Research Letter

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Accurate quantification of CXCR4-using HIV-1 variants by Illumina deep-sequencing

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Next-generation sequencing is a sensitive method for determining HIV-1 tropism but there is a lack of data on the quantification of X4 variants. We evaluated MiSeq and 454 GS-Junior platforms for determining HIV-1 tropism and for quantifying X4 variants. Both platforms were 93% concordant for determining HIV-1 tropism and correlated well for determining the proportion of X4 variants (Spearman correlation, $\rho = 0.748$; $P < 0.0001$). MiSeq Illumina sequencing seems to be well adapted for characterizing X4-containing samples.

The determination of HIV-1 coreceptor tropism is critical for the therapeutic use of CCR5 antagonists [1] but coreceptor usage is also correlated with the disease progression in treated and untreated patients [2–4]. Phenotypic assays are the gold-standard for assessing HIV-1 coreceptor usage and detecting minority CXCR4-using (X4) variants (<15–25% of the virus quasispecies) [5,6]. Although simple genotypic approaches can replace more complicated phenotypic assays, population V3-sequencing is less sensitive for detecting minority X4 variants. Genotypic assays using next-generation sequencing (NGS) are more sensitive, providing levels similar to that of phenotypic assays [7]. However, there is a lack of data on the concordance of X4 variants quantified using different NGS platforms. One study that used the MiSeq platform and mixtures of R5 and X4 viruses overestimated the percentage of X4 variants [8]. We have, therefore, assessed the performance of the MiSeq Illumina platform with reference to the 454 Roche technology for quantifying X4 variants. The samples assayed were from a large panel of clinical baseline plasmas from antiretroviral-naïve individuals enrolled in the ANRS 146 Optimal trial with $CD4^+$ count less than 200 cells/μl or an AIDS-defining event [9].

The median HIV-1 virus load was 5.4 log copies/ml [interquartile range (IQR) 4.1–6.6] and the median $CD4^+$ cell count was 80 cells/μl (IQR 3–148) at baseline. Virus RNA was extracted from them and PCR used to generate amplicons encompassing the V3 *env* region. These were sequenced in parallel on the 454 GS-Junior and MiSeq platforms [8,10]. The sequence reads were aligned with the BaL consensus sequence (GenBank accession no. AY426110.1) and processed using an in-house automated data cleaning strategy (PyroVir, IDDN FR.001.160011.000.S.P.2012.000.31230, Inserm-Transfert) that provides a fast, automated position-specific process for inferring HIV-1 tropism from V3 env deep sequencing data with improved detection of minor variants [10]. The genotypic rule used to predict CXCR4-usage is specific of the virus subtype (subtype B, subtype D and CRF01-AE for which we have developed specific algorithms) [11–14]. We determined specific error rates at each position in V3 by sequencing clones and we constructed a sensitivity threshold matrix at each position of V3 to identify authentic virus variants harboring a point mutation for a given number of reads with $P < 0.001$ (Poisson statistics). The sensitivity for detecting CXCR4-using minority variants was 0.26% for 5000 reads on MiSeq and 0.25% for 2000 reads on the 454 GS-Junior [8].

MiSeq (mean of 14788 reads/sample) successfully genotyped 98 consecutive patient samples and identified CXCR4-using viruses in 36 (proportions of 0.25–72.5%; mean = 20.3%). The 454 GS-Junior (mean of 2087 reads/sample) identified CXCR4-using viruses in 29 samples (proportions of 1.96–100%; mean = 29.2%). Both NGS platforms identified the same 62 exclusive R5 viruses and 29 CXCR4-using viruses (agreement: 93%) but disagreed on seven samples; MiSeq detected CXCR4-using variants (frequencies: 0.25–3.17%) whereas the 454 GS-Junior identified them as CCR5-using. The discrepant samples were analysed using a validated sensitive phenotypic assay (ISO 15189 accreditation) [6]. CXCR4-using variants were detected in five of the seven samples whereas R5 viruses were detected in the other two samples (data not shown). The proportion of X4 variants determined by the MiSeq platform was correlated with the proportion of X4 variants determined by the 454 GS-Junior (Spearman correlation test, $\rho = 0.748$; $P < 0.0001$). Bland–Altman analysis indicated a bias of −4.3% between the two NGS platforms (Fig. 1). Thus, we found no overestimation of the percentage of X4 variants using MiSeq in contrast with a previous study [8], probably because we analysed clinical samples instead of artificial mixtures.

These X4 variant-rich samples were suitable for assessing how well the two assays quantified X4 variants. The MiSeq platform was more sensitive for detecting X4 minority variants probably because it generated seven times more reads than the 454 GS-Junior. Although the MiSeq Illumina was more sensitive than the 454 GS-Junior, both platforms agreed well for determining HIV-1

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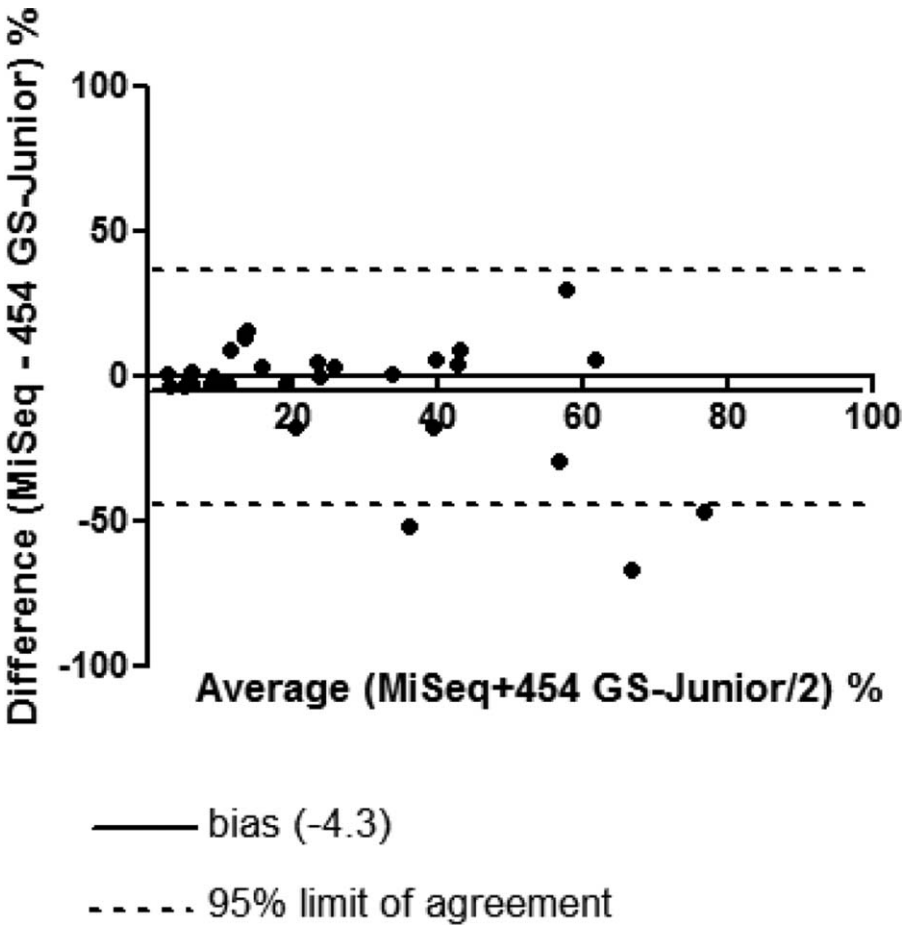

Fig. 1. Two next-generation sequencing methods for quantifying CXCR4-using variants. Bland–Altman diagram comparing the 454 GS-Junior and MiSeq platforms. Each point represents one of the CXCR4-using variants quantified by both platforms. The bias is the mean of the difference between the two methods.

tropism. As the 454-technology is shut down, MiSeq Illumina sequencing seems to be well adapted for characterizing X4-containing samples in virological studies and for determining HIV tropism in clinical practice.

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Conflicts of interest

There are no conflicts of interest.

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