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Toll-Like Receptor (TLR) 9 polymorphism is associated with increased Epstein-Barr virus and Cytomegalovirus acquisition in HIV-exposed infants

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Abstract

Polymorphisms in the Toll-Like Receptor (TLR) 9 1635 locus have been associated with HIV-1 acquisition and progression. Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) acquisition were compared between Kenyan HIV-exposed infants by 1635 genotype. Having 1 or more copies of the 1635A allele was associated with increased CMV acquisition in HIV-infected infants (42% vs 11%, $p=0.03$) and increased risk of EBV acquisition in HIV-exposed uninfected infants ($HR=4.2$, $p=0.02$) compared to 1635GG. Additionally, 1635A was associated with 0.4 log₁₀ copies/ml lower median EBV levels in HIV-infected infants ($p=0.03$). These data suggest a potentially important role for this locus in primary herpesvirus infection.

Keywords

pediatric; HIV; cytomegalovirus; Epstein-Barr virus; Toll-Like Receptor; genetic epidemiology; innate immunity

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Authors' contributions

KBS, RM, and JL conducted the genomics studies and analysis, JAS conducted the herpesvirus studies and analyses, GJS/DW and EM-O designed the cohort study and provided the clinical data and specimens, SG and CC provided expertise in the interpretation of the herpes data and manuscript preparation, JAS and KBS wrote the manuscript together.

Toll-like receptors (TLRs) recognize conserved antigenic motifs, enabling broad non-specific pathogen recognition. Single nucleotide polymorphisms (SNPs) in TLR genes may impact the natural history of viral infections, including HIV [1–8] and human herpesviruses [9]. The TLR9 1635 (rs352140) locus is of interest because having one or more copies of 1635A is associated with increased risk of HIV acquisition in discordant couples [4] and infants [1]. Paradoxically, 1635AA was associated with *lower* HIV viral loads or *slower* CD4 decline/HIV progression compared to 1635GG in some studies [2, 6], including the infant cohort where it was found to be associated with increased HIV acquisition risk [1]. However, other studies found 1635AA to be associated with lower CD4 count or higher HIV viral loads, suggesting differences in among genetically-distinct populations [5, 7].

Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are often acquired in infancy [10–12], establish persistent infection with prolonged shedding, and are opportunistic infections in HIV [10, 13–17]. To date there are few studies of TLR polymorphisms in herpesvirus infections. Mutations in TLR3 increase susceptibility to herpes simplex virus encephalitis in children after primary infection [18]. SNPs in TLR2, -4, and 9 have been examined in the context of congenital CMV; TLR9 1635A was associated with risk in Polish [19–21], but not Japanese infants [22]. We are aware of no published studies examining TLR9 polymorphisms and primary EBV infection.

In a Kenyan infant cohort, having one or more copies of the TLR9 1635A allele was found to be associated with increased HIV acquisition risk and lower HIV viral load [1]. In this secondary analysis, we evaluate whether 1635A is also associated with EBV and CMV risk or viremia. HIV infected mother-infant pairs participated in an observational study in Nairobi, Kenya from 1999–2003 [23][24]. HIV, CMV and EBV DNA levels were assessed from birth to 1 year in HIV-exposed uninfected infants (HEU) and HIV-infected (HIV+) infants, with sampling at birth, 1, 3, 6, 9 and 12 months of age; HIV+ children were followed for an additional year at quarterly intervals. Follow-up [23, 25], and CMV [10] and EBV [11] assays are detailed elsewhere. For host genetic studies, infant DNA was extracted from cryopreserved blood and genotyped for TLR9 1635 (rs352140) and a set of genetic ancestry informative markers (AIMS) using an Illumina Custom Oligo Pooled Assay microarray platform (San Diego, CA) [1].

All analyses were conducted using StataSE 14.0 (College Station, TX), using two tailed tests with $\alpha=0.05$. Based on previous associations between TLR9 and HIV, initial analyses were conducted assuming a dominant model of inheritance; we confirmed this was also the most appropriate inheritance model for CMV and EBV. The Mann-Whitney U test was used to compare the baseline (first detectable) viral DNA level in plasma between genotype groups, because this time-point had the most data and was the peak level for most infants. The analysis for viral acquisition differed for EBV and CMV because the pattern of acquisition differed by virus and HIV status. As EBV transmissions were dispersed over the first 2 years [11], Cox proportional hazards regression was performed to assess the risk of EBV infection, Kaplan-Meier was used to estimate probabilities of infection at 12 months, and the z test was used to compare probabilities between genotypes. As nearly all infants were CMV positive by 3 months of age, there were not adequate time-points for a time-to-event analysis [10]; Pearson's Chi squared test was used to compare the proportion of

infants with CMV detected by 1 month between TLR genotypes. AIMS were homogenous in this population and did not affect point estimates of association; given the small sample size these were excluded from final analyses (data not shown).

The study cohort of 368 infants with host genetic data has been described elsewhere [1]. Prevalence of the 1635A allele was 49% (180/368) in the overall cohort, with 188 (51%) GG, 136 GA (37%) and 44 AA (12%).

CMV and TLR9 genotype data were available in a subset 37 HIV+ infants and 19 HEU. At 1 month of age, 42% of HIV+ infants with 1 or more copies of the 1635A allele had acquired CMV, compared to 11% in infants with the 1635 GG genotype ($p=0.03$; Table 1). We did not detect a difference in baseline CMV viral load by TLR9 genotype in either the HIV+ or HEU infants.

EBV and TLR9 genotype data were available in 62 HIV+ and 42 HEU infants. The probability of EBV infection by 12 months of age was 0.82 in HIV+ infants with 1 or more copies of 1635A, and 0.67 in infants with two copies of the 1635G allele ($p=0.2$). The 1635A variant was not associated with EBV risk, univariately ($HR=1.5$, 95%CI=0.74–2.9; $p=0.3$), or adjusting for maternal CD4 percent at 32 weeks gestation ($aHR=1.4$, 95%CI=0.71–2.7, $p=0.3$). However, infants with 1 or more copies of the 1635A allele had a median baseline EBV viral load that was 0.4 log₁₀ copies/ml *lower* than infants with two copies of the 1635G allele ($p=0.02$).

In HEU infants, having one or more copies of the 1635A allele was associated with a 4.2-fold increased risk of EBV acquisition univariately ($HR=4.2$, 95%CI=1.2–15; $p=0.02$), and adjusting for maternal CD4 percent ($aHR=4.2$, 95%CI=1.2–15; $p=0.03$). The probability of EBV infection by 12 months of age was 0.67 in infants with one or more copies of 1635A and 0.21 in infants with two copies of 1635G ($p=0.003$).

This small study suggests polymorphisms in TLR9 1635 may alter defenses against herpesvirus infections during infancy. Importantly, these effects appear to be modified by HIV infection, which has global and profound impacts on host immunity. Immunosuppression, or unmeasured maternal factors may explain some of the differences observed between HIV+ and HEU infants. The A/G substitution is synonymous and occurs in exon 2 of the TLR9 gene, and it is unknown whether this SNP affects TLR9 expression or reflects changes in another gene in linkage disequilibrium. Studies to determine the effect of 1635A on TLR9 expression and function are needed to understand its impact on acquisition and viral control.

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Table 1

Transmission and viral containment in HIV infected and exposed uninfected infants by TLR9 allele polymorphism.

	HIV+ infants		HEU infants		p value
	TLR9 GA or AA	TLR9 GG	TLR9 GA or AA	TLR9 GG	
CMV infection and containment					
Ever CMV infected ^a	100% (19/19)	89% (16/18)	100% (10/10)	89% (8/9)	0.3
CMV infected by 1 month ^a	42% (8/19)	11% (2/18)	20% (2/10)	22% (2/9)	0.9
Median log ₁₀ CMV DNA copies/ml (IQR) ^b	2.4 (2.1–3.1)	2.9 (2.1–3.1)	2.5 (1.7–3.3)	2.3 (2.1–2.4)	0.8
EBV infection and containment					
Ever EBV infected ^a	69% (24/35)	78% (21/27)	57% (13/23)	16% (3/19)	0.007
Probability of EBV infection at 12 months [95%CI] ^c	0.82 [0.64–0.94]	0.67 [0.47–0.85]	0.67 [0.46–0.86]	0.21 [0.075–0.53]	0.003
Median log ₁₀ EBV DNA copies/ml (IQR) ^b	2.4 (2.0–2.6)	2.8 (2.4–2.9)	2.1 (1.8–2.5)	1.9 ^d	*

Notes.

^a Chi-square test,

^b Mann-Whitney U test,

^c z test comparing infection probabilities at 12 months from Kaplan-Meier model.

^d Only 1 HEU infant was EBV viremic in the GG group, so comparison was not possible.