Amsterdam institute for Infection and Immunity

# A genetic variation in Fucosyltransferase 8 accelerates HIV-1 disease progression indicating a role for N-glycan fucosylation

**Lisa van Pul, Karel A. van Dort, Brigitte Boeser-Nunnink, Irma Maurer, Neeltje Kootstra**
Amsterdam UMC location University of Amsterdam, Experimental Immunology, Meibergdreef 9, Amsterdam, Netherlands
Amsterdam Institute for Infection and Immunity, Infectious diseases, Amsterdam, The Netherlands

## Background

Fucosyltransferase 8 (FUT8) is an enzyme that is uniquely responsible for a process known as N-glycan core fucosylation and is involved in post-translational modification of glycoproteins. Changes in core fucosylation by FUT8 have been shown to affect the immune response. For instance, fucosylation of immunoglobulin G (IgG) alters the antibody binding to Fc receptors. In addition alterations in T-cell receptor (TCR) fucosylation have been shown to influence TCR conformation, TCR signaling, T-cell development and function. Moreover, low level of core fucosylation diminishes PD-1 expression and thereby improves T-cell responses. We hypothesize that genetic variations in FUT8 may affect fucosylation and thus the immune response against HIV-1.

## Aim

Here we investigated the effect of single nucleotide polymorphisms (SNPs) in FUT8 on the outcome of HIV-1 infection and disease course.

## Methods

SNPs in FUT8 were analyzed in HIV-1 infected participants of the Amsterdam Cohort Studies (ACS) on HIV-1 and AIDS. Cox regression and survival analysis was performed to determine the effect of the SNPs on the outcome of untreated infection. Using flow cytometry, the effect of a SNP in FUT8 on T cell surface marker expression was determined 1 year before HIV-1 infection and 1 and 5 years after seroconversion (SC). In a set of blood donors additional flow cytometry analysis was performed to assess T cell surface marker expression. The effect of the SNP on T cell function was analyzed by proliferation assay using CellTrace Violet staining in blood donor PBMC.

## Results: Survival analysis

Genome wide association study (GWA) in the ACS identified 92 SNPs in the FUT8 gene region. SNPs in FUT8 (rs11847263), B4GALT1 (rs12342831), ST6GALT1 (rs11710456), IKZF1 (rs6421315) and LAMB1 (rs2072209) that have previously been reported to affect IgG fucosylation (Lauc et al., 2013; Wahl et al., 2018), had no effect on HIV-1 disease progression. However, the presence of the minor allele (homozygous/heterozygous) of SNP rs4131564 in FUT8 was associated with an accelerated disease progression according to the Centers for Disease Control definition set in 1993 (AIDS-defining events including CD4 counts <200 cells/ul; Figure 1). This was independent of CCR5Δ32 genotype and HLA-B*57 genotype as determined by Cox regression. However, no effect of the SNP was seen on viral load and CD4 T cell counts at setpoint.

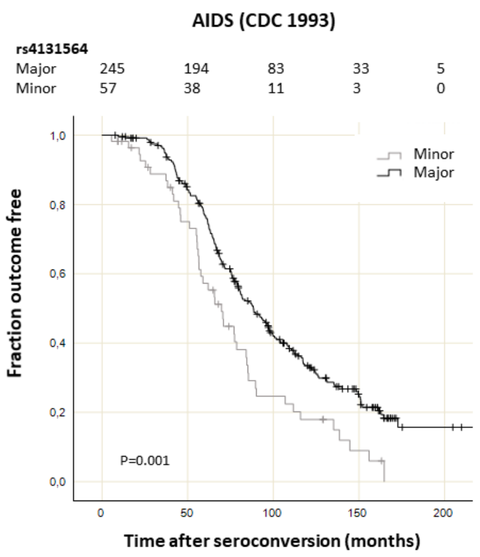


**Figure 1:** Kaplan-Meier survival analysis for SNP rs4131564 in FUT8 with time in months from seroconversion to progression to AIDS as defined by the CDC in 1993. P-value as calculated by the Log Rank test. Carriers of the minor allele showed accelerated disease progression.

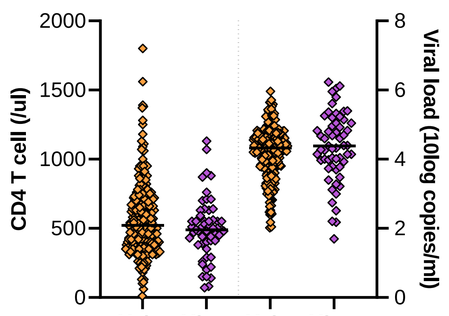


**Figure 2:**. Viral load and CD4 T cell counts at setpoint did not significantly differ between HIV-1 infected ACS individuals homozygous for the Major variant of rs4131564 and individuals carrying the minor allele.

## Results: T cell activation in HIV-1 infected individuals

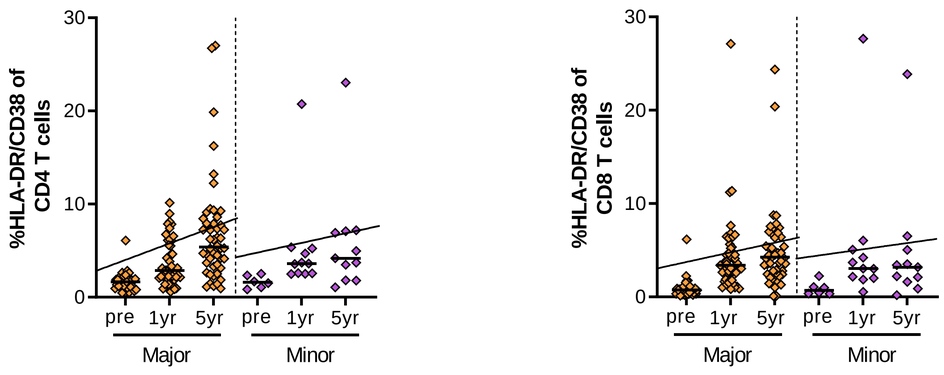


**Figure 3:** To determine whether SNP rs4131564 in FUT8 has an effect on T cell activation levels flow cytometry analysis was performed in PBMC of HIV-1 infected individuals of the ACS. T cell activation was defined as the percentage CD4 or CD8 T cells co-expressing HLA-DR and CD38 at different time points: before seroconversion (pre), 1 year and 5 years post seroconversion. No significant differences in CD4 or CD8 T cell activation are seen at any time point between individuals homozygous for the major variant of rs4131564 and individuals carrying the minor allele. Regression analysis (indicated by the line) revealed an overall significant increase in T cell activation over time in major individuals in both CD4 and CD8 populations (P<0.0001 and P=0.0006 respectively) and not in minor. However, there was no significant difference in the slopes when comparing individuals carrying the minor variant of rs4131564 and individuals carrying the major variant both in the CD4 and CD8 compartment.

## Results: Proliferation marker expression in HIV-1 infected individuals

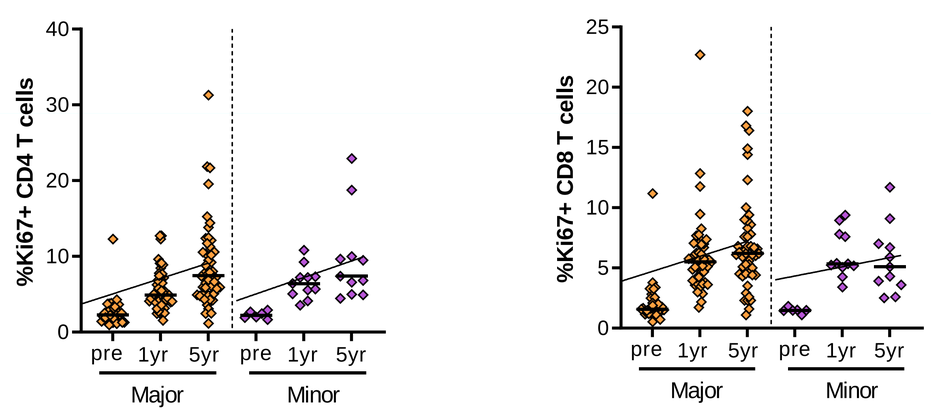


**Figure 4**: To determine the proliferation rate of CD4 and CD8 T cells Ki67 surface expression was assessed in PBMC of HIV-1 infected individuals at three time-points. There were no significant differences in Ki67 expression between individuals carrying the major or the minor allele of rs4131564 at any of the three time-points in either the CD4 or CD8 compartment. There was an overall significant increase of Ki67 expression on CD4 T cells in both groups as determined by linear regression (p<0.0001, p=0.0037 respectively). However, the slopes did not significantly differ between minor and major (p=0.8426). In the CD8 population the increase of Ki67 expression was significant in individuals carrying the major allele (P<0.0001) but not the minor allele (p=0.1017) but the slopes were not significantly different from each other (P=0.4348).

## Results: T cell exhaustion and senescence in blood donors

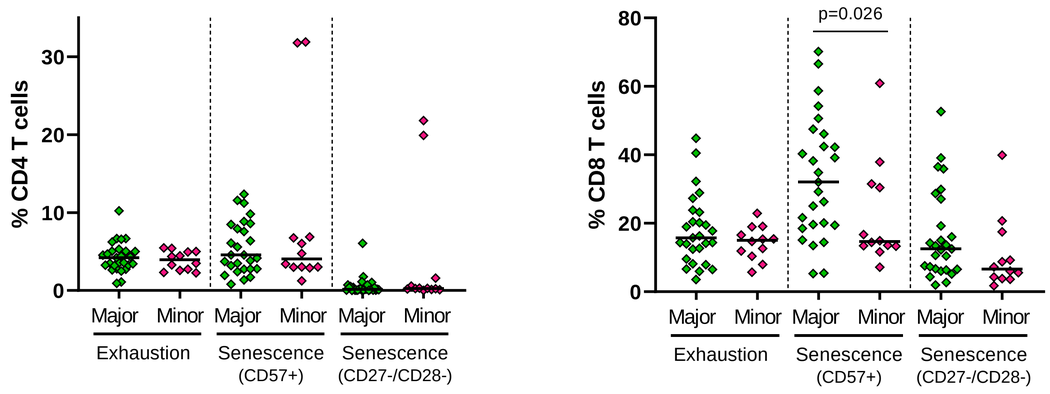


**Figure 5:** In order to assess the effect of SNP rs4131564 on T cell exhaustion and senescence, surface marker expression of PD-1, CD57 and CD27 & CD28 was determined in PBMC of blood donors. T cell exhaustion was defined as expression of PD-1 and T cell senescence was defined as CD57 expression or absence of expression of both CD27 and CD28.
No significant differences in expression of exhaustion or senescence markers were found on the CD4 T cells of donors homozygous for the major allele of rs4131564 (n=27) and donors hetero/homozygous for the minor allele (n=12). CD57 expression on CD8 T cells was significantly lower in individuals carrying the minor allele of the SNP.

## Results: Proliferation assay using CellTrace Violet in blood donors

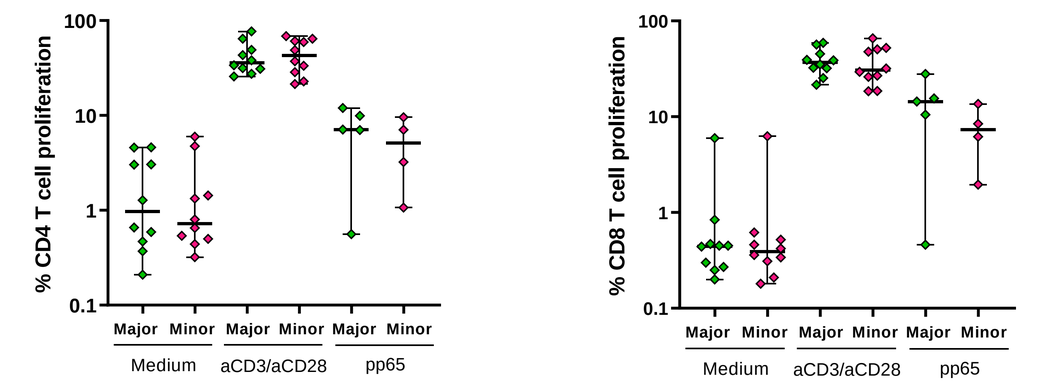


**Figure 6**: To assess whether there is a difference in T cell funtionality in individuals carrying rs4131564 a proliferation assay was performed. PBMC from blood donors homozygous for the major allele or heterozygous/homozygous for the minor allele of rs4131564 were stained with CellTrace Violet and stimulated with anti-CD3/anti-CD28, pp65 (in case of previous CMV infection) and a medium control. No significant differences in CD4 or CD8 T cell proliferative capacity were seen when comparing individuals with to without SNP.

## Conclusions

- We observed that a naturally occurring genetic variation in the FUT8 gene was associated with accelerated HIV-1 disease progression independent of CCR5Δ32 and HLA-B*57 genotpye.
- The SNP was not associated with differences in IgG fucosylation
- T-cell activation, exhaustion, TCR signaling and T-cell proliferation were also not significantly different between individuals with the minor or major variant of rs4131564.

However, these assays were unable to detect minor or combined effects of the SNP on the immune response that might accumulate over time. Our data show that glycoprotein fucosylation plays a role in HIV-1 infection by a yet unknown mechanism.

**Acknowledgements**: Special thanks to the participants of the Amsterdam Cohort Studies (ACS).
**Correspondence**: l.vanpul@amsterdamumc.nl