Do gene expression levels and genetic polymorphisms in HIV Integrase Cofactor LEDGF/p75 have an impact on HIV-1 Disease Progression

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BACKGROUND: Lens epithelium derived growth factor/transcriptional co-activator p75 (LEDGF/p75) is an important cellular co-factor for the HIV enzyme integrase. In the present study, we evaluated if genetic variation in the LEDGF/p75 gene and mRNA expression levels might explain differences in HIV disease progression.

METHODS & RESULTS:

Patients: Samples were derived from a therapy-naïve patient cohort from the Ghent University Hospital and from the long-term-non-progressor patient Spanish RIS cohort, kindly provided by by the HIV BioBank

Table 1: Characteristics of the patients included in this study

| Progression:	Afirican	Caucasian	Total
Elite controllers	2	47	49
Viremic controllers	6	56	62
Non controllers	26	188	214
Total	34	291	325

SNP detection: A comprehensive genomic scan of the coding region (including intronic regions near the exon-intron boundary and 3'UTR) of LEDGF/p75 was performed with high resolution melting (HRM) curve analysis and Sanger sequencing to identify single nucleotide polymorphisms (SNPs). 24 Single SNPs were identified, of which 5 in the coding region, 17 in the non-coding regions and 3'UTR. In addition to these, two known tagSNPs were included. Only the SNPs that were correlated with disease progression, average viral load or CD4 slope are further described (Fig. 1).

Fig. 1: schematic illustration of the DNA, mRNA and protein of LEDGF/p75. Intronic sites in the DNA are grey, and the positions of the four SNPs that are discussed are marked in the DNA.
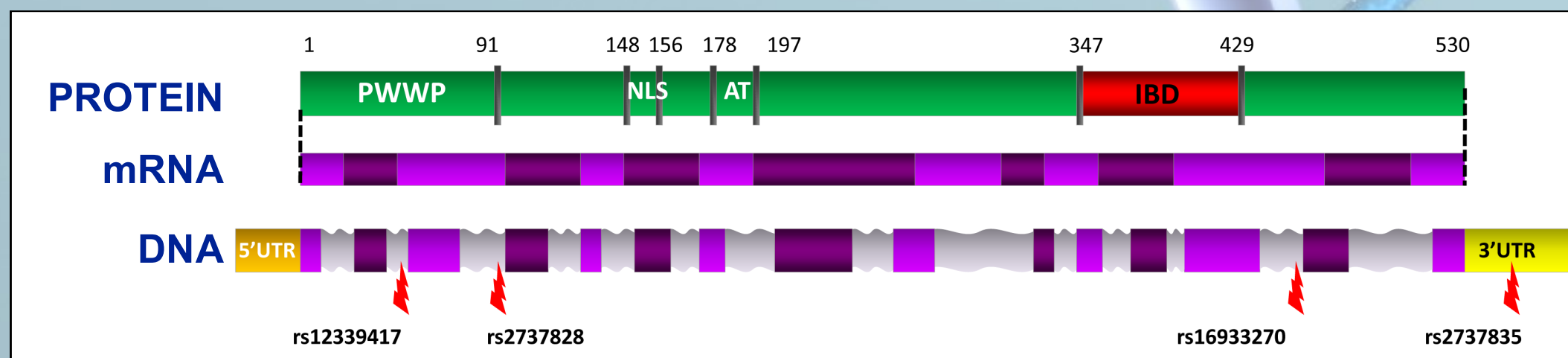
LEDGF mRNA expression: mRNA expression levels were determined using RT-qPCR with validated reference genes for normalization. The optimal number of reference genes was determined as 2 (geNorm V <0.15).

Data analysis: Statistical analysis for minor allele frequencies was performed with Fisher Exact test and compared to Hapmap. Mann Whitney tests were used for correlation analysis. Differential splicing was predicted with MaxEnt Scan and NNSPLICE, and miRNA binding was predicted with the Patrocles Finder.
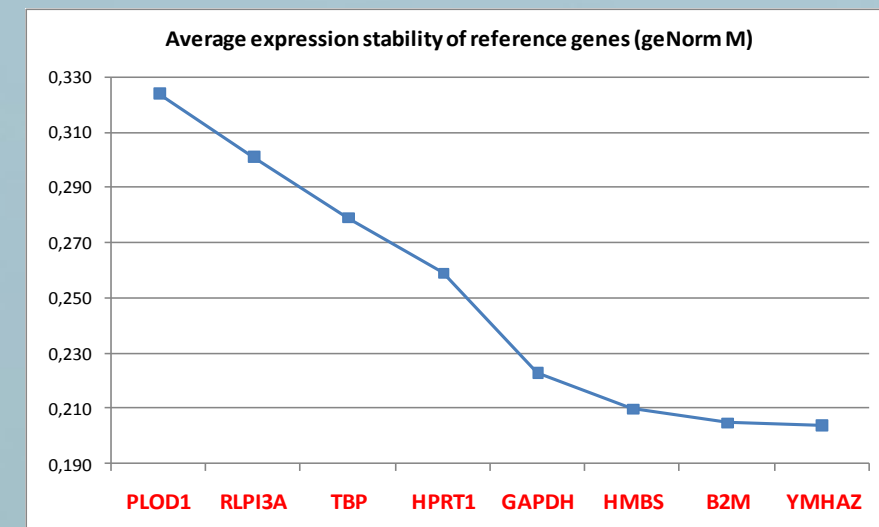

Fig. 2: Exrpression stabilities (M-values) of the tested refernce genes, calculated with GeNorm. The two genes with the lowes M-value, i.e. the most stable genes (B2M and YWHAZ) were used for normalization.

rs2737828
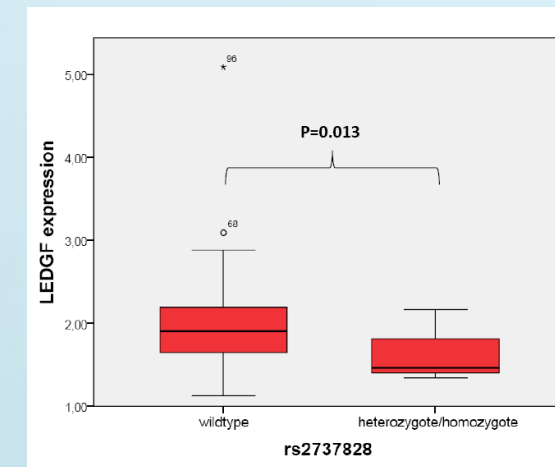
This intron SNP (n= 13) was under-represented in Caucasian HIV patients (P<0.0001) and associated with lower LEDGF/p75 expression (P=0.013). Splice prediction indicated this variation could induce a branch point sequence and alter splicing.
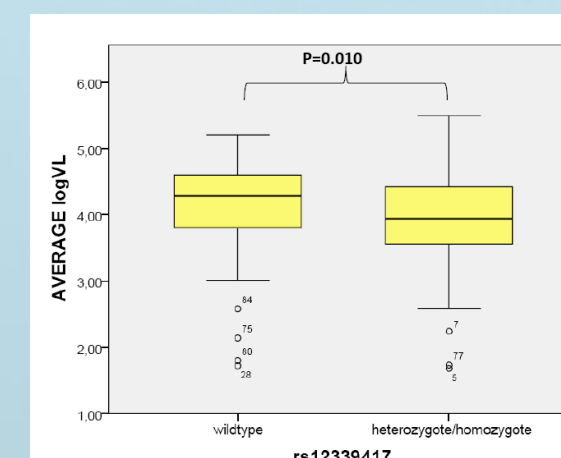

rs2737835
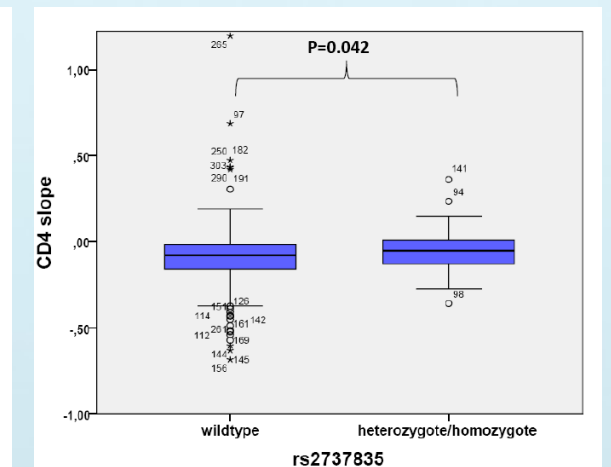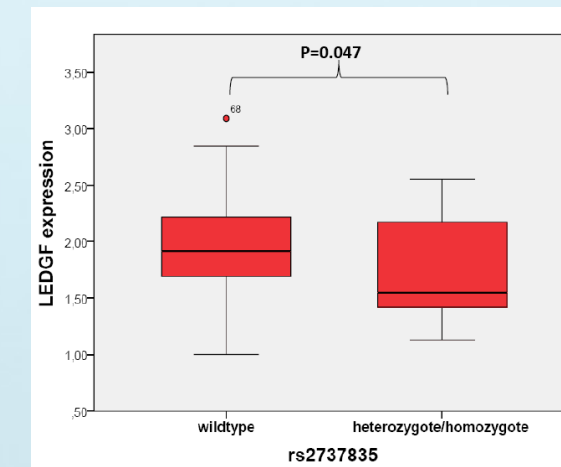
This 5' UTR SNP (n= 46) was more frequent in Caucasian Elite controllers and correlated with slower CD4 decline (P=0.042) and lower LEDGF/p75 mRNA levels (P=0.047). No known gain or loss of miRNA binding sites with this variation could be predicted.

rs16933270
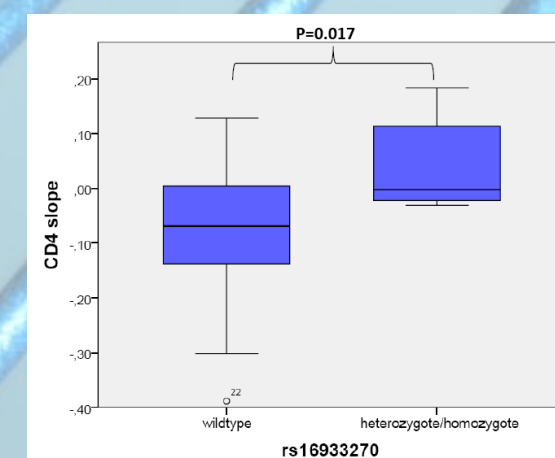
This intron SNP (n= 6) was associated with a slower CD4 decline in Africans (P=0.017). Splice prediction indicated this variation could induce a branch point sequence and alter splicing.

rs12339417

This SNP (n= 95) was not correlated with CD4 slope nor with LEDGF/p75 mRNA levels, but was significantly correlated with a decreased average viral load (p=0.010).

DISCUSSION:

In the investigated cohorts, two SNPs (rs27378235 and rs16933270) were associated with a slower CD4 decline, indicating that genetic variation in LEDGF/p75 can influence disease progression.

SNP rs2737835 and rs16933270 were correlated with lower LEDGF/p75 expression. The finding that SNP rs2737828 was also underrepresented in HIV patients in relation to the expected frequency according to Hapmap suggests that this SNP might influence disease susceptibility.

The correlation of tagSNP rs12339417 with average viral load might be due to sampling bias, as the effect was only significant in the entire cohort, but not within the ethnical groups. This indicates that care should be taken to interpret results, especially in genetically diverse patient cohorts.

CONCLUSIONS: The present study supports the hypothesis that host factors influence HIV disease progression. This is the second known report on LEDGF/p75 SNPs, showing that small genetic differences can influence disease progression.