HLA-C stability and AIDS progression

Stefani Chiara[1]., Sangalli A[1]., Locatelli E[1]., Argañaraz ER[2]., Argañaraz GA[2]., Bosco da Silva CM[3]., da Silva Duarte AJ[3]., Casseb J[4]., Romanelli MG[1]., Zipeto D[1].

1 Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, Italy
2 Lab of Molecular NeuroVirology, Faculty of Helath Science, University of Brasília.DF, Brazil
3 Medical Investigation Laboratory Unit 56 (LIM/56), Faculdade de Medicina FMUSP, Universidade de São Paulo, São Paulo, SP, Brazil
4 Faculty of Medicine, Institute of Tropical Medicine, University of São Paulo, Brazil

Introduction

MHC class I complex is composed of HLA-A/B/C, β2 microglobulin, and a peptide. HLA-C expression is associated with HIV-1 infectivity control. Previous studies reported that less expressed HLA-C variants are associated with poor HIV-1 control and rapid progression to AIDS[1,2]. HLA-C alleles can be grouped in stable and unstable clusters based on their binding stability to $\beta_2$microglobulin/peptide: HLA-C unstable variants release $\beta_2$microglobulin more easily than stable ones[3].To verify if HLA-C unstable alleles correlate with AIDS progression we are preforming HLA-C genotyping by allele specific PCR (ASPCR) in a cohort of 121 AIDS patients form USA, Canada and Brazil. Patients were divided, based on disease progression, into: elite controllers (ECs), long term non-progressors (LTNPs) and progressors (Ps). Our preliminary results suggest an association between HLA-C unstable alleles and a more rapid disease progression.

Flow chart

121 Patients divided into:
- 11 ECs
- 43 LTNPs
- 67 Ps

ASPCR genotyping → YES → Patients genotype

NO → Sanger sequencing and bioinformatic analysis → Patients genotype

Patients genotype → Chi square statistical analysis

Materials and methods

1. HLA-C genotyping

To achieve HLA-C genotype allele specific PCR was carried out. This peculiar PCR enables to amplify highly similar sequences. PCR conditions suitable for each HLA-C allele were established, employing previuosly published primer pairs [4,5,6,7]. An internal control gene (COL5A1) was co-amplified to avoid false negative results. When HLA-C genotype couldn't be determined by ASPCR, Sanger sequencing was performed on HLA-C exon 2 and 3 [8], which are among the most variable regions. The putative individuals genotype were examined with a bioinformatic approach using the entire database of all known HLA-C alleles. To test the association between HLA-C alleles stability and AIDS progression chi-square statistical analysis was employed.

2. HLA-C stable and unstable alleles classification

Stable alleles	Unstable alleles
C*02	C*01
C*05	C*03
C*06	C*04
C*08	C*07
C*12	C*14
C*15	C*17
C*16	C*18

Table 1: HLA-C binding stability to β2 microglobulin/peptide

HLA-C binding stability to β2 microglobulin and peptide [1,3] (**Table 1**). HLA-C unstable alleles are less strongly bound to β2 microglobulin/peptide, facilitating HLA-C "free chains" development, which in turn, enablle HIV-1 Env protein binding thus increasing HIV-1 viral infectivity[9] .

Results

1. Allele specific PCR genotyping

ASPCR for HLA-C*15 allele amplification: samples 6 and 10 tested positive (**Figure 1**).

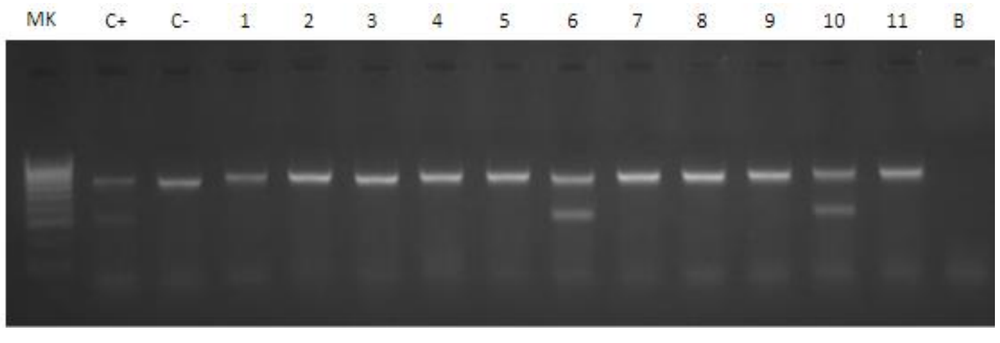

Figure 1: Allele specific PCR results: upper PCR band (COL5A1 internal control), lower PCR band (HLA-C*15 allele). MK: Hyper ladder 100 bp (Bioline); C+: Positive control; C-: Negative control; B: Blank

2. Sanger sequencing analysis

HLA-C exon 2 sequence electropherogram is reported in **Figure 2**. In red boxes are represented two variation points (a SNP in the left box and an in/del in the second one).

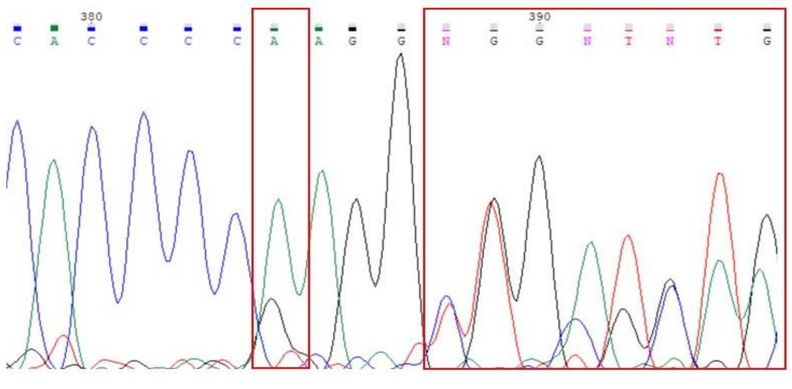

Figure 2: Sanger sequencing: SNP on the left red box; In/del on the right red box

3. Chi square statistical analysis

Groups	Stable alleles (n)	Unstable alleles (n)	Marginal total
P	34	60	94
LTNP	38	28	66
EC	10	12	22
Marginal total	82	100	182

We have so far genotyped 91 patients out of 121. The chi-square analysis indicates that there is an association between the presence of unstable alleles and AIDS progression (p value = 0,027).

Table 2: Contingency table: P: progressors, LTNP : Long term non progressors ; EC: Elite controlers

Discussion

These preliminary results indicates that there is an association between HLA-C genotype and AIDS progression. We have analysed 91 samples and the chi-square statistic test indicates that there is a statistically significant association between HLA-C unstable alleles and a more rapid AIDS progression. HLA-C unstable alleles tend to detach more easily β2 microglobulin/peptide, thus facilitating HLA-C "free chains" development which increase HIV-1 infectivity. This study may clarify HLA-C influence on the rate of AIDS progression.

References

[1] « Stability and expression levels of HLA-C on the cell membrane modulate HIV-1 infectivity » Parolini et al., J Virol., 2017

[2] « Influence of HLA-C expression level on HIV-1 control » Apps et al., Science, 2013

[3] « A single bottleneck in HLA-C assembly » Sibilio et al., J Biol Chem., 2008

[4] « Improvements in HLA-C typing using sequence-specdic primers (PCR-SSP) including definition of HLA-Cw9 and CwlO and a new allele HLA-"Cw7/8v » Bunce et al., Tissue Antigens 1994

[5] « Phototyping: comprehensive DNA typing for DRB5 . & DQBl by PCR with 144 primer HLA-A, B, C, DRB1, DRB3, DRB4, DBR5 and DBQ1 by PCR with 144 primer mixes utilizing sequence-specifiic primers (PCR-SSP) » Bunce et al., Tissue Antigens 1995

[6] « High resolution HLA-C typing by PCR-SSP: identification of allelic frequencies and linkage disequilibria in 604 unrelated random UK Caucasoids and a comparison with serology » Bunce et al., Tissue Antigens 1996

[7] « Molecular typing for HLA class I using ARMS-PCR: Further developments following the 12th International Histocompatibility Workshop » Tonks et al., Tissue Antigens 1999

[8] « Human leukocyte antigen (HLA) typing by DNA sequencing» Lazaro et al., Methods Mol Biol 2013

[9] « HIV-1 Env associates with HLA-C free-chains at the cell membrane modulating viral infectivity» Serena et al., Sci Rep. 2017

chiara.stefani@univr.it

Poster number 77