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Running head: **HIV-1Transmitted Drug Resistance**

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Abstract

Objectives

Transmitted Drug Resistance (TDR) can impair first-line antiretroviral therapy response. It has been shown by Sanger sequencing that TDR was more common among Men having Sex with Men (MSM) in treatment-naive patients chronically infected with HIV type 1 (HIV-1). We aimed to compare the presence of TDR mutations between two groups of HIV-1 transmission.

Methods

We studied, by Sanger Sequencing and UltraDeep Sequencing (UDS), the presence of resistance mutations, both in majority (>20%) and in minority (1-20%) proportions, among 70 treatment-naive MSM and 70 treatment-naive heterosexual patients recently screened positive for HIV-1.

Results

Between the two groups, global prevalence of TDR was not significantly different either by Sanger or by UDS. Nevertheless, a higher frequency of nucleoside reverse transcriptase inhibitors-TDR was observed among heterosexual patients (p=0.04). There was also a trend for a higher frequency of TDR among MSM infected with HIV-1 subtype B compared to MSM infected with HIV-1 subtype non-B (p=0.06).

Conclusions

UDS allows sensitive monitoring of TDR, and highlights some disparities between groups of transmission.

INTRODUCTION

Transmitted Drug Resistance (TDR) detection can impair first-line antiretroviral therapy response. Indeed, the presence of TDR-mutations can conduct to a higher risk of virological failure if the affected drugs are introduced [1]. The surveillance of HIV type 1 (HIV-1) TDR is widely recommended and it has been shown that TDR was more common among Men having Sex with Men (MSM) and among patients infected with subtype B virus in the 2010/2011 French survey study conducted in antiretroviral-naïve chronically HIV-1-infected patients by standard sequencing techniques [2]. Standard sequencing detects viral populations accounting for more than 15-20% of viral population. However, HIV-1 minority resistant variants can be a source of virological failure: it was mainly shown for first line regimens based on first generation Non Nucleoside Reverse Transcriptase Inhibitors (NNRTI) [3,4]. The next generation sequencing technologies are able to detect these minority variants. Nevertheless, few data are available on presence of minority TDR variants in different groups of HIV transmission, treatment-naive patients [5]. The use of UltraDeep Sequencing (UDS) could evidence larger difference of TDR between groups of transmission than Sanger sequencing does.

The aim of the study was to compare the presence of TDR mutations, both in majority (>20%) and in minority (1-20%) proportions, between treatment-naive MSM and treatment-naive heterosexual HIV-1 chronically infected patients.

METHODS

Patients. The study enrolled 70 treatment-naïve heterosexual patients and 70 treatment-naïve MSM recently diagnosed for HIV-1. We performed Sanger Sequencing (n=140; Reverse Transcriptase gene (RT) and Protease gene (PR)) and UDS (n=70 RT and n=70 PR sequences among MSM group; n=54 RT and n=67 PR sequences among heterosexual group). Patients

were followed by Department of Infectious Diseases of Pitié-Salpêtrière and Bichat Claude Bernard hospitals (Paris, France). Informations were obtained from the existing electronic database or medical record. Patients were informed that their demographic and clinical data will be recorded during their follow up and could be used for retrospective studies and gave their consent. This study was approved by the Agence Nationale de Recherches sur le SIDA et les hépatites virales (ANRS) AC11 Ethics Committee.

Sanger sequencing. The first sample of plasma positive for HIV-1 was used for performing genotypic resistance test. RT and PR genotypic analysis was conducted according to the ANRS consensus method [6]. PR and RT mutations were identified using the consensus statement of the list for the TDR genotypic surveillance. [7] Additional interpretations were performed with the International AIDS Society (IAS) list (figure) and the last version of ANRS algorithm (www.hivfrenchresistance.org). Any sequences found to have a mixture of wild type and mutant amino acid residues at single positions were considered to have the mutant.

The subtype determination was performed using the HIV Module of SmartGene (SmartGene, Zug, Switzerland) whose methodology is based on Basic Local Alignment Search Tool (BLAST).

UDS. The steps until pyrosequencing on GS Junior (Roche 454® Life Sciences, Branford, CT, United States) were previously described [8]**.** Primers used are available on *hiv.frenchresistance.org*. Pyrosequencing was performed according to manufacturer recommendations [9]. GS Amplicon Variant Analyzer (Roche 454® Life Sciences, Branford, CT, United States) was used to analyze the UDS results. Alignments were checked.

Statistical Analysis. To compare MSM and heterosexual populations baseline characteristics, Chi 2 and Mann-Whitney tests were used for categorical (subtype) and continuous variables (age, viral load, CD4 cells count), respectively.

Global prevalence and prevalence of TDR mutations by antiretroviral classes were compared by a Chi 2 test between the two groups.

RESULTS

An average of 5198 reads per nucleotide position was amplified and the average error rates in controls (cellular clone 8E5) were 0.0012 and 0.0032 for RT and PR, respectively. These results allowed for accurate detection of variants down to 1% [10, 11].

Characteristics of patients are as follows: the median age was 36.3 years among the 70 MSM patients and 35.4 years among the 70 heterosexual patients (60% female), without significant difference between the two populations. Sixty percent of the patients were infected by HIV-1 subtype B among the MSM group whereas 94% were infected by HIV-1 subtype non-B among the heterosexual group. Among the MSM patients, median viral load (VL) was 4.9 $\log_{10}$ copies/mL (IQR=4.4-5.4) and median CD4 cell count was 498/mm^3 (IQR=347-585). Among the heterosexual patients, median VL was 4.9 $\log_{10}$ copies/mm^3 (IQR=4.3-5.3) and median CD4 cell count was 348/mm^3 (IQR=208-497). The levels of HIV-1 VL were similar between the two groups, but median CD4 cell count was higher among MSM than among heterosexual patients (p= 0.0016).

Global prevalence of TDR and prevalence by drug classes are presented in Table 1. A total concordance was found between Sanger sequencing and UDS for all mutations detected on bulk.

Sanger sequencing detected two Nucleoside Reverse Transcriptase Inhibitors (NRTI)-TDR mutations in virus genome of one MSM patient (M41L and T215C) and one in virus genome of three heterosexual patients (M41L or T215E or M184I/V); one NNRTI-TDR mutation was detected in virus genome of two MSM patients (K103N) and one in virus genome of three heterosexual patients (K103N or Y181I or Y188L) and one Protease Inhibitors (PI)-TDR mutation was detected in virus genome of one heterosexual patient (I85V).

Within each group, we retrieved a higher prevalence of TDR mutations when UDS was performed: prevalence of TDR was 18.6% (95%CI=9.4%-27.7%) with UDS *versus* 4.3% (95%CI=0.0%-9.1%) with Sanger Sequencing among MSM and 22.8% (95%CI=12.7%-33.0%) with UDS *versus* 7.1% (95%CI=0.9%-13.4%) with Sanger Sequencing among heterosexual population.

Among the MSM, the increased TDR detected by UDS was observed especially for NNRTI and PI (10.0% *versus* 2.9% by Sanger sequencing and 7.1% *versus* 0.0% by Sanger sequencing, respectively) while it was mainly for NRTI and PI among the heterosexual patients (14.8% *versus* 4.3% by Sanger sequencing and 10.4% *versus* 1.4% by Sanger sequencing, respectively).

The rate of TDR mutations only detected by UDS was low: between 1.1% and 4.8% for NRTI, 1.1% and 1.5% for NNRTI and 1.1% and 7.0% for PI (Figure 1).

Between the two groups, global prevalence of TDR was not significantly different either by Sanger or by UDS. Nevertheless, a higher frequency of NRTI-TDR was observed among heterosexual patients than among MSM (14.8% *versus* 4.3%, respectively; p=0.04).

We also retrieved a trend for a higher frequency of TDR among MSM patients infected with HIV-1 subtype B compared to MSM patients infected with HIV-1 subtype non-B (26.2% (n=11/42) *versus* 7.1% (n=2/28) with UDS (*p*=0.06), data not shown).

In addition, some resistance mutations, not considered in the list for TDR genotypic surveillance, but considered as major in some genotypic algorithms, were identified as minority variants. For instance, three K65E mutations were detected by UDS. With the last version of ANRS algorithm, the prevalence of resistance to the recommended drugs (European AIDS Clinical Society EACS) detected by UDS for NRTI, NNRTI and IP were 2.9%, 12.8% and 7.1% among MSM patients and 11.1%, 5.6% and 11.9% among heterosexual patients, respectively.

DISCUSSION

Higher prevalence of TDR was observed in antiretroviral-naive chronically HIV-1-infected MSM and heterosexual populations when UDS was used compared to population sequencing. Indeed, these powerful techniques could improve the detection of HIV-1-TDR.

In the present study, UDS allowed to detect a higher frequency of NRTI-TDR among heterosexual population than among MSM patients, whereas based on the results of CD4 cell count, the date of transmission seems to be older in the heterosexual population. Usually, the more time goes by and the less TDR are detected. However, most thymidine analogue mutations were found to be highly stable without selection pressure. It has been shown that NNRTI and PI mutations were, globally, less persistent than NRTI mutations, maybe because of a negative impact on viral fitness [11,12].

Otherwise, UDS evidenced a higher frequency of TDR in subtype B versus subtype non-B viruses among MSM patients. This is consistent with what has been previously shown by Sanger sequencing in the Odyssee study on antiretroviral-naive chronically HIV infected patients and in the study on French patients diagnosed at the time of primary HIV-1 infection [2,13].

We obviously need further investigations for NRTI, second generation NNRTI and PI-based regimen, as the impact of minority TDR mutations on first generation NNRTI is already well known [3,4]. Furthermore, minority TDR mutations on integrase strand transfer inhibitors have not been studied in this work. As few data are available on this subject and still controversial, more studies are needed [14,15]. Nevertheless, an increase of TDR is predictable for this therapeutic class with the extensive use of these drugs and should be monitored in the future.

In conclusion, UDS allows sensitive monitoring of TDR, and is able to evidence some disparities of TDR between HIV groups of transmission. In the present study, next generation sequencing technologies probably detect minority resistant variants that are disappearing. The impact of these minority TDR mutations for certain therapeutic classes and in particular for the more recent drugs is unknown and should be further evaluated.

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Conflicts of interest: All authors declare that they have no conflicts of interest.

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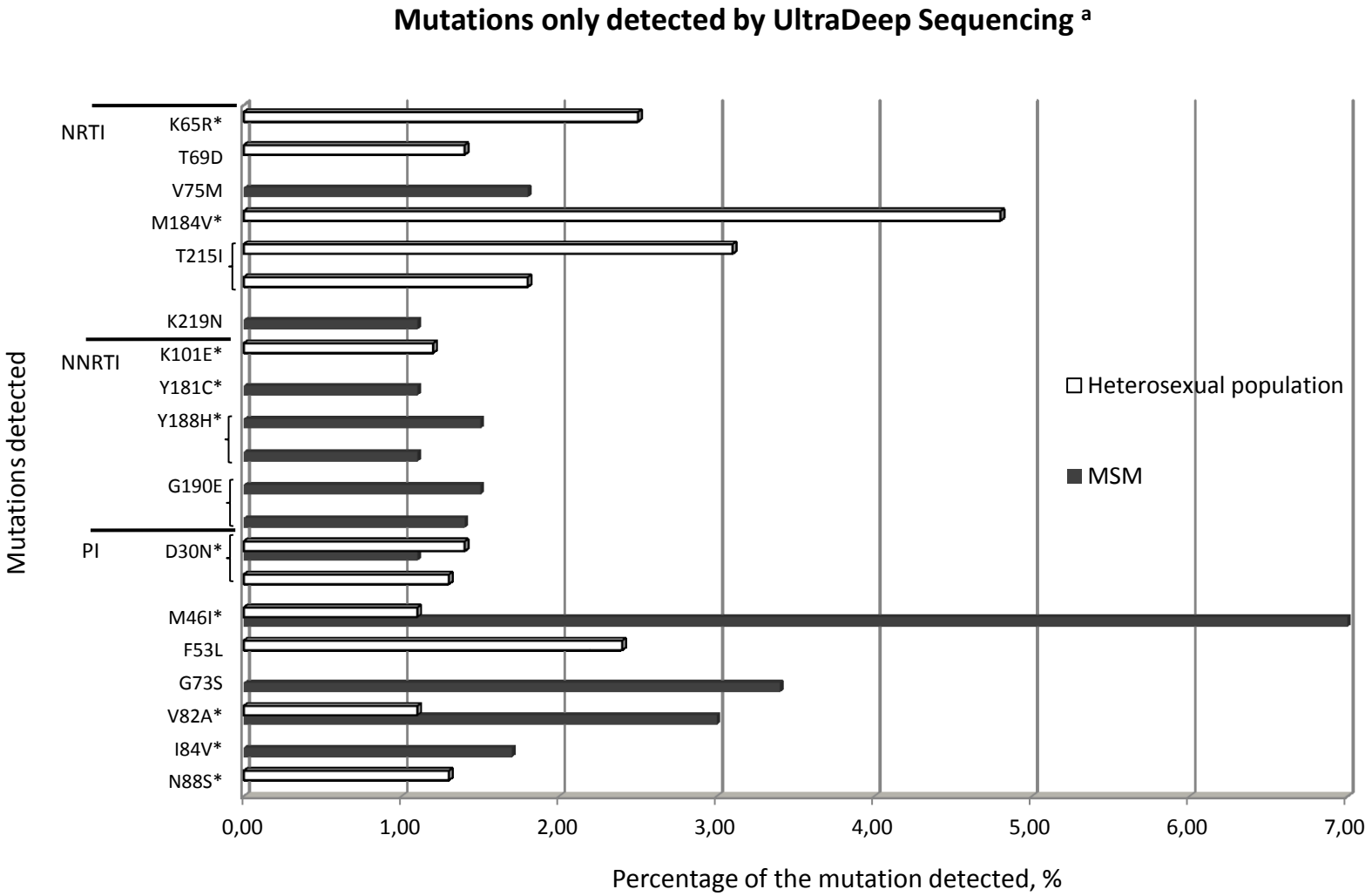
Table 1: Global prevalence of Transmitted Drug Resistance and by drug classes among the two groups: MSM and heterosexual patients.

	Sanger sequencing			**UltraDeep sequencing**		
	MSM n=70	**Heterosexual population n=70**	**p value**	**MSM n=70**	**Heterosexual population RT n=54 Protease n=67**	**p value**
Global prevalence of TDR [95% CI]	n= 3 4.3% [0.0%-9.1%]	n= 5 7.1% [0.9%-13.4%]	0.47	n= 13 18.6% [9.4%-27.7%]	n=16 22.8% [12.7%-33.0%]	0.53
Prevalence of NRTI-TDR [95% CI]	n= 1 1.4% [0.0%-4.2%]	n= 3 4.3% [0.0%-9.2%]	0.31	n= 3 4.3% [0.0%-9.1%]	n= 8 14.8% [5.3%-24.4%]	0.04
Prevalence of NNRTI-TDR [95% CI]	n= 2 2.9% [0.0%-6.8%]	n= 3 4.3% [0.0%-9.2%]	0.65	n= 7 10.0% [2.9%-17.1%]	n= 4 7.4% [0.4%-14.5%]	0.61
Prevalence of PI-TDR [95% CI]	n=0 0.0%	n= 1 1.4% [0.0%-4.3%]	0.32	n= 5 7.1% [1.1%-13.2%]	n= 7 10.4% [3.1%-17.8%]	0.49

CI: Confidence Interval; MSM: Men Men having Sex with Men; NRTI: Nucleoside Reverse Transcriptase Inhibitors; NNRTI: Non NRTI; PI: Protease Inhibitors; TDR: Transmitted Drug Resistance

Figure 1. Percentage of Transmitted Drug Resistance mutations only detected by UDS among the two groups: MSM and heterosexual patients



[a]: Each bar represents one patient; * Major IAS drug resistance mutations
MSM: Men having Sex with Men; NRTI: Nucleoside Reverse Transcriptase Inhibitors; NNRTI: Non NRTI; PI: Protease Inhibitors; UDS: UltraDeep Sequencing