

CCR5 Genotypes and Progression to HIV Disease in Perinatally Infected Children

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The CCR5 molecule, a chemokine receptor, is the most important co-receptor for macrophage-tropic HIV-1. A 32-bp deletion in the gene encoding CCR5 (CCR5-del32) confers nearly complete resistance to HIV-1 infection in homozygotes, and slows the progression to AIDS in heterozygous adults. The aim of this study was to describe the CCR5 genotypes and the characteristics of HIV disease progression in perinatally infected children. From a total of 51 children and adolescents for the CCR5-del32 mutation, 18 (35%) were considered to be rapid progressors, 28 (55%) were moderate progressors and 5 (10%) were slow progressors. A portion of the CCR5 gene was amplified by PCR from genomic DNA and analyzed by agarose gel electrophoresis. Forty-nine children (96%) carried the homozygous wild type genotype for CCR5, 2 (4%) carried the heterozygous wt/del32 genotype. In the population studied, the CCR5 genotype was unable to account for the differences in pattern of the disease progression among the three groups (rapid, moderate and slow progressors), and the allele frequency of CCR5-del32 was too low to allow statistical comparisons with adequate resolving power. Studies on larger populations may help to further elucidate the role of this allele and other host factors in the regulation of HIV-1 pathogenesis in children.

Key-Words: HIV-1, CCR5 co-receptor, HIV disease progression, perinatally infected children.

From the late nineties onwards, expressive advances in healthcare practices concerning HIV-positive children have led to changes in the clinical course of the illness, resulting in lower morbidity and mortality [1].

HIV-1 infection in children takes a variable course, causing early symptoms in approximately 20% (rapid progression) [2]. Most children show moderate progression of the illness, and a small group remains asymptomatic for many years [3]. Several factors contribute to this scenario, mainly linked to viral and host characteristics. Regarding host factors, the pertinent literature emphasizes the role of the CCR5 gene that encodes a cell-surface, chemokine-receptor molecule which serves as a co-receptor for macrophage-tropic strains of HIV-1 [4,5].

Homozygosity for the 32-bp deletion (del32/del32) in the CCR5 gene confers a certain degree of resistance to HIV-1 infection, while heterozygosity (del32/wt) slows the rate of progression to AIDS in adults [6,7].

To better understand the role of the del32 allele in HIV-1 disease progression in children, in the present study, we evaluated the genotypes of the CCR5 co-receptor in children exhibiting different time courses of progression to AIDS.

Materials and Methods

Study Population

The present study was conducted on HIV-infected children attended at the Federal University of São Paulo (SP, Brazil),

enrolled from November 2001 to October 2003, during routine clinical visits, which took place approximately every 2 months. Signed, informed consent was obtained from the parents or guardians of all 51 children enrolled in the study. The present investigation and the protocols comply with the Research Ethics Committee of Federal University of São Paulo.

The demographic characteristics of the studied population are provided in Table 1. All children enrolled had acquired the infection by vertical transmission of HIV, and were analyzed for CCR5 genotypes, CD₄ nadir counts, immunological and clinical categories and pattern of progression of HIV disease. The revised CDC classification [8] was used to define clinical categories and the degrees of immunosuppression based on age-specific CD₄ T-lymphocyte counts. Children were considered to be either rapid, moderate or slow progressors when they exhibited clinical signs and symptoms of the disease, or signs of immunodeficiency, within the first two years of life, between 2-8 years, and after 8 years of age, respectively.

DNA Samples

DNA samples were obtained from PBMC using 4% saponin and the QIAamp Blood kit (QIAGEN Inc, Santa Clarita, C.A., USA), according to the manufacturers.

CCR5 Genotyping

A portion of the CCR5 gene was amplified by PCR utilizing primers that flank the 32-bp deletion (**P1(2975)**, 5'-CAAAAAGAAGGTCTTCATTACACC-3' and **P2(2976)**, 5'-CCTGTGCCTCTTCTTCTCATTTCG-3') [9]. Wild-type and deleted fragments of 189 and 157 bp, respectively, were generated. The presence of both fragments was considered to represent heterozygosity (wt/del32). The PCR reaction mixture contained 0.25 mM dNTPs, 20 pmol of each primer, and 0.5 Unit of Taq polymerase in 1x reaction buffer (Invitrogen). Each PCR amplification consisted of 40 cycles

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as follows: the first 5 cycles at 94°C for 1 min, 55°C for 1 min and 72°C for 1.5 min; 35 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 45 s, were carried out in a MasterCycler thermal cycler (Eppendorf).

A 10 µL aliquot of each 25 µL PCR reaction mixture was run on a 2.5% agarose gel electrophoresis and the appropriately sized products were visualized under UV illumination after staining with ethidium bromide (1 µg/mL). Molecular size marker is indicated at right in basepairs (Figure 1).

Results

Forty-nine (96%) of the 51 infected children were homozygous for the wild-type CCR5 genotype (wt/wt), and 2 (4%) were heterozygous for the del32 genotype (wt/del32). We found no homozygosity for the mutated allele (del32/del32) (Table 2). Between the two heterozygotes, one was a moderately symptomatic rapid progressor and the other was a severely symptomatic moderate progressor. The rapid progressor was a three year-old boy who showed the first symptoms at 7 months of age and the moderate progressor was a seven year-old boy who showed the first symptoms at 3 years of age. Most children showed a pattern of moderate progression (n=28; 55%); 18 children (35%) were rapid progressors, and 5 (10%) were slow progressors.

There were no significant differences among the slow, moderate and rapid progressors in terms of their CCR5 genotypes or in terms of age, CD₄ nadir values and immunological classification. More children classified as rapid progressors had reached clinical category C (n=9; p<0.05), compared to moderate and slow progressors.

Discussion

Several large studies of the effects of CCR5 genotypes on HIV disease progression have been undertaken in adults [6,10,11]; however, less data are available for children [12,13].

There is a high frequency of the CCR5-del32 allele genotype among Caucasians from North America and Europe, 1% of them possessing the homozygous allele, while 10 and 20%, respectively, exhibit the heterozygous allele [14,15]. The frequency of the heterozygous allele among Afro-Americans is 6%, with 7% among Hispanics, 13% among native Americans and 0.6% among Asians [16]. The highest recorded allele frequency is 20.93%, found in the Ashkenazi Jews, known to be highly endogamous [17].

The results of the present study showed that the normal CCR5 genotype was the most frequent among our outpatient HIV-1 children.

The prevalence of the heterozygous allele in this group of children of different ethnic origins was 4%, comparable to the findings for various studies in Brazilian adults (5% in blood donors [18], and 5.1% in patients with falciform anemia [19]). Silva et al. reported a 6.5% incidence of the heterozygous genotype among blood donors from São Paulo city, Brazil.

Many studies have been performed to evaluate whether heterozygosity of the CCR5-del32 allele genotype affects the

vertical transmission of HIV-1, or whether it affects disease progression [6,16,20-22]. One meta-analysis has revealed that perinatal infection is not significantly altered by heterozygosity for CCR5-del32 in children [23]. Another metaanalysis has been performed among 10 studies including 1317 HIV-1 infected children, addressing the effects of CCR5-del32. For progression to clinical AIDS, CCR5-del32 showed an overall non-significant trend for protection (hazard ratio 0.84, 95% confidence interval 0.58-1.23).

However, survival analyses showed a statistically significant time-dependence. The CCR5-del32 genotype was associated with a decreased risk of death among perinatally infected children, although only during the first years of life [21].

Multiple factors may affect HIV-1 disease progression in perinatally infected children, such factors include in utero versus intrapartum infection, maternal disease status at the time of delivery [24], therapeutic and prophylactic treatment of the mother and infant, and host human leucocyte antigen (HLA) genotype 11. In our studied population, the CCR5 genotype was unable to account for the difference in pattern of disease progression among the three groups (rapid, moderate and slow progressors). However, we can not exclude a potential role for these genetic characteristics, since sample size in our study was limited, and the allele frequency of CCR5-del32 was too low to allow statistical comparisons with adequate resolving power. Studies with larger populations may further elucidate the role of this allele and other host factors in the regulation of HIV-1 pathogenesis in children.

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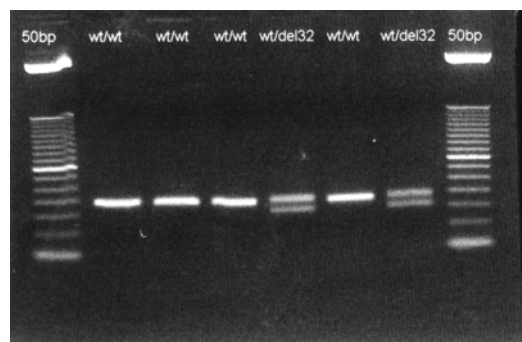
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Table 1. Demographic and clinical characteristics of the study population

Total number of individuals (N)	51	
Gender Female	32 (63%)	
Median age (range)	9 (3-16)	
Median CD ₄ count (range)	842 (20-1735)	
Median count CD ₄ nadir	432 (3-1613)	
Clinical category*	N	%
A	9	18
B	20	39
C	19	37
N	3	6
Immunological category*		
1	9	18
2	19	39
3	21	43

*Centers for Disease Control [9].

Figure 1. CCR5 genotypes among HIV-1 infected children. Lanes 1, 2, 3 and 5 represent the PCR products from samples with the homozygous wild-type genotype (fragments of 189pb/ wt/wt). Lanes 4 and 6 represent the PCR products from 2 samples heterozygous for the del32 genotype (with the presence of both fragments of 189 and 157pb/ wt/del32).**Table 2.** Incidence of CCR5 genotypes among HIV-1 infected children according to the pattern of progression to AIDS

CCR5 genotype	Type of progression to AIDS		
	Rapid	Moderate	Slow
Homozygous wild type (wt/wt)	17	27	5
Heterozygous (del32/wt)	1	1	0
Homozygous mutant (del32/del32)	0	0	0
Total number of cases	18	28	5

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