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Unusual Substitutions in HIV-1 Vif From Children Infected Perinatally Without Progression to AIDS for More Than 8 Years Without Therapy

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The HIV-1 *vif* gene encodes for an accessory protein that is central for virus replication due mainly to its capacity to counteract the antiviral action of host APOBEC3 restriction factors. In order to evaluate whether HIV-1 *vif* alterations account for a delayed progression to AIDS in children infected perinatally, the *vif* genes from a group of 11 patients who exhibited an extremely slow disease progression (slow progressors) were studied by direct sequencing. In addition, the *vif* genes from a group of 93 children with typical disease progression (typical progressors) were analyzed for comparison. Phylogenetic analysis indicated that sequences from slow progressors did not have a common origin, discarding a shared ancestor of reduced virulence. There were no differences in the diversity between the *vif* genes from slow and typical progressors. No gross defects showing a clear distinction among sequences from both groups of children were found. However, in the deduced Vif proteins, changes V13I, V55T, and L81M were observed only in sequences from slow progressors. By analyzing sequences stored in databases, these mutations were determined as unusual substitutions occurring at highly conserved Vif sites across different HIV-1 clades, but were observed with an increased frequency in sequences from elite controllers. These mutations were in the Vif regions reported as relevant for protein activity. These findings suggest that the Vif sequences from slow progressors carry unusual substitutions, which may alter the protein function and may contribute to viral attenuation. ***J. Med. Virol. 84:1844–1852, 2012.***

KEY WORDS: HIV-1; Vif; pediatric AIDS; slow progression

INTRODUCTION

Perinatal infection with HIV-1 leads frequently to the development of AIDS within the first few months of life, although treatment with antiretroviral drugs improves the outcome [Berk et al., 2005]. It has been reported that the rate of clinical progression in children could be influenced by diverse host specific factors. For instance, a protective role has been shown for numerous genetic polymorphisms of chemokine and chemokine receptors that affect cell entry of HIV-1 [Misrahi et al., 1998; Mangano et al., 2000; Tresoldi et al., 2002; Gonzalez et al., 2005]. In addition, different HLA alleles (principally HLA B*57 and B*27) have also been linked to a slow disease progression [Tang et al., 2010; Singh et al., 2011]. However, viral characteristics may also account for a late development of AIDS in children. It has been reported that the presence of HIV-1 strains that carry defective genes could determine a slow course of disease. Viral sequences showing mutations in the

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structural genes (i.e., *gag* and *env*) have been found in pediatric long-term survivors [Alexander et al., 2006], but also a number of alterations affecting the accessory genes (mainly *vif*, *vpr*, and *nef*) have been observed [Alexander et al., 2002; Zhao et al., 2002; Casartelli et al., 2003]. Variations occurring in this latter group of genes are of special interest, since they constitute important determinants of the pathogenicity of HIV-1.

The present study was focused on the HIV-1 *vif* gene, which encodes a 192-amino acid molecule that is central for virus infectivity due to its role against the antiviral action of APOBEC3 proteins (mainly APOBEC3G and APOBEC3F) [reviewed in Albin and Harris, 2010]. These members of the innate host defense system are deoxycytidine deaminases that can be incorporated into nascent HIV-1 virions and edit the viral DNA during preintegration steps. This process may render proviral genomes impaired by an extensive G → A mutation or "hypermutation" in the sense strand sequence, affecting specifically GG → AG (APOBEC3G) and GA → AA (APOBEC3F). APOBEC3 proteins may also inhibit reverse transcription through a deamination-independent mechanism [reviewed in Henriet et al., 2009]. Vif counteracts APOBEC3G and APOBEC3F activity by recruiting both proteins in a Cullin5-based ubiquitin ligase complex responsible for their proteasomal degradation. Vif may also inhibit APOBEC3G translation by binding to its mRNA [Mercenne et al., 2010]. Consequently, the reduced intracellular levels of APOBEC3 proteins prevent their recruitment in the HIV-1 virions.

Although it has been established clearly that the mechanisms described previously constitute the main Vif function, other roles have been described for this HIV-1 accessory protein. It has been shown that Vif interacts with the Gag precursor, the viral protease, and the viral genomic RNA [reviewed in Henriet et al., 2009]. Vif is recruited by a complex interplay with all these factors into the viral particle and is able to modulate its maturation. This participation of Vif in virion assembly might interfere with the packaging of APOBEC3 proteins. In addition, Vif is an integral component of the reverse transcription complex, in which it may stimulate polymerase activity by functioning as a cofactor of the retrotranscriptase [Kataropoulou et al., 2009]. It has been reported that Vif could alter the cell cycle and induce cytopathic changes in infected cells [Sakai et al., 2006; DeHart et al., 2008], reorganize the cytoskeleton [reviewed in Henriet et al., 2009], and disrupt the antiviral response by targeting the interferon regulatory factor 3 (IRF-3) for degradation [Okumura et al., 2008].

Therefore, Vif efficiency in a plethora of activities is important to the HIV-1 replication capacity in infected patients, and its variability may be relevant to the time of progression to AIDS. Although the occurrence of insertions, deletions, premature stop codons, and specific substitutions or altered motifs at the protein level has been reported in patients with a slow progression of disease [Hassaine et al., 2000; Alexander et al., 2002; Farrow et al., 2005; Rangel et al., 2009; Sandonís et al., 2009], few studies have explored the Vif characteristics associated with delayed AIDS development in children [Alexander et al., 2002, 2006].

Thus, the aim of this study was to analyze the HIV-1 *vif* molecular variability in children infected perinatally, looking for alterations that may account for a slow progression to AIDS.

MATERIALS AND METHODS

Study Patients

Two groups of children infected perinatally with HIV-1 were studied: (i) slow progressors (n = 11), patients born between 1987 and 1998 who had not developed AIDS for at least 100 months without receiving antiretroviral therapy and (ii) typical progressors (n = 93), children born between 1994 and 1999 who had shown onset of AIDS (at between less than 1 and 69 months) in 72% of the cases during a median follow-up time of 86 months (3–141 months) and were under HAART in 74% of the cases. Children received medical care at the Hospital de Pediatría "Juan P. Garrahan" (Buenos Aires, Argentina) as members of a pediatric population of around 600 patients infected with HIV-1 that have a recorded clinical follow-up. HIV-1 infection status and AIDS definition were established according to the 1994 criteria of the US Centers for Disease Control and Prevention (CDC) classification for children [Caldwell et al., 1994]. The Ethics Committee and the Institutional Review Board of the hospital approved the study. Written informed consent was obtained from the parents or legal guardians of the children.

HIV-1 *vif* Amplification and Sequencing

Peripheral blood mononuclear cells (PBMC) were isolated from fresh EDTA-anticoagulated blood (5–10 ml) by Ficoll density gradient centrifugation. The samples from slow progressors were collected between 11 and 168 months of age (median of 128 months), while samples from typical progressors were obtained in all the cases within the first year of life. The study was restricted to one sample per patient. The complete proviral *vif* gene (579 bp) was amplified by nested-PCR from the PBMC lysates or purified DNA. The primers used for slow progressors were (5′–3′) Fw-CGGGTTTATTACAGGGACAGC/Rv-TCTCCGCTTCTTCCTGCCATAG for the first round and Fw-CTCTGGAAAGGTGAAGGGGCAGTAG/Rv-CAAGCAGTTTTAGGCTGACTTCC for the second round. The PCR products were purified with the QIAquick purification columns (QIAGEN, Hilden, Germany) and then subjected to bulk sequencing using both second round primers. Sequencing reactions were conducted with the DYEnamic ET Terminator Cycle sequencing kit (Amersham Biosciences,

Buckinghamshire, England), run on an ABI PRISM 310 automated sequencer and analyzed with the DNA Sequencing Analysis Software v3.3 (Applied Biosystems, Foster City, CA). The chromatograms were inspected visually for the presence of multiple nucleotides at a single position using the FinchTV v1.4.0 software (http://www.geospiza.com/Products/finchtv.shtml). For typical progressors, bulk sequences of proviral *vif* were obtained as described previously [De Maio et al., 2011]. Sequences were deposited at GenBank under accession numbers FJ197319–FJ197329 (slow progressors) and JF494923–JF495015 (typical progressors).

Sequence Analysis

Sequences were aligned to reference *vif* genes of different subtype obtained from the Los Alamos National Laboratory (LANL) HIV database (www.hiv.lanl.gov/content/sequence/NEWALIGN/align.html) by using the HIV Align tool (http://www.hiv.lanl.gov/content/sequence/HMM/HmmAlign.html). The RIP 3.0 tool (http://www.hiv.lanl.gov/content/sequence/RIP/RIP.html) was used to assess the viral subtype and identify the recombinant sequences. The phylogenetic tree was constructed by the neighbor-joining method as implemented in MEGA5 [Tamura et al., 2011], using the Tamura-Nei model with 1,000 bootstrap replicates. Pairwise differences per site (p-distance) were calculated with MEGA5. The APOBEC3 mediated hypermutation was evaluated with Hypermut 2.0 (www.hiv.lanl.gov/content/sequence/HYPERMUT/hypermut.html) using the reference sequences according to the subtype. The occurrence of different amino acid substitutions in the LANL HIV database was determined based on 1,599 sequences from the Filtered Web Alignment 2009 for HIV-1 *vif* (all M group, A-K + recombinants). Sequences from our pediatric population previously submitted to GenBank that were included in this curated alignment were omitted for frequency calculations. The LANL HIV database Sequence Search Interface (http://www.hiv.lanl.gov/components/sequence/HIV/search/search.html) was used to retrieve *vif* sequences (one per patient) corresponding to elite controllers (50 sequences in August 2011). The presence of specific HLA-B anchor residue motifs in Vif proteins was evaluated with the Motif Scan tool (http://www.hiv.lanl.gov/content/immunology/motif_scan/motif_scan).

HIV-1 Plasma Viral Load and CD4+ T Cell Counts

Plasma HIV-1 RNA was quantified using commercial methods according to their availability at the institution at different times. HIV-1 RNA QT Nuclisens (Organon Teknika, Boxtel, The Nederlands) was used from May 1998 to June 2005, Amplicor HIV-1 Monitor test v1.5 (Roche Diagnostics Systems, Branchburg, NJ) from June 2005 to October 2007, and HIV-1 RNA Cobas TaqMan 48 (Roche Diagnostic Systems) from October 2007 up to the present. CD4+ T cell counts were measured using flow cytometry (FACS Sorter; Becton Dickinson, San Jose, CA) on whole blood samples.

HLA-B Genotyping

HLA-B alleles were genotyped at the molecular level by using a fluorescent bead-based assay with the Luminex platform (Luminex, Austin, TX) for high resolution. Briefly, the LIFEMATCH_System (Gen-Probes, Stamford, CT) for HLA-B typing is based on the simultaneous detection of multicolored beads in suspension. In the present study, one tube reaction containing the PCR-amplified specific HLA-B product was hybridized with a set of probes attached to the fluorescent beads and discrimination of positive hybridization was allowed using streptavidin–phycoerythrin binding to PCR products carrying original biotin-labeled primers. HLA-B alleles were assigned using the LIFEMATCH v2.5.2 software provided by the company. Genotyping was performed at the Department of Medicine, University of Texas, Health Science Center, San Antonio, TX, USA.

Statistical Analysis

Differences in plasma viral load and CD4+ T cell percentage were evaluated with the Wilcoxon rank sum test (Statistix v7.0). Pairwise distances were compared using the Standard two-sample *t*-test (R statistical package v2.10.1). Mutation frequencies were compared with Pearson chi-square (Statistix v7.0).

RESULTS

To evaluate whether HIV-1 *vif* defects contribute to a delay of the onset of pediatric AIDS, proviral *vif* sequences from a group of children exhibiting a slow rate of disease progression were obtained (Table I). This group comprised 11 patients infected perinatally with HIV-1 (including a pair of siblings), who were selected based on the fact that they had not developed AIDS for more than 8 years in the absence of antiretroviral therapy. It should be noted that these slow progressors represent less than 2% of cases of the total pediatric patients infected with HIV-1 at this hospital. Therefore, these children could provide important information on the factors that may account for viral attenuation. Proviral *vif* genes from children considered as typical progressors were used for comparison. This second group included 93 patients infected perinatally with HIV-1 without delayed progression of disease, independently of the antiretroviral therapy.

Based on all available determinations for each patient, slow progressors showed significantly reduced values of highest viral load (Wilcoxon rank sum test $P < 0.001$) and a tendency to increased values of lowest CD4+ T cell percentage (Wilcoxon rank sum

TABLE I. Epidemiological, Biochemical, and Clinical Data of Slow Progressors

Slow progressor	Gender	Date of birth (month/year)	Date of last visit (month/year)	Viral load		CD4+ T cells		Clinical AIDS (months)[b]	Antiretroviral therapy			*vif* sequence	
				Range (log)	Median (n)[a]	Range (%)	Median (n)[a]		Initiation (months)[b]	Drugs		Code	Sample collection (months)[b]
JDM	F	11/92	09/07	<1.7–5.1	4.1 (15)	10–23	19 (13)	146	105	3TC	AZT EFV	F856-JDM	125
VA[c]	M	12/91	01/08	<1.7–5.3	4.6 (17)	21–41	30 (21)	—	179	3TC	AZT EFV	I842-VA	168
VT[c]	M	01/96	01/08	<1.7–4.8	3.2 (14)	13–37	27 (16)	—	117	3TC	AZT EFV	I270-VT	113
GI	M	10/90	04/03	2.8–3.8	3.1 (6)	26–55	33 (9)	—	—			C303-GI	116
BML	F	07/87	05/07	<1.7–5.4	3.1 (20)	21–66	52 (34)	—	115	ddI		C997-BML	162
PFS	M	01/94	10/07	<1.7–4.4	2.6 (28)	25–39	32 (35)	—	—			F83-PFS	104
SGR	F	12/92	05/07	<1.7–4.1	2.8 (20)	20–39	27 (20)	123	149	3TC	AZT EFV	H129-SGR	137
EHL	M	08/95	11/07	<1.7–4.9	3.8 (22)	18–34	23 (26)	—	141	3TC	AZT EFV	K97-EHL	128
GLOA	F	11/98	02/08	<1.7–4.0	2.9 (29)	21–43	36 (33)	—	13[d]	AZT	ddI NFV	B693-GLOA	11
SCJ	M	06/95	06/06	3.3–3.5	3.4 (2)	27–31	29 (2)	—	—			K100-SCJ	130
FSA	F	01/95	04/06	4.0–4.9	4.3 (6)	13–24	22 (12)	—	—			1661-FSA	130

F, female; M, male.
[a]Total number of all available determinations.
[b]Age of patient.
[c]Siblings.
[d]Treatment interrupted after 5 months.

Fig. 1. HIV-1 RNA levels and CD4+ T cells in children. Scatter plots of highest viral load (log) in slow progressors (3.5–5.4, median of 4.8) and typical progressors (3.1–7.0, median of 5.7) (**a**). Scatter plots of lowest CD4+ T cell percentages in slow progressors (10–27%, median of 21%) and typical progressors (0.3–44%, median of 17%) (**b**). Medians and *P* values are depicted.

test $P = 0.064$) in relation to typical progressors (Fig. 1).

To address whether viruses infecting slow progressors derive from a common ancestral HIV-1 strain of reduced virulence, the 104 *vif* sequences obtained from slow progressors and typical progressors were analyzed phylogenetically. In the phylogenetic tree, *vif* genes were intermingled together irrespective of the disease progression rate and similarly distributed between the subtype B and subtype F1 clusters (Fig. 2) (a sole A1 sequence and different B/F1 recombinants were also detected). There was no evidence for an epidemiological link that may explain the clinical status shared by slow progressors.

The examination of *vif* genes from slow progressors revealed no major alterations, with no insertions, deletions, premature termination codons or lack of initiation codon in any case. Hence, no gross defects in *vif* showing a clear distinction between sequences from slow progressors and typical progressors were found.

Next, *vif* heterogeneity was studied by calculating the nucleotide sequence distances (using p-distance) within and between the groups of slow and typical progressors. In subtype F1 cases (5 slow progressors and 76 typical progressors), the median value for pairwise distance was 0.041 within slow progressors, 0.039 within typical progressors, and 0.041 between the slow and the typical progressing children (Fig. 3).

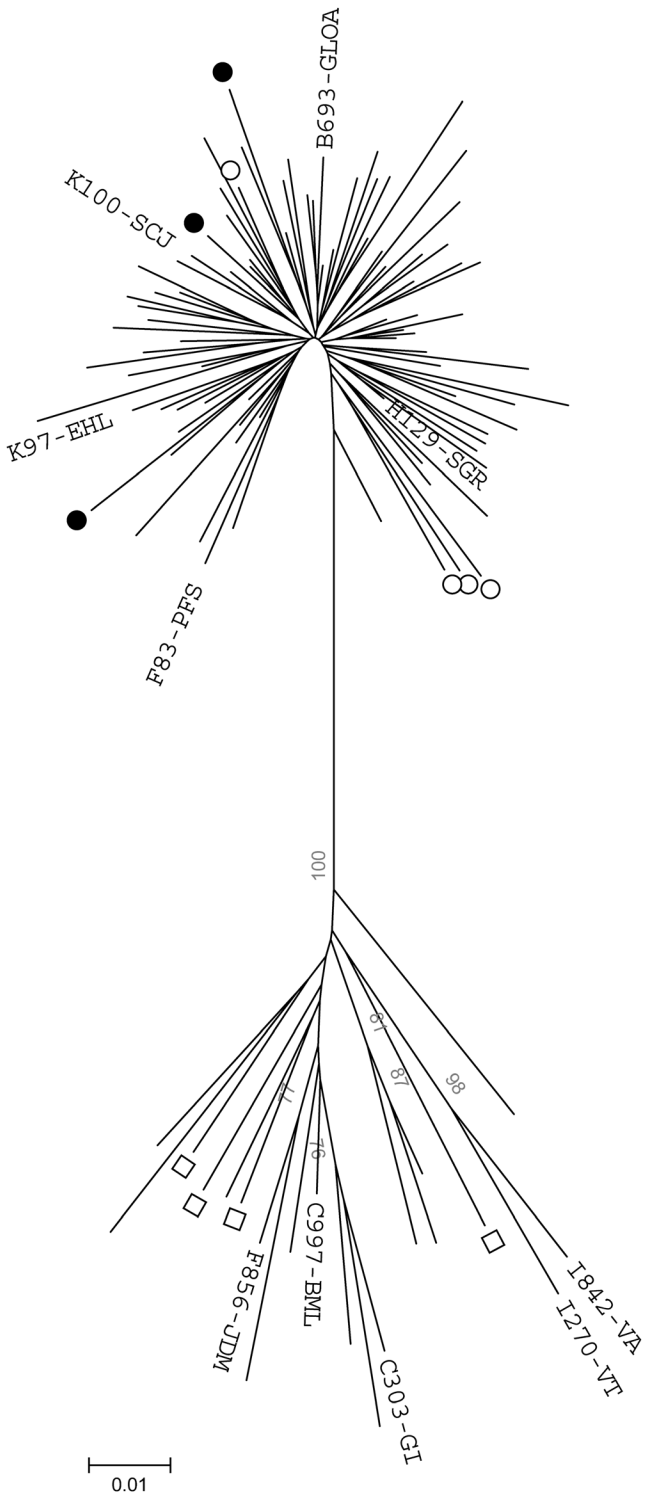

Fig. 2. Phylogenetic analysis of HIV-1 *vif* from children. The resulting neighbor-joining tree, including 10 sequences from slow progressors (codes depicted, 1 recombinant was omitted) and 87 from typical progressors (5 recombinant genes and a sole A1 sequence were omitted), is shown. Reference sequences of different HIV-1 clades were obtained from the LANL HIV-1 database (white circles: subtype F1 references, black circles: CRF_12 BF references, white squares: subtype B references). Bootstrap values of 70% or higher are indicated.

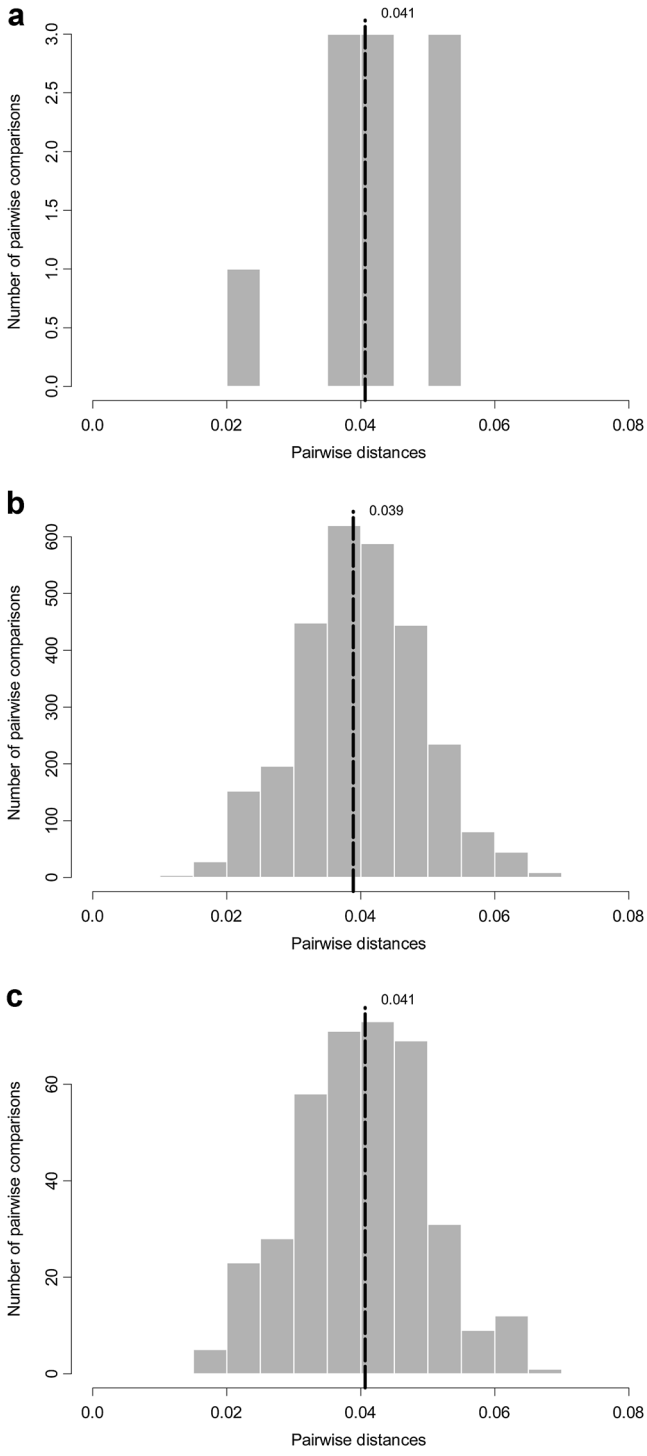

Fig. 3. Heterogeneity of the *vif* gene. Distribution of nucleotide pairwise distances (p-distance) of subtype F1 sequences for comparisons within the group of slow progressors (**a**), within the group of typical progressors (**b**), and between the group of slow progressors and the group of typical progressors (**c**). Medians are indicated.

There were no statistically significant differences (Standard two-sample t-test $P > 0.05$). This was also observed in subtype B cases (5 slow progressors and 11 typical progressors), although higher median values were found for distances within and between groups (around 0.070, not shown). Thus, at the *vif* gene, slow and typical progressors exhibited similar levels of heterogeneity and could not be distinguished in terms of nucleotide diversity.

Besides, the incidence of APOBEC3-mediated editing in *vif* genes was investigated. The Hypermut 2.0 program did not indicate any sequence as hypermutated, evaluating either general (APOBEC3G and APOBEC3F) or specific (APOBEC3G or APOBEC3F) editing patterns.

The deduced proteins were then studied to identify amino acid substitutions that could account for an altered activity in those Vif variants observed in slow progressors. For this purpose, a position-by-position analysis was conducted. This analysis showed that 85 sites had no variation in the whole set of Vif sequences from slow and typical progressors, 70 sites had variation in both groups, 33 sites had variation only in typical progressors and, interestingly, that 4 sites had variation restricted to slow progressors. Thus, the following analysis was centered in single changes found at the latter four positions. Mutations detected were M8L, V13I, V55T, and L81M, which lay separately in Vif sequences from patients EHL, GI, FSA, and PFS (Fig. 4).

To estimate the possible relevance of these mutations to Vif function, their occurrence in 1,599 sequences from the LANL HIV-1 database Filtered Web Alignment 2009 was studied. While M8L was observed commonly in Vif of certain viral clades (mainly subtype C and diverse recombinants), changes V13I, V55T, and L81M presented frequencies of 1.1%, 0.1%, and 0.8%, respectively, and all of them affected highly conserved sites (predominant residue frequency of 98% or above) (Fig. 5). Therefore, three out of the four changes identified initially in slow progressors could be considered as unusual substitutions at positions exhibiting very low variability across Vif sequences of different HIV-1 subtypes.

The role of the Vif mutations V13I, V55T, and L81M in the rate of disease progression was investigated by analyzing the full set of 50 sequences (one per patient) from elite controllers (individuals with non-progressive disease that control HIV-1 replication) retrieved from the LANL HIV-1 database. Change V13I was found in two (4%) cases, V55T in one (2%) case, and L81M in two (4%) cases. The frequency in elite controllers for these mutations was significantly higher than that observed in sequences from the LANL HIV-1 database Filtered Web Alignment 2009 (Pearson chi-square $P < 0.001$).

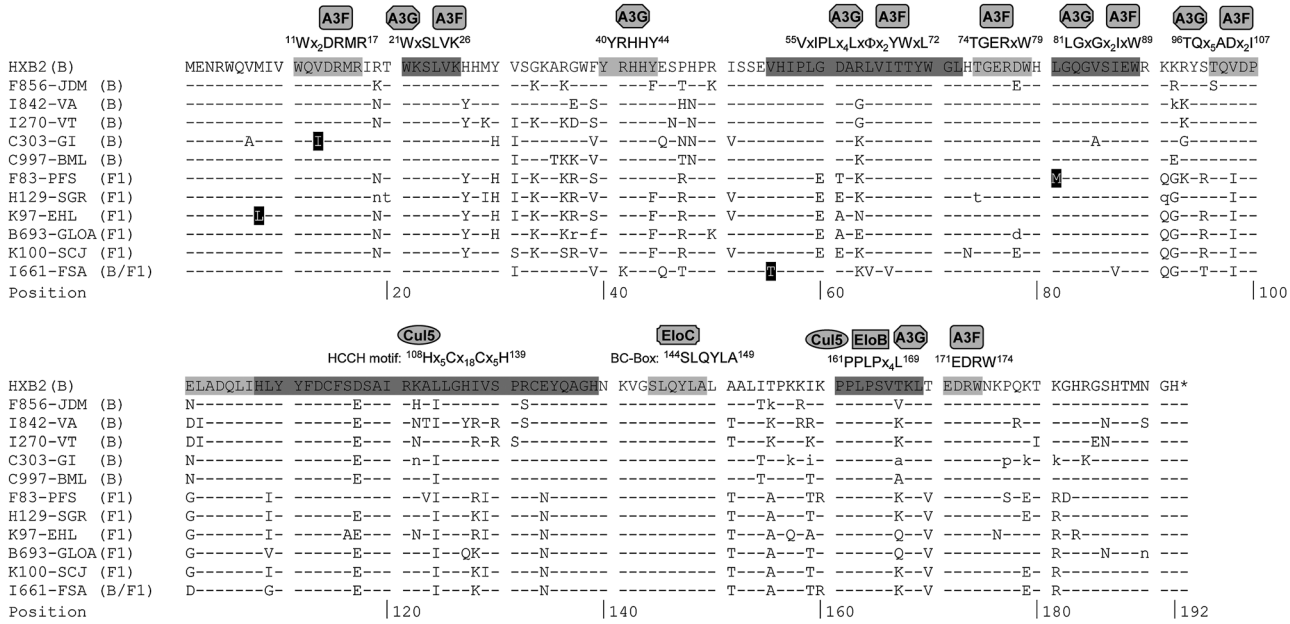

Fig. 4. HIV-1 Vif sequences from slow progressors. The subtype (B, F1, or B/F1 recombinant) is indicated for each sequence. The proteins are compared to the subtype B reference HXB2 (K03455) on top. Changes at sites with variation restricted to slow progressors are depicted in black. Amino acids in lower case indicate the sites where a mixed population was detected (the alternative of higher frequency for the corresponding subtype is shown). The Vif N-terminal region mainly includes domains important for recognition of APOBEC3G (A3G) and/or APOBEC3F (A3F), whereas the C-terminal region contains domains required for recruitment of ElonginC (EloC), ElonginB (EloB), and Cullin5 (Cul5) in an ubiquitin ligase complex [depicted according to Dang et al., 2010b and He et al., 2008].

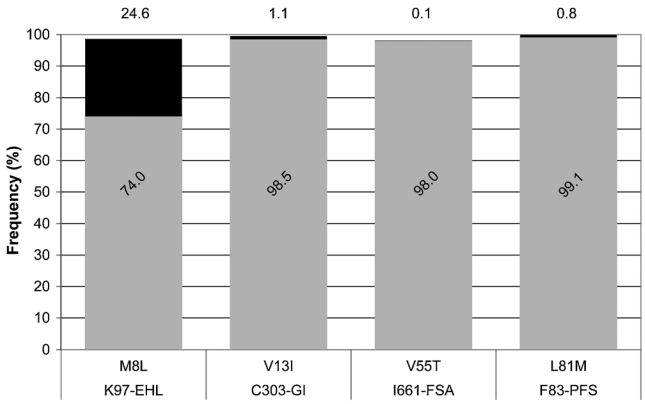

Fig. 5. Occurrence of changes detected in Vif sequences from slow progressors. The frequency in LANL HIV-1 database (1,599 sequences from the Filtered Web Alignment 2009) of the predominant (gray bar) and mutant (black bar) residue is depicted for substitutions M8L, V13I, V55T, and L81M.

The potential effect of substitutions V13I, V55T, and L81M was then explored by analyzing whether they were in domains previously reported as relevant to Vif activity (Fig. 4). Interestingly, these mutations were located in three distinct Vif motifs important for the interaction with APOBEC3G/APOBEC3F [Russell and Pathak, 2007; He et al., 2008; Dang et al., 2010a,b].

The presence of HLA variants that could select positively for these unusual Vif substitutions and contribute to slow progression was also evaluated. To this end, an analysis focused on the HLA-B alleles was performed. Genotypes of patients GI, PFS, and FSA were determined as B*1302/B*1402, B*1402/B*4801, and B*1517/B*3801, respectively. A protective role during HIV-1 infection has not been reported for the HLA-B alleles found, except for B*1302 [Honeyborne et al., 2007]. Mutations V13I, V55T, and L81M did not disrupt CD8+ T cell epitopes, as could be predicted based on the corresponding host HLA-B genotype. Thus, in our analysis, Vif sequences carrying unusual substitutions that were observed in slow progressors seemed not to constitute immune escape variants.

DISCUSSION

The study of HIV-1 Vif from children infected perinatally led to identifying that changes V13I, V55T, and L81M occur differentially in patients with a slow progression of disease. The analysis of sequences stored in the LANL HIV-1 database indicated that these substitutions have an extremely low frequency (0.1–1.1%) and affect highly conserved Vif sites across diverse HIV-1 clades. In addition, database sequences of Vif from elite controllers appeared to be enriched in these specific mutations, which may be interpreted as an external validation of our findings. Interestingly, residues at sites 13, 55, and 81 are within Vif regions important for the suppression of APOBEC3 proteins. However, Vif has been reported to have a variety of functions and many of its domains are expected to have diverse binding partners other than APOBEC3G/APOBEC3F or the ubiquitination machinery. Therefore, the precise consequences of the mutations detected in children with slow progression are difficult to infer due to the complex role of Vif during the viral replication cycle.

None of the 104 patients infected perinatally with HIV-1 that were studied (independently of the rate of disease progression) exhibited a clearly defective *vif* gene. This is in agreement with previous reports pointing to a high functional conservation of *vif* during mother-to-child transmission [Yedavalli et al., 1998; Yedavalli and Ahmad, 2001].

It has been proposed that in some individuals (adults and infants) infected with HIV-1 a delayed AIDS onset could be at least partially explained by the presence of viruses carrying altered *vif* genes. Certain Vif mutations, like R132S (associated with low viral loads) [Hassaine et al., 2000; Rangel et al., 2009], S130I (in viruses from three long-term non-progressors) [Sandonís et al., 2009], and a two-amino acid insertion (detected in a non-progressing mother–child pair) [Alexander et al., 2002], have been found in common among a number of cases displaying a slow disease progression. On the other hand, a collection of dissimilar *vif* defects that could account for particular cases of viral attenuation, including deletion of extensive genetic segments [Rangel et al., 2009; Sandonís et al., 2009], protein truncation due to premature stop codons [Rangel et al., 2009], and modification of Vif motifs that regulate subcellular localization [Farrow et al., 2005], have also been described. In the pediatric patients infected with HIV-1 included in this study, this variety of alterations was not observed as a distinctive feature of sequences from children showing a slow course of disease. However, a slow progressor condition seems attributable, in some cases, to non-shared amino acid changes occurring in distinct Vif regions, as revealed by the unusual substitutions V13I, V55T, and L81M identified. It should be noted that these alterations do not represent difficult-to-revert mutations (like insertions or deletions). Thus, it might be hypothesized that host factors selecting for these changes may favor their predominance in the viral swarm. In that sense, the substitutions were analyzed regarding the patient's HLA-B genotype, but no evidence was found supporting these Vif variants as immune escape forms.

Some of the factors that may account for the differences observed in Vif from slow progressors and typical progressors include discrepancies at the time of blood sampling regarding age, stage of disease or previous therapy [Adekale et al., 2005]. However, none of these can easily explain the remarkably low frequency in the LANL HIV-1 database of changes that were actually observed only in the group of pediatric slow progressors. In addition, no differences between *vif* sequences from both groups were found in terms of

nucleotide heterogeneity or diversity. Thus, the relevance of the unusual substitutions V13I, V55T, and L81M seems not to be diminished by the abovementioned issues.

Although direct sequencing has been demonstrated to be an appropriate way to study hypermutation [Pace et al., 2006; Land et al., 2008], no hypermutants were observed among the proviral *vif* sequences analyzed in the present work. However, it should be noted that the *vif* genes studied showed no major defects that could favor severe APOBEC3 editing in the bulk of proviral population. Further studies are necessary to determine the relevance of hypermutation in the course of pediatric HIV-1 infection.

In conclusion, this study provides new clues about the nature of unusual Vif substitutions that may be linked to HIV-1 attenuation in patients infected perinatally. Apparently minor alterations in distinct Vif domains could determine proteins with suboptimal activities, thus affecting the disease progression rate. These results must be considered in those analyses aimed to establish the concurrent factors implied in a delayed onset of AIDS.

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