Perforin Gene PRF1 c.900C>T Polymorphism and HIV-1 Vertical Transmission

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Abstract

Perforin-1, component of the immune system, is able to control Human Immunodeficiency Virus-1 (HIV-1) replication and it could be involved in HIV-1 mother-to-child transmission (MTCT). This study aims at evaluating the role of c.900C>T PRF1 gene (encoding for perforin-1) polymorphism (rs885822) in HIV-1 MTCT. PRF1 c.900C>T polymorphism was genotyped in 331 children from Zambia using Taqman probe on Real Time PCR platform. PRF1 c.900C>T C/T genotype was more
frequent amongst HIV-1 exposed but non-infected children than in HIV-1 positive cases and the results were confirmed among children infected during breastfeeding. PRF1 c.900C>T correlated with protection against HIV-1 MTCT, suggesting its role in HIV-1 vertical transmission.

**Keywords:** PRF1, perforin, HIV-1 susceptibility.

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Perforin-1 (pore forming protein) is a protein present in the granules of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells (Heintel et al., 2002). In the presence of calcium, Perforin-1 polymerizes and forms channels in the target cell membrane through which other components of lytic granules including granzyme A, granzyme B and granulysin may enter the cells (Shresta et al., 1998; Stenger et al., 1999). Therefore, Perforin-1 is one of the fundamental components of the death machinery of CTLs. CTLs possess anti-viral activity and in Human Immunodeficiency Virus-1 (HIV-1) infection they could concur in the control of viremia both during the initial and the persistent phases of infection (Musey et al., 1997; Ogg et al., 1998). The fraction of perforin-expressing HIV-1 specific CD8+ T cells inversely correlates with the peripheral blood CD4+ T cell count thus being a marker for disease progressions (Shresta et al., 1998).

Perforin-1 expression in *ex vivo* HIV-specific CD8+ T cells was described as higher in healthy controls compared to patients with uncontrolled viral replication and an inverse correlation between perforin-1 expression in HIV-specific CD8+ T cells and viral load was observed (Migueles et al., 2008; Hersperger et al., 2010). Perforin-1 is encoded by the *PRF1* gene, located at 10q22, and one polymorphism, namely c.900C>T (rs885822), was previously associated with HIV-1 vertical transmission in a Brazilian population (Padovan et al., 2011). In the present study the *PRF1 c.900C>T* was analysed in a population of HIV-1 exposed (infected and uninfected) children from Zambia in order to replicate previous findings contributing to disclose its possible involvement in HIV-1 mother to child transmission (MTCT).

In this study a subset of the population enrolled in the Zambia Exclusive Breastfeeding Study (ZEBS, Lusaka Zambia ClinicalTrials.gov Identifier: NCT00310726) was recruited. Briefly, ZEBS is a randomized clinical trial implied in the investigation of the relationship between the time of breastfeeding (i.e. exclusive breastfeeding up to 4 months or breastfeeding with a median of 16 months) and the risk of HIV transmission and child mortality. Between May 2001 and September 2004, 958 HIV-positive women were followed during
pregnancy up to the delivery and until 24 months postpartum (PP) with their infants. Newborns were tested for HIV. All women were counseled to breastfeed to at least 4 months, and then half of them were randomized to stop breastfeeding and the other half to continue it. Women received only a single-dose nevirapine as prophylaxis to prevent HIV-1 MTCT.

For this analysis, 331 infants were selected, 22 had intrauterine (IU) transmission (defined as a positive polymerase chain reaction (PCR) result within 2 days of birth), 25 had intrapartum (IP) MTCT (defined as a positive PCR result within 42 days of birth with an earlier negative result), and 38 had postnatal (breastfeeding) MTCT (defined as a positive PCR results older than 42 days with a negative earlier result in a breastfed child), 256 were HIV-exposed but uninfected children (designed as HIV-). Only 99 mothers were randomly selected for genotyping. All women provided a written informed consent allowing children to participate to the study. All the experiments and procedures involved in this experiment 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 research (protocol L-1106 1 May 2010).

DNA was extracted as reported by Segat et al. (Segat et al., 2014). The PRF1 polymorphism was detected using TaqMan SNPs genotyping C___1799201_10 assays and TaqMan® GTXpress™ Master Mix with the ABI7900HT Real Time PCR platform (Applied Biosystems - Life Technologies Carlsbad California U.S.A.) following manufacturer’s instructions.

The PRF1 allele and genotype frequencies were calculated by direct counting. Fisher’s exact test was used for pairwise comparison of allele and genotype. Logistic regression and Wald’s test were conducted to examine the association between polymorphism genotypes and the risk of HIV-1 MTCT. The statistical tests were performed with the free software R version 3.1.3 (R core Team, 2018). Post-hoc power calculations were performed with G*Power software version 3.1.9.2 using post-hoc calculation using Fisher’s exact test (Faul et al., 2007).

The PRF1 c.900C>T C/T genotype was more frequent among HIV- respect to HIV+ children and was associated with decreased risk of acquiring HIV-1 infection (p=0.03; CI=0.23-0.94; OR=0.47 power=0.68 table 1 and supplementary table 1) also after adjustment for maternal CD4 cells count and HIV-1 plasma viral load (p=0.01 OR=0.40 CI=0.19-0.81; data not shown). When children were subdivided according to the route of transmission, C allele and C/T genotype correlated with protection towards HIV-1 MTCT in the group of PP infected children (C allele: p=0.02 OR=0.35 CI=0.11-0.90 power=0.64 and C/T genotype: p=0.01
OR=0.22 CI=0.04-0.74 power=0.50; table 1 and supplementary table 1) also after adjustment for maternal CD4 cells count and HIV-1 plasma viral load (p=0.009 OR=0.19 CI=0.05-0.66; data not shown).

Our results partially agree with those of Padovan et al. (Padovan et al., 2011), in fact both studies observed an increased frequency of c.900 T allele in the HIV-1 positive children group if compared to the group of HIV-1 exposed but not infected children. Our study found the c.900 T/T genotype to be more frequent among HIV+ respect to T/C genotype, while in the study of Padovan et al. T/T was more frequent compared to C/C homozygous genotype. The study of McIIrioy et al.(McIIroy et al., 2006) also analysed this PRF1 gene polymorphism in a cohort of French HIV+ sero-converters. They observed that PRF1 c.900C>T polymorphism seemed not to alter the amino acidic sequence of perforin-1 protein and it was not associated with HIV-1 infection or progression. The different mode of HIV-1 transmission and different ethnic genomic background could account for the divergent results.

In the current study, we observed an association of PRF1 polymorphism with the susceptibility to HIV-1 in the HIV+ group, but intriguingly, it was confirmed only in the infants that present PP MTCT, thus indicating a protective effect of the variants at birth and not during the pregnancy or the delivery.

The functional effect of this polymorphism on the protein and its possible influence in HIV-1 vertical transmission were not yet reported. A possible hypothesis was suggested by Padovan et al. (Padovan et al., 2011): the PRF1 c.900C>T polymorphism might exert effects on protein expression which might in turn influence NK functionality. Indeed NK response plays a pivotal role in preventing HIV-1 vertical transmission: higher HIV-1 specific NK response was found in HIV-1-infected non transmitter mothers and exposed-uninfected children respect to transmitter mothers and exposed-infected children (Tiemessen et al., 2009). However, this speculation should be confirmed by functional analysis which have not been performed in our study due to the fact that the sole biological material available were dried blood spots.

We are aware that the small sample size of our population could have influenced the statistical analysis, especially in the subgroups classified according to the route of MTCT; however, the quite high power of the statistically significant associations allows us to be enough confident about the statistical relevance of the results. We also decided to not perform correction for multiple tests in order to unravel all the possible associations that could be significant, especially in an infectious disease where role of genetic polymorphisms should be small and since applying multiple test correction our significance will be lost.

Another point that could be taken into account is the MTCT even when the viral HIV-1 RNA was undetectable, the risk MTCT still exists (see for example (Reliquet et al., 2014)), however, the modern test for virologic diagnosis did not reveal the possible infections so possibly creating a bias in our analysis.
Considering our findings and the comparison with the two other studies analyzing the role of PRF1 variants in the context of HIV-1 infection, further association studies in populations of different ethnic backgrounds are necessary in order to disclose the effective role of perforin-1 in HIV-1 MTCT susceptibility.

Conflict of interest
The authors declared no conflict of interest.

Acknowledgements
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References


**Supplementary material**

The supplementary material will be available in the final version of the article.
Table 1 - *PRF1* polymorphism 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.

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<th>IU</th>
<th>IP</th>
<th>PP</th>
<th>HIV-</th>
<th>HIV+ vs. HIV-</th>
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<th>IP vs. HIV-</th>
<th>PP vs. HIV-</th>
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<td>0.12</td>
<td>0.07</td>
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<td>p=0.83; Cl=0.43-2.55; OR=1.11</td>
<td>p=0.54; Cl=0.23-1.68; OR=0.68</td>
<td>p=0.02; Cl=0.11-0.90; OR=0.35</td>
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</table>

HWE = Hardy Weinberg equilibrium

HIV- = HIV-1 exposed but not infected children

HIV+ = HIV-1 infected children

IU = intrauterine HIV-1 mother to child transmission

IP = intrapartum HIV-1 mother to child transmission

PP = postpartum HIV-1 mother to child transmission

OR= odds ratio

CI= confidence interval