

Infant CD4 C868T polymorphism is associated with increased human immunodeficiency virus (HIV-1) acquisition

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Summary

The C868T single nucleotide polymorphism (SNP) in the CD4 receptor encodes an amino acid change that could alter its structure and influence human immunodeficiency virus (HIV-1) infection risk. HIV-1-infected pregnant women in Nairobi were followed with their infants for 1 year postpartum. Among 131 infants, those with the 868T allele were more likely than wild-type infants to acquire HIV-1 overall [hazard ratio (HR) = 1.92, 95% confidence interval (CI) 1.05, 3.50, $P = 0.03$; adjusted HR = 2.03, 95% CI 1.03, 3.98, $P = 0.04$], after adjusting for maternal viral load. This SNP (an allele frequency of ~15% in our cohort) was associated with increased susceptibility to mother-to-child HIV-1 transmission, consistent with a previous study on this polymorphism among Nairobi sex workers.

Keywords: CD4 receptor, genetics, HIV, mother-to-child transmission, single nucleotide polymorphism

Introduction

Mother-to-child transmission of human immunodeficiency virus (HIV-1) is a major problem in sub-Saharan Africa, where transmission rates remain 5- to 10-fold higher than in developed countries [1]. While lack of medical access and resources contribute to high transmission rates, genetic factors may also influence maternal infectivity and infant susceptibility. The most heavily studied genetic polymorphisms relevant to HIV-1 are in genes encoding the CCR5 HIV-1 co-receptor [2–6]. Polymorphisms in the CD4 receptor, the primary receptor for HIV-1, may also influence directly HIV-1 transmission and progression.

C868T (rs28919570), one of only five non-synonymous single nucleotide polymorphisms (SNPs) in CD4, results in an amino acid substitution of tryptophan for arginine in the third domain. C868T was first discovered by an observation showing that individuals of African descent had CD4 molecules that were unable to bind to OKT4 antibody, which recognizes an epitope in the third domain (D3) of CD4 [7]. This single amino acid change in the D3 region may modify

the tertiary structure of CD4, resulting potentially in altered HIV-1 acquisition.

This hypothesis was tested in a study among HIV-1 uninfected Kenyan commercial sex workers (CSW) [8]. Women who were heterozygous for CD4 C868T were more than twice as likely to acquire HIV-1 than those with the wild-type CD4 allele [8]. In addition, the rate of CD4 decline was faster among women heterozygous for the polymorphism than those homozygous for the wild-type allele. These data suggest that in a heterosexual HIV-1 transmission cohort CD4 C868T is associated with increased susceptibility among HIV-1-uninfected individuals and more rapid progression among infected individuals.

In our study, we determined whether infant and maternal CD4 C868T increased risk of vertical HIV-1 transmission overall, by 1 month of age, or late via breast milk in a cohort of HIV-1 infected women and infants in Kenya. A secondary objective was to define the association between this SNP and maternal plasma, genital and breast milk HIV-1 RNA levels, as well as CD4 count.

Methods

Study setting and subjects

HIV-1-seropositive pregnant women were recruited from antenatal clinics in Nairobi and provided written informed consent. This study received ethical approval from the Institutional Review Boards of the University of Washington, University of Manitoba and the Kenyatta National Hospital. Study participants were enrolled at 32 weeks gestation and began taking zidovudine twice daily at 34–36 weeks of pregnancy, and continued through delivery following Kenyan national guidelines at the time of the study [9].

Women were seen in clinic antenatally, at delivery, 2 weeks after birth, and then monthly for the subsequent 12 months. Maternal blood specimens were collected at 32 weeks gestation and at delivery. Cervical swabs were collected at 32 weeks gestation, and breast milk was collected 1 month postpartum. Infants were examined at birth, week 2, and then monthly for 12 months. Infant blood specimens were collected at birth, 2 weeks of age and 1, 3, 6, 9 and 12 months after birth to determine HIV-1 infection status.

Laboratory procedures

HIV-1 RNA viral load was quantified in plasma, cervical secretions and breast milk using the Gen-Probe Transcription Mediated Amplification assay (Gen-Probe Incorporated, San Diego, CA, USA) [10,11]. Filter paper HIV-1 DNA assays and plasma HIV-1 RNA assays (described above) were performed to determine infant infection. The filter paper assay detects HIV-1 *gag* DNA using polymerase chain reaction (PCR) and is sensitive for detection of HIV-1 subtypes A, C and D [11,12]. HIV-1 infection status was determined by either a positive filter paper DNA or plasma RNA assay on two consecutive dates or a single positive filter paper or plasma RNA assay if it was the last available sample.

Genotyping was performed by sequencing analysis, as described previously [8]. Infant DNA was extracted from blood spots using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) according to the dried blood spot protocol. Maternal DNA was extracted from plasma using the AIAamp DNA Mini Kit. CD4 DNA was then amplified using a nested PCR protocol (due to low DNA yield) with two sets of primers: outer amplifying primers 5-GTCCAGGAATCC TAAGGACAGC-3' and 5-CCACCAGGTTCACTTCCTGATG-3; inner amplifying primers 5-GTGGCCTGCTGTAGG AAAATGC-3' and 5-CACCAGGTTCACTTCCTGATGC-3'. The PCR product (50 μ l) was then purified using Montage PCR Centrifugal Filter Devices (Millipore, Billerica, MA, USA) according to the AGTATCTG-3' and 5-TTCCTGTT TTCGCTTCAAG-3' primers. Genotypes were determined by manual inspection of the SNP locus.

Statistical analysis

Timing of vertical transmission was defined as follows: (1) early transmission occurred within 1 month of birth and (2) late transmission occurred between 1 month and 1 year of age among breast feeders. Independent *t*-tests were used to compare maternal CD4 count, and log₁₀-transformed HIV-1 viral loads in plasma, cervical secretions and breast milk among mothers with and without the C868T polymorphism. Kaplan–Meier survival analysis and Cox's proportional hazard regression were used to determine associations between the CD4 polymorphism and HIV-1 transmission overall, and logistic regression was used for early and late transmission. All multivariate analyses were adjusted for maternal plasma HIV-1 load. Analyses were performed using infant genotypes in two models: (1) a co-dominant (allele–dose) model, which assumed that there was a constant change in transmission risk with the number of T alleles; and (2) a dominant model, which assumed that risk was the same in infants with either one or two T alleles. All analyses were specified a priori and data were analysed in STATA version 9.2 (College Station, TX, USA).

Results

Cohort characteristics

Infant and maternal polymorphism data with infant HIV-1 infection status were available for 131 mothers and their singleton infants. At 32 weeks gestation, median maternal CD4 cell count, plasma and cervical HIV-1 RNA load were 412 cells/ μ l [interquartile range (IQR) 295–582], 4.72 log₁₀ copies/ml (IQR, 4.22–5.20) and 2.22 log₁₀ copies/ml (IQR, 1.79–3.37), respectively. At delivery, median maternal plasma viral load was 4.0 log₁₀ copies/ml (IQR, 3.35–4.74) and was correlated strongly with maternal viral load antenatally (Pearson's correlation coefficient, 0.76, *P*-value < 0.001). At 1 month postpartum, for 93 breast-feeding mothers median maternal breast milk HIV-1 RNA load was 2.45 log₁₀ copies/ml (IQR, 1.95–3.41).

Among 131 infants, 30 (23%) were infected during 12 months of follow-up. Of these infections, 23 infants (77%) were detected within 1 month of birth and were classified as early transmission events; seven infants (23%) were detected after 1 month of age and were classified as late or breast milk transmission events (Table 1).

Association between C868T and maternal CD4 and HIV-1 RNA levels

Of the 131 infants, 92 (70%) were wild-type (C/C); 37 (28%) were heterozygous variants (C/T); and two (2%) were homozygous variants (T/T). One hundred and twenty-one mothers had polymorphism data, of whom 89 (74%) women were wild-type; 25 (20%) heterozygous; and seven (6%) homozygous for the variant allele.

Table 1. Description of the cohort.

Characteristics	<i>n</i> = 131*
	Median (interquartile range) or number (%)
Maternal	
Age (years) [†]	24 (21–27)
CD4 ⁺ T cell count at 32 weeks of gestation (cell/ μ l) [‡]	412 (295–582)
Plasma HIV-1 viral load (log ₁₀ copies/ml)	
32 weeks of gestation [§]	4.72 (4.22–5.20)
Delivery [¶]	4.00 (3.35–4.74)
Cervicovaginal HIV-1 viral load (log ₁₀ copies/ml)**	2.22 (1.79–3.37)
Breast milk HIV-1 viral load (log ₁₀ copies/ml) ^{††}	2.45 (1.95–3.41)
Infant	
Gender (percentage male) ^{‡‡}	66 (53%)
Birth weight (kg) ^{§§}	3.1 (2.8–3.4)
Number of HIV-infected infants	30 (23%) ^{†††}
Early transmission ^{¶¶}	23 (77%) ^{†††}
Late or breast milk transmission ^{***}	7 (23%) ^{§§§}

*Number of infants with single nucleotide polymorphism (SNP) data; [†]data available for 125 mothers; [‡]data available for 124 mothers; [§]data available for 124 mothers; [¶]data available for 85 mothers; **data available for 87 mothers; ^{††}data available for 93 mothers; ^{‡‡}data available for 125 infants; ^{§§}data available for 121 infants; ^{¶¶}early transmission occurring within 1 month of birth; ^{***}late or breast milk transmission occurring between 1 month and 1 year of age; ^{†††}percentage of human immunodeficiency virus (HIV)-infected infants with mothers with SNP data; ^{§§§}percentage of early transmission from all HIV-infected infants; ^{§§§}percentage of breast milk transmission from all HIV-infected infants.

When women with wild-type alleles were compared to a combination of heterozygous and homozygous variants using linear regression, no difference was seen in maternal CD4 count at 32 weeks gestation, plasma HIV-1 viral load at 32 weeks gestation and at delivery and cervical or breast milk HIV-1 viral load.

Potential correlates were also analysed using linear regression in a co-dominant (allele–dose) model (data not shown),

which assumed a constant change in association with the number of T alleles. No associations were seen in this model.

Infant C868T polymorphism and risk of vertical transmission of HIV-1

Of the 131 mother–infant pairs, HIV-1 transmission occurred at any time-point in 16 (17%) of 92 wild-type infants, in 13 (35%) of 37 heterozygous infants and in one (50%) of two homozygous variant infants (Table 2). In the co-dominant model, heterozygous variant infants showed a ~two-fold increased risk of HIV-1 acquisition overall compared to wild-type genotype infants, and this retained significance after adjusting for viral load at 32 weeks [adjusted hazard ratio (aHR), 2.03; 95% confidence interval (CI), 1.03, 3.98; *P* = 0.04]. Also, a significant increase in acquisition risk was seen in homozygous variant infants compared to wild-type infants. Because there was only one infection out of two homozygous variant infants, these results need to be interpreted with caution due to the small size. In a dominant model, homozygous variant and heterozygous infants showed a significant increase in acquisition risk compared to wild-type infants [HR, 2.18 (95% CI, 1.06, 4.47); *P* = 0.03]. After adjustment for viral load at 32 weeks, there was a trend towards increased acquisition for infants with the variant T allele [aHR, 2.02 (95% CI, 0.95, 4.27); *P* = 0.07].

Early transmission

Early HIV-1 acquisition occurred in 13 (14%) of 92 wild-type infants, 10 (27%) of 37 heterozygous infants, and none of two homozygous variant infants. In both co-dominant and dominant models, the presence of the infant variant allele showed no significant association with early transmission risk.

Late or breast feeding transmission

Transmission via breast feeding occurred in three (5%) of 62 wild-type infants, three (13%) of 23 heterozygous infants

Table 2. Risk of mother–child human immunodeficiency virus (HIV-1) transmission by infant C868T polymorphism.

Models	Genotype	HIV-1 positive/total (%)	Unadjusted HR/OR* (95% CI) [†]	Adjusted HR/OR (95% CI) [‡]
Co-dominant model [§]	C/C	16/92 (17%)	Reference	Reference
	C/T	13/37 (35%)	1.92 (1.05, 3.50)	2.03 (1.03, 3.98)
	T/T	1/2 (50%)	3.69 (1.10, 12.25) <i>P</i> = 0.03	4.12 (1.06, 15.84) <i>P</i> = 0.04
Dominant model [¶]	C/C	16/92 (17%)	Reference	Reference
	C/T or T/T	14/39 (36%)	2.18 (1.06, 4.47) <i>P</i> = 0.03	2.02 (0.95, 4.27) <i>P</i> = 0.066

For both models, Cox regression was used. *Hazard ratio/odds ratio; [†]confidence interval; [‡]adjusted for maternal viral load at 32 weeks of gestation; [§]co-dominant (allele–dose) model, which assumes that the risk decreased directly with the number of T alleles; [¶]dominant model, which assumes that the decrease in risk was the same in mothers with one or two T alleles.

and one (50%) of two homozygous infants. Because the numbers of homozygous infants and the number of total late transmissions were small, there was not sufficient power to make any conclusions regarding late transmission.

Maternal C868T polymorphism and risk of vertical transmission of HIV-1

Analyses for vertical transmission were also performed for maternal genotype for overall, early and late transmission. We used both dominant and co-dominant models, and the presence of maternal genotype allele did not show a significant change in transmission risk.

Discussion

The presence of infant C868T allele was associated with increased risk of vertical HIV-1 acquisition. These results are consistent with the only previous study on this polymorphism, which found that C868T heterozygosity was associated significantly with increased risk of seroconversion compared to the wild-type among Nairobi CSWs [8]. No significant association was seen with infant C868T polymorphism and early transmission. While we observed a significant increased risk for acquisition of HIV-1 among homozygous infants, the number of infants infected via breast milk was small, making it difficult to draw definitive conclusions on the role of the C868T polymorphism in late transmission events. In addition, maternal C868T polymorphism was not associated significantly with vertical transmission, nor was it associated with maternal CD4 or maternal HIV-1 viral levels in plasma, cervical secretions or breast milk, suggesting that the C868T polymorphism affects host-susceptibility to vertical acquisition of HIV-1 rather than transmitter infectivity.

In our study, the C868T allele frequency in mothers and infants was ~15%, which is consistent with allele frequency findings from previous studies investigating the polymorphism. Allele frequencies in Kenyan CSW (17%) and among African Americans (19%) were higher than what has been observed in Caucasian cohorts (<1%) [8,13]. If this polymorphism plays a role in HIV-1 acquisition or progression, the large difference in C868T allele frequency in different races may be one mechanism to explain racial disparities in HIV prevalence.

The mechanism for increased overall acquisition among heterozygous infants compared to wild-type genotype infants is unknown. One possibility is that the C868T polymorphism changes the tertiary structure of the CD4 receptor, thereby altering the affinity of gp120 for CD4 [14]. This altered affinity may decrease the threshold for HIV acquisition and explain increased overall vertical transmission. Another possibility is that the presence of the infant polymorphism allele may modify signal transduction pathways necessary for HIV-1 replication [8]. The presence of C868T

allele may lead to more activated CD4 T cells, leading to an increased likelihood for HIV infection.

In summary, in this cohort, presence of infant CD4 868T allele was associated with increased risk of infant HIV-1 acquisition. We conclude that a better understanding of genetic co-factors associated with HIV-1 acquisition and progression, especially those related to CD4 and HIV-1 co-receptors, may lead to improved or innovative HIV-1 interventions in therapy and prevention.

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Disclosure

None.

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