

**Is long term virological response related to CCR 5 Δ32 deletion in HIV infected patients started on highly active antiretroviral therapy?**

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Short title: CCR5 Δ32 in HIV treated patients

Abstract: 242 words

Keywords : HIV, antiretroviral therapy, CCR5 Δ32 deletion, virological response

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## Abstract

**Objective:** To examine whether CCR5  $\Delta$ 32 deletion is associated with long-term response to combination antiretroviral treatment (cART) in HIV-1 infected patients.

**Methods:** The genetic sub-study of ANRS CO8 APROCO-COPILOTE cohort included 609 patients who started a protease inhibitor-containing cART in 1997-99. Patients were considered to have a sustained virological response if all plasma HIV-RNA measurements between month 4 and years 3-5 were  $<500$  copies/ml, allowing for a single blip. Virological response was compared between patients heterozygous for CCR5  $\Delta$ 32 ( $\Delta$ 32/wt) and wild-type patients (wt/wt) from month 4 to year 3 and month 4 to year 5. Logistic regression analysis was used to adjust for baseline demographical data, HIV-RNA, CD4 cell counts, antiretroviral naive status, time spent on antiretroviral therapy at year 3 and 5 and adherence to treatment (month 4 to year 3 and 5).

**Results:** Sustained virological response was better in  $\Delta$ 32/wt than in wt/wt patients: 66% versus 52% up to year 3 ( $p=0.02$ ), nearly significant after adjustment to potential cofounders ( $p=0.07$ ).  $\Delta$ 32/wt patients had a better virological response, up to year 5, 48% versus 35% ( $p=0.01$ ), and remained significantly better, after adjustment, associated with a better virological response up to 5 years post initiation of cART ( $p=0.04$ ). There was no association with CD4 response.

**Conclusion:**  $\Delta$ 32/wt deletion is associated with a beneficial virological response to cART on the long-term. Whether this association can be a direct effect of  $\Delta$ 32/wt deletion remains questionable and needs confirmation in other observational studies.

## 1 **Introduction**

2 The CCR5 receptor for the  $\beta$ -chemokines RANTES (CCL5), MI-1 $\alpha$  (CCL3), and MIP-1 $\beta$   
3 (CCL4) is the primary co-receptor for macrophage-tropic, non-syncytium inducing (NSI)  
4 strains of HIV-1 entry into CD4 cells [1]. The CCR5  $\Delta$ 32, an allele that contains a 32-base  
5 pair deletion encoding for a non-functional receptor, protects against infection in  
6 homozygotes patients and is associated with delayed disease progression and death in  
7 heterozygous untreated patients [2, 3, 4,5].

8 Since 1996, the widespread use of combination antiretroviral therapy (cART) has improved  
9 the prognosis of HIV-1 infected patients [6,7]. Several studies, whether immunological or  
10 virological, have focused on the association between CCR5 genotype and response to cART, ,  
11 at different time frames and have yielded controversial results [8, 9, 10, 11, 12, 13, 14, 15  
12 ,16,17].

13 In the setting of the ANRS CO8 APROCO-COPILOTE cohort, we studied the association  
14 between the presence of the deletion and the virological response to cART up to 5 years.  
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## 16 **Patients and methods**

### 17 **Patients**

18 The ANRS CO8 APROCO-COPILOTE cohort is a prospective observational study that  
19 enrolled 1281 HIV-1 infected adults in 47 hospitals in France starting a protease inhibitor  
20 (PI)-containing antiretroviral regimen for the first time in 1997-1999 [18]. Patients had  
21 physical and laboratory examinations at enrolment, after one and four months of cART, and  
22 every four months thereafter. Sera and cells were collected at enrolment and at follow-up  
23 visits. A DNA bank was set up in 2002 to study genetic factors associated with response to  
24 treatment or tolerability of the antiretroviral drugs. The study was approved by the Ethics  
25 Committee of Hospital Cochin and informed consent was obtained from all participants.

26

## 27 **Methods**

28 Polymerase chain reaction amplification of CCR5 sequences was done using genomic DNA  
29 extracted from cryo-preserved lymphocytes.  $\Delta 32$  deletion in the CCR5 gene was detected by  
30 amplifying part (735 base pair) of the coding region [3]. CCR5  $\Delta 32$  heterozygous ( $\Delta 32/wt$ )  
31 patients were compared to wild-type patients ( $wt/wt$ ) for their baseline characteristics. Chi-  
32 square and Wilcoxon tests were performed to analyze categorical and quantitative variables,  
33 respectively. The study was performed in 2005.

34 CCR5  $\Delta 32$  heterozygous ( $\Delta 32/wt$ ) patients were compared to wild-type patients ( $wt/wt$ ) for  
35 their long term virological and immunological response to cART. The long term virological  
36 response to cART was analysed up to year 3, then to year 5 by logistic regression. To be  
37 included in the year 3 and year 5 analyses, patients should have had respectively at least one  
38 data at year 3 +/- 4 months and one at year 5 +/- 4 months. First, a stable sustained virological  
39 response was defined as a plasma HIV-1 RNA measurement below the threshold of detection  
40 of 500 copies/ml at all measurements between month 4 (M4) and year 3, and between M4 and  
41 year 5. Patients experiencing only one plasma HIV-1 RNA above 500 copies/ml were  
42 considered as meeting the definition of sustained virological response in this  
43 analysis. Secondly, immunological response was assessed using the proportion of patients who  
44 achieved a CD4 cell count greater than 500/mm<sup>3</sup> at year 3 and at year 5 [19]. Both models  
45 were adjusted for the following baseline characteristics: HIV-1 RNA, CD4 cell count, history  
46 of antiretroviral treatment at baseline (naïve cART) and during the follow-up (month 4 to year  
47 3 and 5) (median cumulative time under cART between M4 and year 3 or 5), adherence to  
48 treatment (month 4 to year 3 and 5) and demographical data (sex, age, country of birth and  
49 group of contamination). The portion of the follow-up spent without treatment was compared  
50 in the two groups:

51 - for the 3-year analysis, patients spent 2.5% of the follow-up without treatment (0.3%  
52 for CCR5  $\Delta$ 32 heterozygous patient and 2.9% for wild type patient p=0.18).

53 - for the 5-year analysis, patients spent 3.8% of the follow-up without treatment (2.1%  
54 for CCR5  $\Delta$ 32 heterozygous patients and 4.1% for wild type patients p=0.50).

55 Assessment of adherence was made by self-administrated questionnaire every year of follow-  
56 up defined in the cohort[20High adherence refers to patient who always declared being  
57 adherent, moderate adherence refers to patient who declared at least one moderate adherence,  
58 low adherence refers to patient who declared one time a non adherence and non adherence  
59 refers to patient who declared more than one time a non adherence.

60 Quantitative variables with clinically relevant threshold were analyzed as categorical  
61 variables, ie CD4 cell count categorized as  $\leq 200$  / 200-350 / 350-500 /  $> 500$ . Regarding other  
62 quantitative variables quartiles, median were considered. These potential confounding factors  
63 were used in virological and immunological analyses. Variables were included in the initial  
64 multivariable if they were associated with virological or immunological success in each  
65 univariable analysis separately with a  $P < 0.25$ . Reduced models resulted from stepwise  
66 selection retaining only variables associated with virological or immunological success at the  
67 0.05 significance level.

68 Statistical analysis was performed using Statistical Analysis System software (SAS, version  
69 8.2).

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## RESULTS

74 Among 1281 patients initially enrolled in the cohort, 609 (48%) participated in the genetic  
75 study set up in 2002. The reason for non-participation was lost to follow-up or withdrawal

76 from the cohort (n=259), death (n= 84), refusal (n=51), inability to amplify (n=42) or  
77 unknown (n=236). As the selection was important, we compared baseline characteristics  
78 according to whether patients were selected or not for this study. Regarding CD4 cells count  
79 and undetectable HIV1-RNA at enrolment, no significant difference was noticed between  
80 these two groups. Regarding baseline CD4 cells count, participating patients had a median of  
81 272/mm<sup>3</sup> vs 277/mm<sup>3</sup> among non participating patients (p=0.60). For HIV1-RNA,  
82 participating patients had a median of 4.5 copies/ml vs 4.5 copies/ml among non participating  
83 patients (p=0.13). Among the 609 patients included in the analysis, 97 (16%) were  
84 heterozygous for the CCR5-Δ32 deletion, 512 (84%) were wt/wt, and none were  
85 homozygous. At baseline, as compared to wt/wt patients, Δ32/wt patients were less frequently  
86 born in Africa and were older (table 1). They had a lower median HIV-1 RNA and a higher  
87 but not significant CD4 cell count (Table1).

88  
89 Patients were followed for a median duration of 76.3 months (Interquartile range 71.5-84.6)  
90 and experienced a median of 3 therapeutic new lines among heterozygous patients versus 4  
91 among wt/wt (p=0.05). 2679 episodes of treatment modification have been reported among  
92 577 patients. 374 among 90 Δ32/wt patients (93%) and 2305 among 487 wt/wt patients  
93 (95%). In the database, reasons are reported for 1975 of them. Among these, virological  
94 failure was mentioned in 165 cases whose 50 patients with at least one virological failure (4  
95 for Δ32/wt patients (4%) and 46 for wt/wt patients (9%)).  
96 601 and 576 patients were included respectively in the year 3 and year five analysis. Patients  
97 had a median of 9 available HIV-1 RNA measurements (IQR[8-9]) between M4 and M36 and  
98 15 available HIV-1 RNA measurements (IQR[13-15]) between M4 and M60.  
99 From M4 to year 3, 63 patients have a stable virological response (66%) among patients with  
100 the Δ32 deletion, and 264 (52%) among those without the deletion (p = 0.02). When

101 extended follow-up (M4 to year 5), respectively 44 patients (48%) and 168 patients (35%)  
102 have a stable virological response (p=0.01).

103 At year 5, these differences were also noticed among  $\Delta 32$ /wt and wt/wt in the two subgroups  
104 adjusting for cART-naïve patients (51% vs 45% respectively) and pre treated patients (46%  
105 vs 27%) (this difference was significant  $p_{\text{Mantel Haentzel}}=0.02$ ). The proportion of patients with a  
106 CD4 cells count greater than  $500/\text{mm}^3$  at year 3 did not significantly differ between the  $\Delta 32$   
107 and wild type patients: 55% and 49 % respectively (p=0.26), nor at year 5: 52% and 54%,  
108 respectively (p=0.73).

109

110 After adjustment for confounding factors,  $\Delta 32$  deletion significantly associated with a  
111 sustained virological response during the period M4 to 5 years post-enrolment (p=0.04), and a  
112 near significantly associated with a sustained virological response during the period from M4  
113 to 3 years post-enrolment (p=0.07)(Table 2).

114 For immunological response  $\Delta 32$  deletion was not a significant factor associated with a CD4  
115 count greater than  $500/\text{mm}^3$  at year 3 (p=0.78), nor at year 5 (p=0.15).

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## DISCUSSION

119 Among 609 HIV-1 infected patients started on a PI containing treatment, the  
120 frequency of heterozygous patients for CCR5  $\Delta 32$  was 16%: it was 4% in patients born in  
121 Africa, and 19% in patients born in Europe, similar to previous studies carried out in similar  
122 populations [12, 14, 16, 17]. The CCR5  $\Delta 32$  deletion was associated to a better virological  
123 response to cART up to 3 and 5 years. Better virological response was not translated into a  
124 significantly better immunological response at any term of the study.

125 At baseline, patients with  $\Delta 32$  deletion, were older, had higher CD4 cell count and  
126 lower HIV RNA than others. This might be explained by the effect of CCR5  $\Delta 32$  deletion on  
127 the natural evolution of HIV infection before these patients started cART. Indeed, previous  
128 studies have shown that the presence of an allele with CCR5  $\Delta 32$  confers a delayed  
129 progression to the HIV-1 disease in the absence of cART [3, 4]. Furthermore, the effect of the  
130 deletion may have contributed to a possible selection bias [19]. Indeed, the patients who could  
131 be included in the genetic bank study were those who had survived from 1997 to 2002, and  
132 were younger. This bias limits the interpretation of our results, since only those with a better  
133 prognosis could be included.

134 Previous studies relating to the effect of virological response to cART according to  
135 CCR5- $\Delta 32$  deletion provided contradictory results: some found a protective role of the  
136 CCR5- $\Delta 32$  deletion on virological response to cART [8, 9, 10, 11, 16, 17] while others did  
137 not find any difference [12, 13, 14, 15]. All have studied relatively short term response, no  
138 longer than 2 years. Moreover the characteristics of the patients, clinical and biological status  
139 of HIV infection, geographical origin, whether they were pretreated or naive of cART,  
140 adherence to treatment, definition of the virological response (e.g 50 or 500 copies/ml) and  
141 time for assessment, varied among the studies.

142 Our study is probably the first to assess the impact of this deletion over a long follow-up of a  
143 large number of treated patients, and shows a significant better response after 5 years of  
144 treatment in the heterozygous patients. The longest follow-up was 24 months in the study of  
145 Bogner et al. [11], which found a better virological response to cART, among adherent  
146 caucasian patients, naïve of antiretroviral treatment.

147 The discrepancy between short-term and long-term virological response to cART in  
148 our study might explain some of the differences between previous studies. The interpretation  
149 of such a moderate effect of the deletion on response to cART would be in favour of either the

150 absence of an effect among treated patients, or a limited effect only detectable after extensive  
151 follow-up.

152 In order to take into account differences existing at baseline or occurring during  
153 follow-up that might also influence response to cART, the multivariable analysis was adjusted  
154 for potential confounders. Yet, we found that long term virological response was more  
155 pronounced in the heterozygous patients, reflecting a true independent effect of the CCR5  
156  $\Delta 32$  deletion in the context of a multifactorial determinism of long term virological response.  
157 Therefore, the potential disadvantage of the innate wild-type profile might be counterbalanced  
158 by the beneficial effect of high adherence level and appropriate time for starting cART.  
159 In view of conflicting results in the previous studies, a meta-analysis including other  
160 observational cohorts would bring more evidence on the long-term effect of this mutation.

**Acknowledgements : APROCO-COPILOTE ANRS CO8 study group**

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**Data monitoring and statistical analysis:**

J. Biemar, S. Boucherit, AD Bouhnik, C. Brunet-François, M.P. Carrieri, F. Couturier, J.L. Ecobichon, , V. Guiyedi, P. Kurkdji, S. Martiren, M. Préau, C. Protopopescu, C. Roy, J. Surzyn, A. Taieb, V. Villes, C. Wallet

**Promotion:** Agence Nationale de Recherches sur le Sida et les hépatites virales (ANRS, Action Coordonnée n°7).

**Other supports:** Collège des Universitaires de Maladies Infectieuses et Tropicales (CMIT ex APPIT), Sidaction Ensemble contre le Sida, et laboratoires : Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, Glaxo- SmithKline Gilead Sciences, Pfizer, Roche.

**Clinical Centers (coordinators):** Amiens (Pr JL. Schmit), Angers (Dr JM. Chennebault), Belfort (Dr JP. Faller), Besançon (Pr JL. Dupond, Dr JM. Estavoyer, Dr Drobachef), Bobigny (Pr O. Bouchaud), Bordeaux (Pr M. Dupon, Pr Longy-Boursier, Pr P. Morlat, Pr JM. Ragnaud), Bourg-en-Bresse (Dr P. Granier), Brest (Pr M. Garré), Caen (Pr R. Verdon), Compiègne (Dr D. Merrien), Corbeil Essonnes (Dr A. Devidas), Créteil (Pr A. Sobel), Dijon (Pr H. Portier), Garches (Pr C. Perronne), Lagny (Dr P. Lagarde), Libourne (Dr J. Ceccaldi),

Lyon (Pr D. Peyramond), Meaux (Dr C. Allard), Montpellier (Pr J. Reynes), Nancy (Pr T. May), Nantes (Pr F. Raffi), Nice (Pr JG Fuzibet, Pr P. Dellamonica), Orléans (Dr P. Arsac), Paris (Pr E. Bouvet, Pr F. Bricaire, Pr P. Bergmann, Pr J. Cabane, Dr J. Monsonogo, Pr P.M. Girard, Pr L. Guillevin, Pr S. Herson, Pr C. Leport, Pr MC. Meyohas, Pr J.M. Molina, Pr G. Pialoux, Pr D. Salmon), Poitiers (Pr B. Becq-Giraudon), Reims (Pr R. Jaussaud), Rennes (Pr C. Michelet), Saint-Etienne (Pr F. Lucht), Saint-Mandé (Pr T. Debord), Strasbourg (Pr JM Lang), Toulon (Dr JP. De Jaureguiberry), Toulouse (Pr B. Marchou), Tours (Pr JM. Besnier).

The authors would like to thank Rodolphe Thiebaut for his helpful suggestion concerning the statistical methodology.

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