Genotyping of CCR5 Gene, CCR2b and SDF1 Variants Related to HIV-1 Infection in Gabonese Subjects

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Key Words

CCR5 gene · CCR2b · Gabon · HIV-1 · SDF1

Abstract

Objective: Given the magnitude of the HIV epidemic infection, many viral and human factors were analyzed, and the most decisive was the variant CCR5-Δ32. The presence of a low HIV prevalence (1.8%) in Gabon in the 1990s, compared to neighboring countries, represents a paradox that led us to search for viral and human genetic variants in this country. In this study, only variants of coreceptors and chemokines were investigated. **Methods:** Variants of the coding region of the CCR5 gene were analyzed by denaturing gradient gel electrophoresis, and then variants of SDF1 and CCR2b were determined by polymerase chain reaction-restriction fragment length polymorphism. **Results:** Four rare variants of the CCR5 coreceptor were found, while CCR5-Δ32 and CCR5m303 variants were not found. No association with CCR2b-V64I (17%) and SDF1-3'A (2%) variants was determined in relation to HIV-1 infection in Gabonese patients. **Conclusion:** The paradox of HIV seroprevalence in Gabon, which ended in the 2000s, was not caused by human genetic variants but rather by environmental factors.

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The exponential increase of HIV infection over the past 30 years has been mainly observed in sub-Saharan Africa. Many reasons have been proposed to explain this widespread phenomenon. These reasons include the existence of several viral forms and human, socioeconomic and environmental factors. In Gabon, an epidemiological study conducted between 1986 and 1994 in Libreville reported a very low HIV-1 prevalence (1.7% in 1989) in comparison to values observed in other central African countries [1]. Another epidemiological study in 1996, including 453 subjects residing in Franceville, showed a low prevalence of syphilis (8.3%) and hepatitis B (13.6%), while there was a very low HIV-1 prevalence (1.8%) [2], confirming results observed in Libreville.

In fact, the low HIV-1 prevalence in Gabonese subjects from Franceville, but also from Libreville and Port-Gentil, contrasted with a high prevalence observed in neighboring countries (Congo, Democratic Republic of Congo, Central African Republic and Cameroon) [2], and, therefore, the 'HIV Gabon paradox' emerged. In a previous study, we showed that this paradox was not due to HIV strain diversity [3]. As viral factors were not involved in assessing the HIV Gabon paradox, host genetic factors may explain this paradox as shown in many studies among Caucasians [4, 5]. The CCR5-Δ32 genetic variant

was found to confer a strong resistance to HIV-1 infection in the Caucasian population [4, 5]. The CCR2b and the SDF-1 (stromal cell-derived factor-1) genetic variants were also found to be associated with a resistance to HIV-1 infection [4, 5]. The latter result suggests that the low HIV-1 prevalence displayed in Gabon may be due to the presence of known and unknown human genetic variants that would influence HIV-1 infection susceptibility.

The SDF-1 molecule, a major ligand for α -chemokine receptor CXCR4 (receptor used by T-tropic HIV strains), has a polymorphic gene in the 3' noncoding region. Two studies reported that the homozygous status for the SDF1-3'A variant showed evidence of a slow and a rapid HIV progression in infected patients [4, 5]. These conflicting results require further investigations.

CCR5 (β -chemokine receptor 5) is the major coreceptor for the entry of human macrophage-tropic HIV strains. CCR5- Δ 32, a first and most important mutant allele of the CCR5 gene, bears a 32-bp deletion in a region corresponding to the second extracellular loop of the coreceptor [6–8]. For the homozygous status, the CCR5- Δ 32 allele present in the Caucasian population in Europe, with an average allele frequency of 9.1% and an increasing gradient from South to North [6], confers a resistance to HIV infection in almost all studies [7, 8].

The HIV virus does not directly use CCR2 for host cell entry, so CCR2 is considered a minor coreceptor for HIV infection. Although the CCR2b-V64I allele (a valine to isoleucine substitution at position 64 in the first transmembrane domain of CCR2), common in all types of populations, proved to have a slowing effect on HIV-1 infection in African-Americans, this was not the case in the Caucasian population [9]. Studies on putative consequences of the CCR2b-V64I variant in the HIV-infected population do not all agree yet.

The main goal of this work was to assess in Gabonese subjects the genetic variants which modulate the entry of HIV-1 virus in target cells (susceptibility to HIV-1 transmission).

The two specific objectives of this study were (1) to establish CCR5- Δ 32 (main coreceptor), CCR2b (accessory coreceptor) and SDF1 (CXCR4 chemokine coreceptor) genotypes and (2) to investigate other mutations in the CCR5 gene by exploring the coding region of this gene, without any a priori hypotheses. These genetic variants were classified into two groups of subjects in Gabon according to HIV-1 seronegative and seropositive categories.

This study was conducted in three major Gabonese cities in 1997. In fact, HIV-1-infected patients were recruit-

ed through sexually transmitted disease centers in Libreville, Port-Gentil and Franceville. Subsequently, two groups of subjects were distinguished based on their HIV status. The HIV seronegative group comprised about 234 subjects recruited in Port-Gentil. These were soldiers from different ethnic groups and from different regions of Gabon. The HIV seropositive group consisted of 102 subjects recruited from sexually transmitted disease centers of Libreville, Port-Gentil and Franceville. At the time of recruitment and serology, these subjects had no symptoms of AIDS. HIV seropositivity tests were then performed by the Western blotting technique using HIV antibodies (Sanofi Diagnostics Pasteur, Paris, France).

The investigations for genetic variants were performed in two ways: (1) the detection of known polymorphisms was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and (2) the exploration of other sequence changes was performed using denaturing gradient gel electrophoresis (DGGE) followed by sequencing.

In the CCR5 gene's coding region, a DNA fragment of 801 bp length was explored; this region comprises a portion of exon 4, including most of the open reading frame. This 801-bp fragment also comprises sequences encoding for the first six transmembrane domains of the CCR5 protein. A sequence from GenBank (HSU95626) was used as a reference to allow the location of the explored region to be in the range of 61,464–62,264. The targeted area was spliced into three partially overlapping zones in order to optimize the discovery of a mutation using the DGGE technique. The exploration conditions of the CCR5 gene using DGGE (primer sequences, GC tail added and PCR conditions) are listed in table 1.

PCR-RFLP, DGGE and sequencing allowed the identification of six mutations (table 2), which were further investigated and characterized. Only four of these mutations have been found in subjects with heterozygous status. Two out of four nucleotide substitutions were non-synonymous, leading to an amino acid change: R60S and V134G. The majority of mutations are rare (3/4), but only the S75S one reaches up to a polymorphic rate. Unfortunately, its low frequency cannot be statistically correlated with the susceptibility or resistance to HIV-1.

SDF1 and CCR2b gene polymorphisms revealed by PCR-RFLP are listed according to the HIV-1 seronegative and seropositive groups in table 3. No association was found between the SDF1-3'A variant and HIV-1 infection ($p = 0.974$) as well as between the CCR2b-V64I variant and HIV-1 infection ($p = 0.858$). The distribution of SDF1-3'A genotypes and CCR2b-V64I alleles shows no

Table 1. Optimized conditions of the PCR-DGGE method for detecting mutations

Sequence	GC clamp	Annealing	Gradient	Migration time
5'atgcacaggggtgaacaagatggat3' 5'catagtgagcccagaaggggacagt3'	0 bp 40 bp	57°C	30–70%	9 h at 180 V (DGGE1)
5'tgaagagcatgactgacatctacct3' 5'gagagctgcaggtgtaatgaagac3'	5 bp 50 bp	54°C	30–70%	8 h at 180 V (DGGE2)
5'gcgtctctcccaggaatcatct3' 5'ggaaggtgttcaggagaaggaca3'	0 bp 50 bp	56°C	20–60%	8 h at 180 V (DGGE3)

Table 2. Distribution of mutations in the CCR5 gene-coding region among Gabonese patients

Mutation	HIV-1 seronegative heterozygote patients, n/total n (allelic frequency)	HIV-1 seropositive heterozygote patients, n/total n (allelic frequency)
CCR5-Δ32	0/234	0/102
CCR5-m303	0/234	0/102
CCR5-P35P (CCG→CCA)	2/234 (0.43%)	0/102
CCR5-R60S (AGG→AGT)	1/234 (0.21%)	0/102
CCR5-S75S (TCT→TCC)	7/234 (1.5%)	2/102 (1%)
CCR5-V134G (GTG→GGG)	1/234 (0.21%)	0/102

Table 3. Distribution of SDF1-3'A and CCR2b-V64I variant genotypes in HIV-1 seronegative and seropositive Gabonese patients

Variant	Subjects	Wt/Wt	Wt/Mut	Mut/Mut	Allelic frequency	HIV- vs. HIV+, p value
SDF1-3'A	HIV- obs (theoretical)	225 (225)	9 (9)	0 (0)	0.02	0.974
	HIV+ obs (theoretical)	98 (98)	4 (4)	0 (0)	0.0196	
CCR2b-V64I	HIV- obs (theoretical)	157 (158)	71 (68)	6 (8)	0.177	0.858
	HIV+ obs (theoretical)	71 (71)	28 (28)	3 (3)	0.167	

Wt = Wild type; Mut = mutant; obs = observed.

deviation from Hardy-Weinberg equilibrium of observed and theoretical values among HIV-1 seronegative and seropositive subjects.

The exploration of the CCR5 gene-coding region revealed several rare mutations and one polymorphic mutation. This polymorphism (S75S) leads to a nonsynonymous mutation, allowing to have an unmodified functional impact, unless there is a preferential codon reading, a creation of a cryptic splice site or a destabilized DNA secondary structure. The low frequency of this polymor-

phism did not allow us to perform any association study. The R60S mutation, occurring in the present study as a rare mutation, is controversially associated with HIV infection [10].

The CCR5 gene-coding region in Gabonese subjects does not have a deletion of 32 bp and a m303 mutation, confirming results found in other African populations [11, 12], but instead has a high rate of rare mutations. This pattern observed in the CCR5 gene is similar to that seen in the South African population, which also shows a high

rate of rare mutations but no association with HIV infection [9]. All these results suggest that the CCR5 gene-coding region does not seem to play a role in the resistance to HIV infection and that further investigations should be performed in the promoter region of the gene.

The allele frequency of the SDF1-3'A variant in the Gabonese population (2%), which are typical Bantu, is consistent with the values observed in the subregion of Central Africa (0–7.1%) [12] as well as with those found in South African Xhosa (2%) [13]. So far, three situations in connection with the SDF1-3'A variant have been established in black African populations: (1) a nonassociation with HIV-1 according to the present study; (2) an association with age in Cameroonian seronegative subjects but no association with HIV infection in Cameroon [12] and, finally, (3) an association with a high risk of HIV infection in the Xhosa population of South Africa [13].

With 17% in the Gabonese population, the CCR2b-V64I variant has an allele frequency similar to those observed in other African populations [11, 12]. It should be noted that this variant is found two times more frequently in Asian populations [11]. Consistent with previous results in African populations of western Kenya [14] and Cote d'Ivoire [15], no association was observed between the CCR2b-V64I variant and HIV infection in the Gabonese population. It thus seems that in African populations, the CCR2b-V64I variant is not associated with resistance to HIV infection, though a high risk for HIV infection was found in Cameroonian men but not in Cameroonian women [12]. Linkage disequilibrium found between the CCR2b-V64I and CCR5 promoter region can interfere with the associations of the CCR2b-V64I variant in HIV infection.

The low prevalence of HIV-1 observed in the Gabonese population before 1999 (1.8%) could be attributed to specific viral variants. But our study on strain diversity in Franceville produced results with a predominant HIV-1 subtype A (49%) and a diversity of HIV-1 strains (A, A/G, B, B/D, C, C/G, D, F, G and H) of group M [3]. This diversity of strains does not support any protective effect against HIV infection by viral variants. The results of the present study on human genetic factors reveal a lack of genetic variants functioning as protectors. Our previous study on human genetic variants of mannose-binding lectin (MBL) detected variants associated with a susceptibility to HIV-1 infection [16], predicting the spread of HIV infection in Gabon.

After excluding human and viral genetic factors, environmental factors appear to better explain this contradictory situation. Indeed, the HIV seroprevalence in the Gabonese population reached 6% in 2008 [17], marking

the end of the 'HIV Gabon paradox'; Gabon now has the same average prevalence values as other countries in the subregion of Central Africa.

The situation registered before 1999 may be explained by a more important geographical isolation at the time, thus preventing the Gabonese population from mixing with other populations and preventing the spread of HIV strains. This isolation was mainly due to bad road conditions between major cities, costly air flights and the fact that only one waterway between Libreville and Port-Gentil and one railway between Libreville and Franceville have always existed. Environmental factors that have changed between the 1990s and the 2000s are essentially the mixing of populations. Indeed, in Gabon, the road network was poorly developed in the 1990s, leading to little mixing of populations. However, in the 2000s, the road network has developed considerably bringing about the mixing of a larger population. Besides, Gabon is one of the countries in the subregion to attract people from the surrounding countries by offering substantially higher wages. This resulted in a greater flow of migration in the 2000s rather than in the 1990s.

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Disclosure Statement

The authors declare that there are no conflicts of interest.

References

- 1 Delaporte E, Janssens W, Peeters M, Buve A, Dibanga G, Perret JL, Ditsambou V, Mba JR, Courbot MC, Georges A, Bourgeois A, Samb B, Henzel D, Heyndrickx L, Franssen K, van der Groen G, Larouze B: Epidemiological and molecular characteristics of HIV infection in Gabon, 1986–1994. *AIDS* 1996;10:903–910.
- 2 Bertherat E, Nabias R, Georges-Courbot MC, Renaud A: Seroprevalence of HIV, hepatitis B, and syphilis in an urban population and isolated villages in Gabon. *Sex Transm Infect* 1999;75:271.

- 3 Makuwa M, Souquière S, Apetrei C, Tevissen C, Bedjabaga I, Simon F: HIV prevalence and strain diversity in Gabon: the end of a paradox. *AIDS* 2000;14:1275–1276.
- 4 Martin MP, Dean M, Smith MW, Winkler C, Gerrard B, Michael NL, Lee B, Doms RW, Margolick J, Buchbinder S, Goedert JJ, O'Brien TR, Hilgartner WM, Vlahov D, O'Brien SJ, Carrington M: Genetic acceleration of AIDS progression by a promoter variant of CCR5. *Science* 1998;282:1907–1911.
- 5 Mummidi S, Ahuja SS, Gonzalez E, Anderson SA, Santiago EN, Stephan KT, Graig FE, O'Connell P, Tryon V, Clark RA, Dolan MJ, Ahuja SK: Genealogy of the CCR5 locus and chemokine system gene variants associated with altered rates of HIV-1 disease progression. *Nat Med* 1998;4:786–793.
- 6 Lucotte G, Mercier G: Distribution of the CCR5 gene 32-bp deletion in Europe. *J Acquir Immune Defic Syndr Hum Retrovirol* 1998;19:174–177.
- 7 Hogan CM, Hammer SM: Host determinants in HIV infection and disease. Part 2: genetic factors and implications for antiretroviral therapeutics. *Ann Intern Med* 2001;134:978–996.
- 8 Kristiansen TB, Knudsen TB, Ohlendorff S, Eugen-Olsen J: A new multiplex PCR strategy for the simultaneous determination of four genetic polymorphisms affecting HIV-1 disease progression. *J Immunol Methods* 2001;252:147–151.
- 9 Petersen DC, Kotze MJ, Zeier MD, Greenwood A, Pretorius D, Vardas E, van Rensburg EJ, Hayes VM: Novel mutations identified using a comprehensive CCR5-denaturing gradient gel electrophoresis assay. *AIDS* 2001;15:171–177.
- 10 Arenzana-Seisdedos F, Parmentier M: Genetics of resistance to HIV infection: role of co-receptors and co-receptor ligands. *Semin Immunol* 2006;18:387–403.
- 11 Su B, Sun G, Lu D, Xiao J, Hu F, Chakraborty R, Deka R, Jin L: Distribution of three HIV-1 resistance-conferring polymorphisms (SDF1-3'A, CCR2-64I, and CCR5-Δ32) in global populations. *Eur J Hum Genet* 2000;8:975–979.
- 12 Ma L, Marmor M, Zhong P, Ewane L, Su B, Nyambi P: Distribution of CCR2-64I and SDF1-3'A alleles and HIV status in 7 ethnic populations of Cameroon. *J Acquir Immune Defic Syndr* 2005;40:89–95.
- 13 Petersen DC, Glashoff RH, Shrestha S, Bergeron J, Laten A, Gold B, van Rensburg EJ, Dean M, Hayes VM: Risk for HIV-1 infection associated with a common CXCL12 (SDF1) polymorphism and CXCR4 variation in an African population. *J Acquir Immune Defic Syndr* 2005;40:521–526.
- 14 Brouwer KC, Yang C, Parekh S, Mirel LB, Shi YP, Otieno J, Lal AA, Lal RB: Effect of CCR2 chemokine receptor polymorphism on HIV type 1 mother-to-child transmission and child survival in Western Kenya. *AIDS Res Hum Retroviruses* 2005;21:358–362.
- 15 Adjé CA, Bilé CA, Kestens L, Kobravi-Dem S, Ghys PD, Maurice C, Kalou-Badrou M, Kabran N, Ekpini RE, Roels TH, Wiktor SZ, Nkengasong JN: Lack of effect of chemokine receptor CCR2b gene polymorphism (64I) on HIV-1 plasma RNA viral load and immune activation among HIV-1 seropositive female workers in Abidjan, Côte d'Ivoire. *J Med Virol* 2001;64:398–401.
- 16 Mombo LE, Lu CY, Ossari S, Bedjabaga I, Sica L, Krishnamoorthy R, Lapoumeroulie C: Mannose-binding lectin alleles in sub-Saharan Africans and relation with susceptibility to infections. *Genes Immun* 2003;4:362–367.
- 17 Caron M, Lekana-Douki SE, Makuwa M, Obiang-Ndong GP, Biba O, Nkoghe D, Kazanji M: Prevalence, genetic diversity and antiretroviral drugs resistance-associated mutations among untreated HIV-1-infected pregnant women in Gabon, central Africa. *BMC Infect Dis* 2012;12:64–70.