotypes of DEFB1 and risk of HIV-1 infection, according to three genetic models.

SNP	Genetic Model	Uninfected children n	Infected children n	OR	95% CI		p	Corrected p	
					LCL	UCL			
-44(rs1800972)	Codominant	CC	119	90	1				
		CG	54	27	0.66	0.39	1.13	0.03	0.10
		GG	9	1	0.15	0.02	1.18		
	Dominant	CC	119	90	1				
		CG + GG	63	28	0.59	0.35	0.99	0.04	0.13
	Recessive	CC + CG	173	117	1				
GG		9	1	0.16	0.02	1.31	0.03	0.10	
-52(rs1799946)	Codominant	AA	29	26	1				
		GA	79	61	0.86	0.46	1.61	0.03	0.10
		GG	74	31	0.47	0.24	0.92		
	Dominant	AA	29	26	1				
		GG + GA	153	92	0.67	0.37	1.2	0.190	0.57
	Recessive	GA + AA	108	87	1				
GG		74	31	0.52	0.31	0.86	0.010	0.03	

OR, odds ratio; CI, confidence intervals; LCL, lower confidence limit; UCL, upper confidence limit; p , p -value. Statistically significant values after Bonferroni correction are marked in bold.

Values of maternal viral load at delivery were available in a subgroup of 96 infants (median 11295 HIV-1 RNA copies/ml, range 200-2106650 HIV-1 RNA copies/ml). After adjustment for viral load, the -52GG genotype remained significantly associated with a lower risk of HIV-1 infection (OR = 0.28, 95% CI 0.09 - 0.83, $p = 0.03$).

Estimation of haplotype frequencies (Figure 2) identified the most frequent haplotype among infected children to be -44C/-52A, while the -44C/-52G haplotype was the most frequent in uninfected children. The -44G/-52G haplotype had a low frequency, but was more prevalent in uninfected than infected infants. No case of a -44G/-52A haplotype was found.

Figure 2. Haplotype frequencies of DEFB1 in HIV-1-uninfected and HIV-1-infected children born to HIV-1-seropositive mothers.

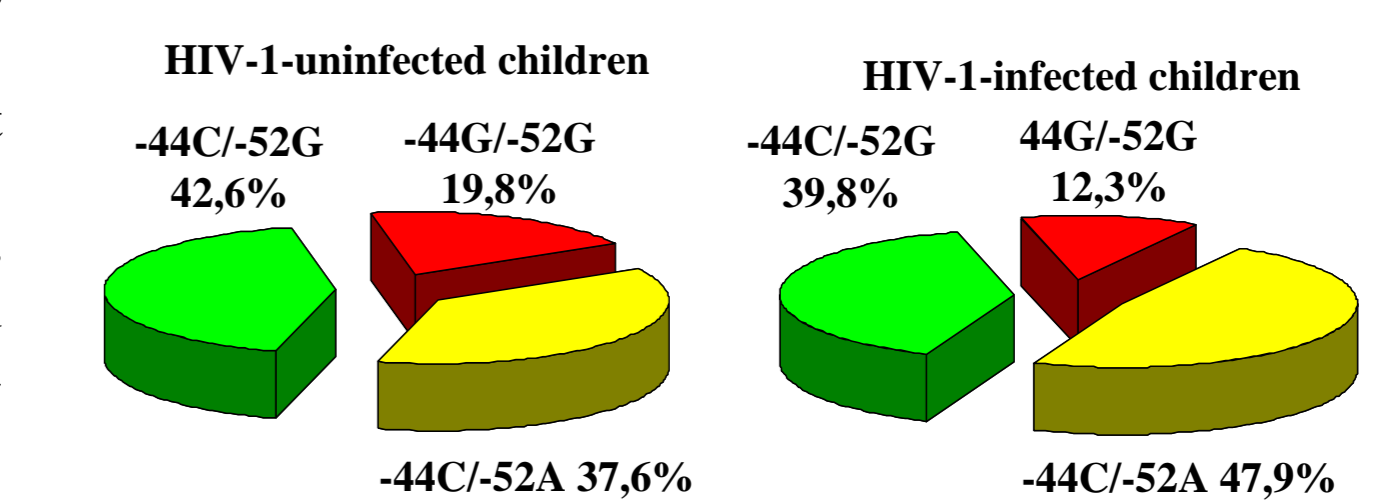


Table 3. DEFB1 haplotypes and risk of HIV-1 infection.

	-44(rs1800972)	-52(rs1799946)	OR	95% CI		p
				LCL	UCL	
1	C	A	1			
2	C	G	0.75	0.52	1.07	0.11
3	G	G	0.49	0.30	0.79	0.007

OR, odds ratio; CI, confidence intervals; LCL, lower confidence limit; UCL, upper confidence limit; p , p -value. Bold characters represent statistically significant values.

The analysis of haplotype frequencies, using the most common haplotype (-44C/-52A) as a reference, showed a significant association between the -44G/-52G haplotype and lower risk of HIV-1 infection. (Table 3).

CONCLUSIONS

The innate immune system is the first line of defense against invading pathogens. It has been suggested that in newborns, in whom the acquired immune response is not fully developed, the innate immune system might play an important role in conferring resistance to infections (9). Several studies support the hypothesis that polymorphisms in genes encoding defensins influence their expression and therefore susceptibility/resistance to infection.

In this study, we investigated the role of two SNPs, -44C/G (rs1800972) and -52G/A (rs1799946), in the DEFB1 gene in susceptibility/resistance to MTCT of HIV-1. The study, performed in Italian HIV-1-infected and exposed-uninfected children all born to HIV-1-seropositive mothers, demonstrated that the -52GG genotype was highly protective against MTCT of HIV-1. This protective effect tended to be significant using the codominant genetic model, but was stronger and statistically significant even after multiple test correction in the recessive genetic model. A weaker correlation was found for the -44GG genotype; p values in the tested genetic models did not remain statistically significant after multiple test corrections. However, its tendency to be protective was supported by the finding that the haplotype -44G/-52G played a strongly protective role against infection.

These results partially agree and extend previous data concerning the interplay between the two DEFB1 SNPs and the risk of MTCT of HIV-1. A study performed in Italian HIV-1-infected and unexposed-uninfected children (6) indicated that the -44CC genotype significantly increased susceptibility to infection, while the -52G/A SNP was not significantly associated with the risk of MTCT. In contrast, a study performed in Brazilian HIV-1-infected and exposed-uninfected children indicated a strong association of the -52G/A SNP with risk of MTCT, while the -44C/G SNP was not significantly involved, partially due to the low frequency of the -44G allele in the Brazilian population (7). Our study performed on a large number of exposed-infected and exposed-uninfected infants confirms the weakly protective role of the -44GG genotype and supports a strongly significant relationship between -52G/A polymorphism and risk of MTCT in an Italian population.

The 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 (3). Interestingly, the -52GG genotype remained significantly associated with a lower risk of infection, even after adjustment for maternal viral load. This finding suggests that DEFB1 may protect against HIV-1 infection through multiple pathways. DEFB1 is mainly produced by epithelial and mononuclear cells in several tissues; it may be important in protecting skin and mucosa of newborns during delivery.

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