RESEARCH BRIEF

Genetic Factors Associated with Slow Progression of HIV among Perinatally-Infected Indian Children

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Objectives: To study the association between common AIDS restriction genes and slow disease progression among perinatally-infected children in India.

Methods: ART-naïve children were identified and selected host factors including CCR5-Δ32, SDF1-3'A, CCR5-59029G, HLA-B*27, B*57 were studied using allele-specific PCR-RFLP and SSPGo HLA typing kits.

Results: Among 165 children, 10 (6%) long-term non-progressors and 8 (5%) slow progressors were identified. For comparison, 12 children with normal progression of HIV were included. The frequencies of CCR5-Δ32 deletion, SDF1-3'A and CCR5-59029G did not differ significantly. HLA-B*27 and B*57 were observed only in long-term non-progressors or slow progressors, who also harbored either SDF1-3'A and/or CCR5-59029G.

Conclusions: There is an association between host genetic factors and slow disease progression in this population.

Keywords: *Genetics, Human Immunodeficiency virus, Outcome, Progression*.

The disease course of HIV is varied in perinatally-infected children; those who succumb to terminal illnesses soon after infection are termed as rapid-progressors. Others termed as long-term non-progressors (LTNP) often survive up to 25 years or longer without antiretroviral therapy (ART) [1]. This variability is related to differences in viral biological properties, host genetics and host immune responses [2]. Several immunoregulatory genes – AIDS restriction genes (ARGs) - play a pivotal role in entry of the virus into the host cell [2]. Mutations in these receptors or their ligands, have been associated with natural resistance to HIV-1 and delayed progression of HIV-1 disease [3]. Two HLA-class I alleles, HLA-B*27 and HLA-B*57 have been associated with slower HIV-1 disease progression in adults [4]. Most studies assessing the role of host genetic factors in LTNPs have been carried out on Caucasian or African populations; studies in India are limited [5-7].

Our study aimed at correlating five commonly reported ARGs, CCR5-Δ32, SDF1-3'A, CCR5-59029G, HLA- B*57 and B*27, with disease progression among perinatally-infected Indian children.

Methods

The study participants were selected from a cohort of 165 children living with perinatally-acquired HIV-1 infection obtaining care at the Pediatric Infectious Disease Clinic, St. John's Hospital, Bangalore. Written informed consent was obtained from the caregivers, and verbal assent was obtained from children ≥8 years of age. The study was approved by the Institutional Ethical Review Board at St. John's Medical College Hospital, Bangalore. Perinatal acquisition of HIV was confirmed by history and documentation of maternal HIV-positive status, and absence of other risk factors of HIV infection acquisition in the child. The following definition was used to screen for LTNPs: ART-naïve, asymptomatic, HIV-1 infection for >10 years, and CD4 T-cell count >500 cells/mm^3. Slow progressors (SP) were defined as children who were ART-naïve, asymptomatic, with known HIV-1 infection for >10 years, and CD4 count<500 cells/mm^3 [1]. Normal progressors (NP) constituted those children who had HIV-1 infection for less than 10 years with CD4 cell count <500 cells/mm^3. The time of onset of HIV infection was considered as the date of birth of the child, and the length of infection was the same as child's age. Clinical features and CD4 counts were documented for this study, and viral load was performed using Abbott m2000RT Real Time PCR assay. Genomic DNA was extracted from whole

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blood using QIAmp DNA Blood Mini Kit (Qiagen, US). SDF1-3'A and CCR5-59029G genotypes were identified by PCR-based restriction followed by fragment length polymorphisms (PCR-RFLP) using *MspI* and *BsaBI*. CCR5-Δ32 was amplified by PCR using the primers described previously [8]. HLA-B*57 and B*27 allele polymorphisms were determined by SSPGo-HLA Typing Kits (Biofortuna, Bromborough, UK) as per manufacturer's instructions.

RESULTS

Among the 165 children who were initially screened, we identified 6% LTNPs and 5% SPs. We also included 12 NPs, and the final study sample consisted of 30 children (14 males). Mean (SD) CD4 absolute count was 482 (397) cells/mm^3, and mean (SD) CD4 percentage was 19% (4%). Mean (SD) viral load was 5.3 (0.7) log copies/mL. The majority of the children were asymptomatic, and 5% had minor illnesses that constituted features consistent with WHO Clinical Stage 2 illnesses. Among the NPs, two each had previous WHO clinical stage 3 or 4 conditions, and all were on anti-retroviral treatment (ART).

The clinical features and allelic distribution of ARGs among the three groups is presented in ***Table* I**. None of the children had CCR5-Δ32 deletion; all had wild-type CCR5. Among the SPs and LTNPs together (collectively called "long term survivors" (LTS)), the SDF1-3'A allele was found either in the heterozygous or homozygous form in 44% (8/18). Among NPs, it was found in 25% (3/12) (P=0.279), Similarly, the CCR5-59029G allele was found in 89% (16/18) among LTS and 92% (11/12) among NPs (P=0.804). The HLA-B*57-positive SP was also heterozygous for SDF1-3'A and CCR5-59029G while the HLA-B*57-positive LTNP was homozygous for SDF1-3'A and heterozygous for CCR5-59029G. The HLA-B*27-positive LTNP was also heterozygous for CCR5-59029G.

DISCUSSION

The progression to AIDS was observed to vary widely in our HIV-1-infected pediatric cohort. It is noteworthy that a tenth of all the children being followed in the clinic belonged to the LTNP or slow progressor category.

The prevalence of LTNPs has been reported to be 3% in children of Spanish and Italian origin [9], and 9% in children of Italian origin [10]. These studies demonstrated the occurrence of known ARGs (CCR5-Δ32 and SDF1-3'A) among LTNPs at low frequencies. Previous studies in India have analyzed the prevalence of ARGs among exposed seronegative HIV-1 populations and healthy controls [6,11]. A study from a French cohort of adults previously reported that the CCR5-D32 and SDF1-3'A along with HLA-genotypes in combination, portray the synergistic effects of ARGs in disease progression [12]. HLA class I alleles have been closely associated with immune control in HIV among humans [13]. The observation that HIV-infected HLA-B*27-positive individuals can mount strong cytotoxic T lymphocyte

TABLE I CLINICAL, DEMOGRAPHIC AND ALLELIC FREQUENCY OF AIDS RESTRICTION GENES AMONG PERINATALLY-INFECTED CHILDREN LIVING WITH HIV-1, CLASSIFIED BASED ON THEIR RATE OF DISEASE PROGRESSION

Parameter	*Normal progressors (n=12)*	*Slow progressors (n=8)*	*Long-term non-progressors (n=10)*
Age; mean (SD)	6.3 (2.6)	12.8 (1.7)	11.8 (2.0)
Boys; No. (%)	6 (50%)	4 (50%)	4 (40%)
Viral load, log copies/mL; Mean (SD)	5.35 (0.73)	5.37 (0.66)	5.11 (0.78)
CD4 count, cells/mm^3; Mean (SD)	282 (105)	243 (118)	913 (415)
SDF1-3'A prevalence, n (%)			
Heterozygous (3'G/3'A)	1 (8%)	3 (38%)	2 (20%)
Homozygous (3'A/3'A)	2 (17%)	1 (13%)	2 (20%)
Wild Type (3'G/3'G)	9 (75%)	4 (50%)	6 (60%)
CCR5-59029G prevalence, n (%)			
Heterozygous (G/A)	4 (33%)	4 (50%)	4 (40%)
Homozygous (G/G)	7 (58%)	3 (38%)	5 (50%)
Wild type (A/A)	1 (8%)	1 (13%)	1 (10%)
*HLA-B*27 prevalence, n (%)*	0	0	1 (10%)
*HLA-B*57 prevalence, n (%)*	0	1 (13%)	1 (10%)

WHAT THIS STUDY ADDS?

- There is a possible association of HLA alleles with other host factors such as AIDS restriction genes in predicting HIV disease outcome in perinatally HIV-infected children in India.

(CTL) responses against the immunodominant epitope showed that specific HLA class I alleles can influence the outcome of HIV infection [14]. There is also a gag epitope (corresponding to the gag amino acids 240-249) which is the main target of the acute CD8+T-cell response in HIV-infected subjects who have the HLA-B*57 allele [15].

Our study findings are limited by a cross-sectional design and small sample size. The host genetic studies were also restricted to a small number of factors; other host genetic factors are yet to be studied in this population.

Our study highlights the proportion of Indian children with slow disease progression among the cohort of children living with HIV. These preliminary results of host genetic studies suggest an association between known ARGs, as well as a possible combined effect of host factors such as chemokine-receptors and their ligands, and HLA-class molecules on the outcome of disease progression. A larger sample size and a more extensive list of factors, including host chemokine co-receptor polymorphisms and viral alterations associated with HIV-1 disease non-progression may reveal better insight into the protective correlates among the Indian HIV-1 population.

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REFERENCES

1. Poropatich K, Sullivan DJ, Human immunodeficiency virus type 1 long-term non-progressors: The viral, genetic and immunological basis for disease non-progression. J Gen Virol. 2011;92(Pt 2):247-68.
2. O'Brien SJ, Nelson GW. Human genes that limit AIDS. Nat Genet. 2004;36565-74.
3. Ioannidis JP, Contopoulos-Ioannidis DG, Rosenberg PS, Goedert JJ, De Rossi A, Espanol T, *et al*. Effects of CCR5-delta32 and CCR2-64I alleles on disease progression of perinatally HIV-1-infected children: An international meta-analysis. AIDS. 2003;17:1631-8.
4. UK Collaborative HIV Cohort Study Steering Committee. HLA B*5701 status, disease progression, and response to antiretroviral therapy. AIDS. 2013;27:2587-92.
5. Sharma G, Kaur G, Mehra N. Genetic correlates influencing immunopathogenesis of HIV infection. Indian J Med Res. 2011;134:749-68.
6. Rathore A, Chatterjee A, Sivarama P, Yamamoto N, Singhal PK, Dhole TN. Association of CCR5-59029 A/G and CCL3L1 copy number polymorphism with HIV type 1 transmission/progression among HIV type 1-seropositive and repeatedly sexually exposed HIV type 1-seronegative North Indians. AIDS Res Hum Retroviruses. 2009;25:1149-56.
7. Chaudhary O, Rajsekar K, Ahmed I, Verma R, Bala M, Bhasin R, *et al*. Polymorphic variants in DC-SIGN, DC-SIGNR and SDF-1 in high risk seronegative and HIV-1 patients in Northern Asian Indians. J Clin Virol. 2008;43:196-201.
8. Su B, Sun G, Lu D, Xiao J, Hu F, Chakraborty R, *et al*. Distribution of three HIV-1 resistance-conferring polymorphisms (SDF1-3'A, CCR2-641, and CCR5-delta32) in global populations. Eur J Hum Genet. 2000;8:975-9.
9. Romiti ML, Colognesi C, Cancrini C, Mas A, Berrino M, Salvatori F, *et al.* Prognostic value of a CCR5 defective allele in pediatric HIV-1 infection. Mol Med. 2000; 6:28-36.
10. Tresoldi E, Romiti ML, Boniotto M, Crovella S, Salvatori F, Palomba E, *et al*. Prognostic value of the stromal cell-derived factor 1 3'A mutation in pediatric human immunodeficiency virus type 1 infection. J Infect Dis. 2002;185:696-700.
11. Kaur G, Singh P, Rapthap CC, Kumar N, Vajpayee M, Sharma SK, *et al.* Polymorphism in the CCR5 gene promoter and HIV-1 infection in North Indians. Hum Immunol. 2007;68:454-61.
12. Magierowska M, Theodorou I, Debre P, Sanson F, Autran B, Riviere Y, *et al*. Combined genotypes of CCR5, CCR2, SDF1, and HLA genes can predict the long-term nonprogressor status in human immunodeficiency virus-1-infected individuals. Blood. 1999;93:936-41.
13. Goulder PJ, Watkins DI. Impact of MHC class I diversity on immune control of immunodeficiency virus replication. Nat Rev Immunol. 2008;8:619-30.
14. Feeney ME, Tang Y, Roosevelt KA, Leslie AJ, McIntosh K, Karthas N, *et al*. Immune escape precedes breakthrough human immunodeficiency virus type 1 viremia and broadening of the cytotoxic T-lymphocyte response in an HLA-B27-positive long-term-nonprogressing child. J Virol. 2004;78:8927-30.
15. Goepfert PA, Lumm W, Farmer P, Matthews P, Prendergast A, Carlson JM, *et al*. Transmission of HIV-1 Gag immune escape mutations is associated with reduced viral load in linked recipients. J Exp Med. 2008;205:1009-17.