Linkage between disease status and a naturally-arising mutation in functional region of HIV-1 Nef.

P-E7
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INTRODUCTION

-HLA-I downregulation by Nef has been shown to protect infected cells from CTL killing, thereby contributing to viral persistence in vivo.

-HLA-I-restricted CTL-mediated immune pressure leads to the accumulation of escape variants which imposes functional constraint in Nef.

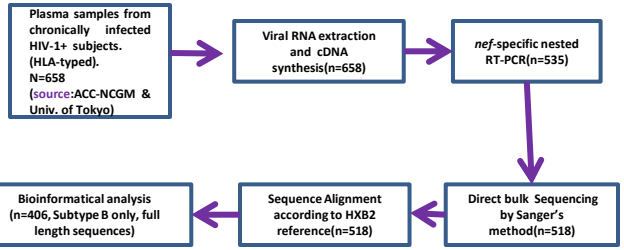
-This HLA-I downregulation function of Nef is achieved through a number of highly conserved sequence domains.

Therefore, naturally- arising polymorphisms, within the highly conserved sequence domains responsible for HLA-I down-regulation in NEF, may affect CTL-mediated virus killing and subsequently influence patients' disease status

AIMS

1. To identify significant naturally-arising polymorphism(s) within HLA-I downregulation functional domains in *nef* and evaluate their influence on clinical parameters or in vivo disease status.
2. To identify, (if any) associating HLA, for any identified polymorphism(s).

MATERIALS AND METHODS

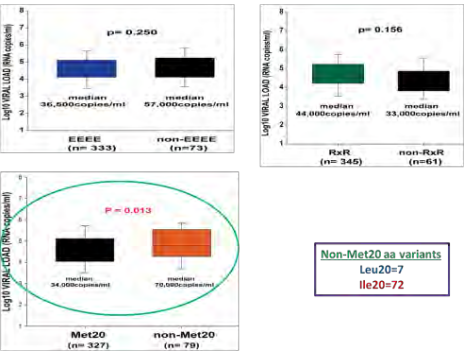


DATA ANALYSES AND RESULTS

1. Functional Domains' conservation in our cohort

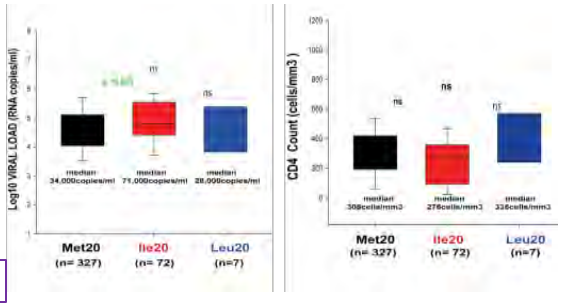
Nef Domain	Role	Conservation Frequency(%)
$R_{17}xR_{19}$	β-COP	85.0
M_{20}	AP1 and MHC-interaction	80.5
$E_{62}EEE_{65}$	PACS-1/2	82.0
P_{78}	SH3 binding	100

2. Functional domains' plasma viral load comparism

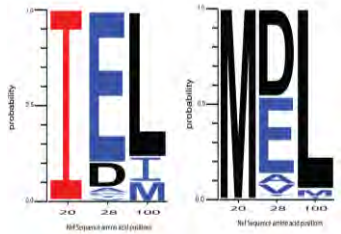


Non-Met20 aa variants
Leu20=7
Ile20=72

3. Ile20 is associated with high plasma viral load



4. Glu28 and Ile100 are positively associated with Ile20 but have no influence on plasma viral load



Position	Amino acid	Median(With)	Median(Without	N(With)	N(Without)	p value	q value
28	E	36000	41500	188	206	0.8563	0.951006
100	I	54000	42000	14	377	0.4597	0.751193

5. Enrichment of *HLA-C0303* in Ile20 Harboring patients.

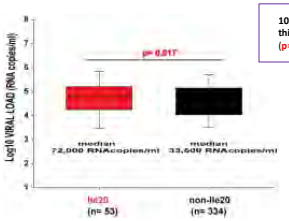
Chi-square Contingency Table

p=0.0161	not-Ile20	Ile20	Total
C0303	62	23	85
not-C0303	272	49	321
Total	334	72	406

6. CONSIDERING LINEAGE EFFECT (Removing phylogenetic cluster)

Chi-Square contingency table

P = 0.3583	Ile20	not-Ile20	Total
C*0303	13	64	77
non-C0303	40	270	310
Total	53	334	387

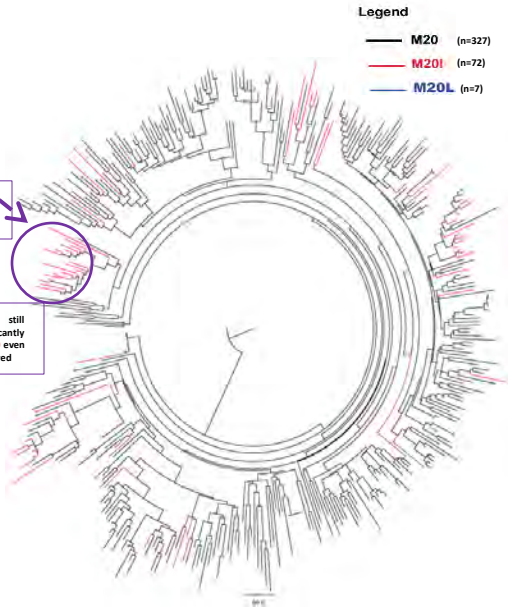


10 out of 19samples within this cluster is *HLA*C0303*+ (p=0.0014)

plasma viral load still remains significantly associated with Ile20 even when cluster is removed

PATIENTS' NEF PHYLOGENETIC TREE

Subtype-B with full length sequences (n=406)



SUMMARY

Ile20 is a deleterious polymorphism in Nef, by virtue of it's propensity to enhance in vivo viral load.

Other associating codons with Ile20 (ie Glu28 and Ile100) did not show any contributory effect on Ile20's ability to enhance in vivo viral load.

*HLA-C*0303 showed* an enrichment in subjects harboring Ile20. However, this association was lost when phylogenetic lineage effect was considered.

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