IMMUNOLOGY AND HOST GENETICS P-D04

Poster 0274

ADDITIVE DETRIMENTAL EFFECT OF *B*35/39* TYPES IN A LARGE MEXICO/CENTRAL AMERICA COHORT

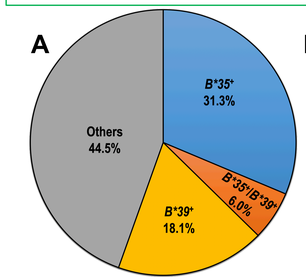
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BACKGROUND

- Polymorphism within the human leukocyte antigen (HLA) class I loci represents the strongest genetic modifier of HIV disease progression.
- Additive and/or synergistic effects between HLA alleles in the context of HIV disease outcome have been described.
- A unique HLA context has been described in a large cohort of persons living with HIV from Mexico and Central America (MEX/CAM, N=3,213) expressing HLA types common to Amerindian and Hispanic populations.
- The *B*35* and *B*39* subtypes are highly frequent in MEX/CAM, with a characteristic high allele diversity compared to Caucasian populations.
- The *B*35* and *B*39* subtypes feature novel risk associations with HIV disease outcome in MEX/CAM, including the Amerindian *B*35:12/14* and *B*39:01/05/06*.

A



B

MEX/CAM HLA-HIV associations		
CANONICAL	B*57:01/02/03, B*27:05	PROTECTIVE
	B*35:01/02	RISK
NOVEL	B*39:02	PROTECTIVE
	B*35:12/14, B*39:01/05/06	RISK

Figure 1. Highly frequent *HLA-B*35* and *B*39* subtypes associated to risk. (A) Pie chart showing the proportion of Mestizo MEX/CAM individuals (n=3,213) carrying any *B*35* (blue), *B*39* (yellow) or both *B*35/B*39* (orange) alleles. (B) Summary table of both canonical and novel HLA-HIV associations in MEX/CAM.

AIM

As the *B*35* and *B*39* subtypes featured prominently in both frequency and worse HIV outcome associations in a cohort of individuals with Amerindian/Hispanic ancestry, we investigated possible HLA additive effects and in the context of *B*35*-PX/PY grouping.

METHODS

- HLA sequence-based typing was performed on 3,213 chronically HIV-1 clade B-infected, ART-naïve individuals from Mexico (n=1,679), Guatemala (n=418), Nicaragua (n=254), Honduras (n=402), Panama (n=316), Belize (n=102), and El Salvador (n=42).
- Univariate and multivariate analyses were performed using Generalized Linear Model (GLM) to evaluate additive effects between *B*35/39* subtypes or between *B*35*-PX/PY groups. Cofounding variables in multivariate analyses includes but are not limited to age, gender, geographic location of recruitment (n-1 binary variables), and *B*57/27*.
- For *B*35*-PX/PY analyses, only *HLA-B* heterozygous individuals were compared in order to exclude confounding effects resulting from HLA homozygosity.

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RESULTS

MEX/CAM individuals express a **high diversity** of **unstudied *HLA-B*35*** and ***B*39* subtypes** described in **Amerindian** and **Hispanic** populations.

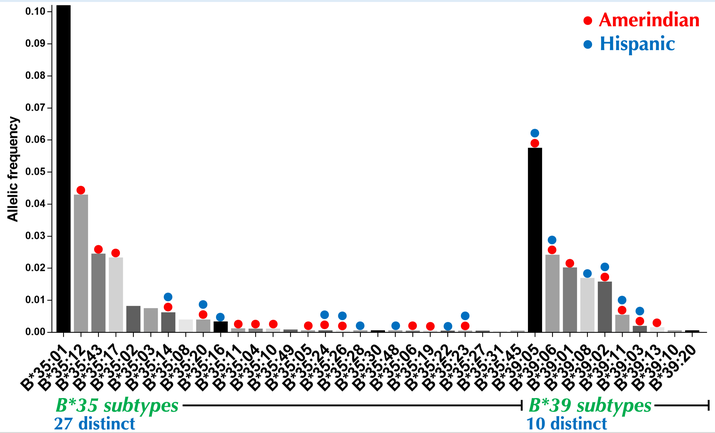


Figure 2. Diversity *HLA-B*35* and *B*39* subtypes in MEX/CAM cohort. Graph bar showing allele frequency (AF) of the diversity of *HLA-B*35* and *B*39* subtypes. AF were calculated by direct gene count (denominator 2n). HLA ethnicity were advised with IMGT/HLA Allele Ethnicity Tool (https://www.ebi.ac.uk/ipd/imgt/hla/ethnicity.html).

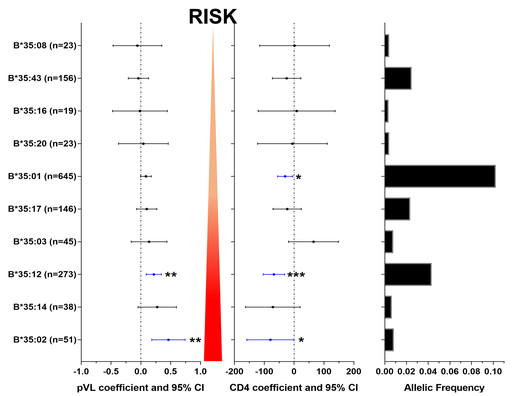


Figure 4. Impact of *B*35* subtypes on HIV clinical parameters. Univariate linear regression of *B*35* subtypes (AF>0.005) with respect to pVL and CD4. Correction for multiple comparisons was performed with False Discovery Rate (q-value). Coefficients and 95% confidence intervals are shown. *p-value<0.05, **p-value<0.005, ***p-value<0.0005.

Ranking of *B*35* subtypes according to linear regression coefficients and 95% CI:

- ✓ The second most frequent *B*35* subtype, not previously described in the PX/PY context, and of **Amerindian** origin, ***B*35:12*** was significantly associated to both **higher pVL** and **lower CD4 count**.
- ✓ The frequent ***B*35:01*** (**PY**, and associated to risk in some settings) was associated to lower CD4 counts.
- ✓ ***B*35:02*** **(PX)** was associated to lower CD4 count and to the highest pVL of all *HLA-B* alleles.

Next, we aimed to **test the *B*35*-PX/PY hypothesis** in MEX/CAM. Historically, ***B*35* alleles** have been classified into **two groups** based on the **amino acid preference at P9** of the peptides they present.

Allele	Epitope anchor preference	
	P2	P9 (F-pocket)
B*35:02	P	X
B*35:03	P	X
B*35:04	P	X
B*35:01	P	Y
B*35:08	P	Y

X= leucine, methionine or valine; Y= tyrosine

*B*35*-PX → Rapid rate of disease progression

*B*35*-PY → Neutral association to disease progression

Both in **univariate** and **multivariate** analyses:

- ✓ Expressing **one or two-copies** of **any *B*35* or *B*39*** was associated to a significant **increase in pVL** and a **decrease in CD4 count.**
- ✓ **pVL** and **CD4** linear regression **coefficients (β)** were **one-fold larger** in those groups of individuals that **co-express 2-copies of any *B*35/39*** subtypes in comparison with those subjects that express **1-copy of any *B*35/39***, **suggesting an additive detrimental effect between *B*35* and *B*39* subtypes.**

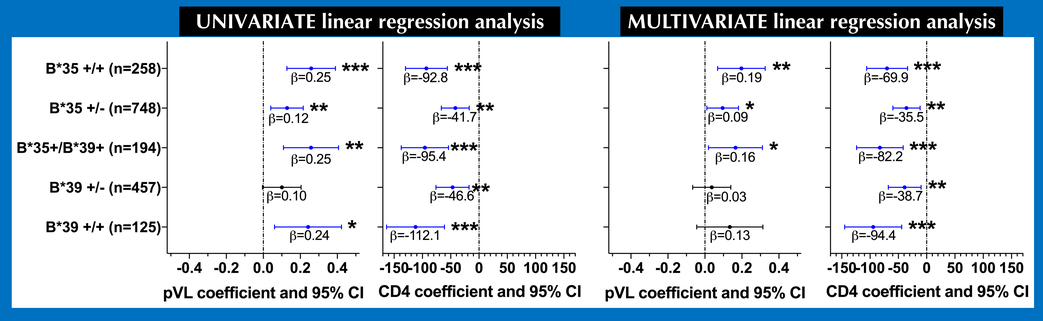


Figure 3. *HLA-B*35* and *B*39* additive effects on HIV disease outcome in large MEX/CAM cohort (n=3,13). Univariate linear regression analysis (Generalized Linear Model; GLM) was used to compare plasma viral load (pVL) and CD4 T cell counts between individuals carrying any *B*35* or *B*39* subtypes in MEX/CAM (left panel). Multivariate analyses (GLM) were performed accounting for possible cofounding factors including age, gender, geographic location of recruitment (n-1 binary variables) and *B*57/27* (right panel). Coefficients and 95% confidence intervals are shown. *p-value<0.05, **p-value<0.005, ***p-value<0.0005.

CONCLUSIONS

Our results suggest an additive detrimental effect between *B*35* and *B*39* subtypes, highly frequent in the Mesoamerican Mestizo population. Our results also challenge the *B*35*-PX/PY hypothesis, indicating that PY alleles can be disease-susceptible and also that differences exist in disease associations within PX/PY grouping.

To test whether adding further **putative *B*35*-PX and PY** alleles would yield **significant differences on pVL and CD4 counts**, we sorted the unclassified *B*35* alleles as PX or PY, based on positions 114 and 116 of the HLA peptide binding groove (that predicts amino acid preference in P9) to classify *B*35* alleles as putative PY or PX; in both univariate and multivariate analyses:

- ✓ We **confirmed** the previously observed **association of PX group** with **poor HIV outcome** (both with pVL and CD4 counts).
- ✓ By virtue of its similarity with other PX members, **Amerindian *B*35:12*** represents **a putative member** of the established **PX HIV risk group**.
- ✓ ***B*35*-PY alleles** were associated to **significant lower CD4 counts**.

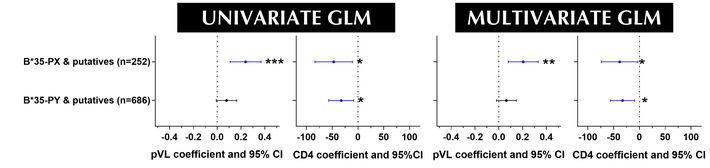


Figure 5. Impact of *B*35*-PX/PY grouping on HIV clinical parameters. Univariate and multivariate linear regression analyses were performed between *B*35*-PX or PY groups and HIV outcome, including previously unclassified B*35 alleles sorted as putative PX or PY according to residues found at positions 114 and 116. Multivariate analysis (GLM) accounted for: age, gender, geographic location of recruitment (n-1 binary variables) and *B*57/27* (right panel). Coefficients and 95% confidence intervals are shown. *p-value<0.05, **p-value<0.005, ***p-value<0.0005.

FUNDING

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