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A NOVEL POLYMORPHISM IN THE 5' UNTRANSLATED REGION (5'UTR) OF CD28 GENE IS ASSOCIATED WITH SUSCEPTIBILITY TO HIV-1 INFECTION

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BACKGROUND

CD28 plays an essential role in determining the effectiveness of T-cell immune responses as it is a critical co-stimulatory molecule for the optimal activation of CD4⁺ and CD8⁺ T-cells via CD3-T-cell receptor complex. In addition, CD28 co-stimulation of CD4⁺ T-cells confers resistance to in vitro infection by R5-tropic HIV-1 strains, due to a down-regulation of CCR5 molecule and an up-regulation of CCR-chemokines (1-3), while it increases cell-surface CXCR4 expression, facilitating the infection by X4 strains(4). We hypothesized therefore that immunogenetic variants in CD28 might exist that could influence resistance/susceptibility to HIV-1 infection and its progression.

(1)Levine B et al. Science 1996; 272:1939. (2) Riley JL et al. J. Immunol 1997; 158: 5545. (3) Carrol RG et al. Science 1997; 276:273.

(4) Secchiero P et al. J. Immunol. 2000; 164:4018.

OBJECTIVE

To analyze the presence of possible polymorphisms in the 5'UTR region of CD28 and its relationship with resistance/susceptibility to HIV-1 infection and its progresión to AIDS

Methods: Cold Single-Strand Conformation Polymorphism (SSCP) by 4-20% gradient PAGE at 15° C. DNA Sequencing and cloning.

Exposed but uninfected individuals (EU): 1) EU-Sex (n=32), exposed through persistent and current unprotected sex with HIV+ partners. 2) EU-Hem (n=25), Hemophiliacs who received presumably HIV-contaminated clotting factor concentrates between 1980-1985; all of them became infected by hepatitis C virus (HCV).

HIV+ individuals: 1) **LTNP (n=82)**, Long-term non progressors, i.e., asymptomatic individuals with CD4+ T cell counts > 500 cells/microL, after > 10 years of known seroprevalence, without antiretroviral therapy. 2) **Progressors (N=78)**, at different stages of HIV disease with high plasma viral load (PVL) >30,000, CD4 T cells >200 cells/microL and no opportunistic infections. 3) **Advanced stage (AS) (n=15)**, with CD4+ T cell counts <100 cells/microL, high PVL (>200,000 copies/mL), who had developed an opportunistic infection after a known seroprevalence and follow-up of 5-10 years.

Healthy controls (HC) (n=77), negative for HIV, HCV and other infections and with normal biological parameters as screened for altruistic blood donors, matched in sex and age with the EU and HIV+ groups.

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AMPLIFIED PRODUCT IN CD28 5' UTR

Amplified product

1 ccctttcctt ttttctctct **ccccttcctt** ccttcttttc ttcttttctt ttcttttctt
 61 ttcttttctct ctttcttttct gtcttttcttt **tctcattctg** **ttgccctggc** **tggagtgag**
 121 **tggcatgac** **tcggctcata** **gcagcctcca** **cctcctgggt** **tcaagcgatt** **ctcctgcctt**
 181 **agccctccct** **agtagctgga** **ttacaggtac** **ccaccatgat** **gcctggctaa** **ttttttgtat**
 241 **tttcaatgga** **gacgggggtt** **caccatgttg** **gccaggctcg** **tcttgacctc** **ctggcctcaa**
 301 **atgatccacc** **cactttggcc** **tcccaaattg** **ctggcattac** **aggcgtgagc** **cactgcaccc**
 361 ggctgttcc ttcttaagaa cactttgtct ccctttaat ctctgctgga ttcaagcac
 421 cccttttaca caactcttga tatccatcaa taaagaataa ttcccataag cccatcatgt
 481 agtgaccgac tatttttca **tgacaaaa** **aaagtcttta** **aaaatagaag** **ttaaagtcta**
 541 **aagtcatcaa** **aacaacgtta** **tatcctgtgt** **gaaatgtgc** **agtcaggatg** **ccttgtggtt**
 601 tgagtgcctt gatcatgtgc cctaagggga tgggtggcggg ggtgggtggcc gtggatgacg
 661 gagactctca ggcttggca ggtgcgtctt tcagttcccc tcacacttctg ggttcctcgg
 721 ggaggagggg ctggaaccct agcccatcgt caggacaaag **atgctcaggc** **tgctcttggc**
 781 **tctcaactta** **ttcccttcaa** **ttcaagtaac** **aggtaaacaa** tgttaatgtc tttctttctg
 841 taaatatttt ttgaggtctt ccaattggct tagtttattt taaatttcta acaatgtgtg
 901 aaatttgaac atttgaagtg tagttttgct gtaatagggc aatgtgttat tttgaaaatc
 961 attgattctc agactacata taga

Alu-family repeat

AP-1-like

Initiation of transcription region

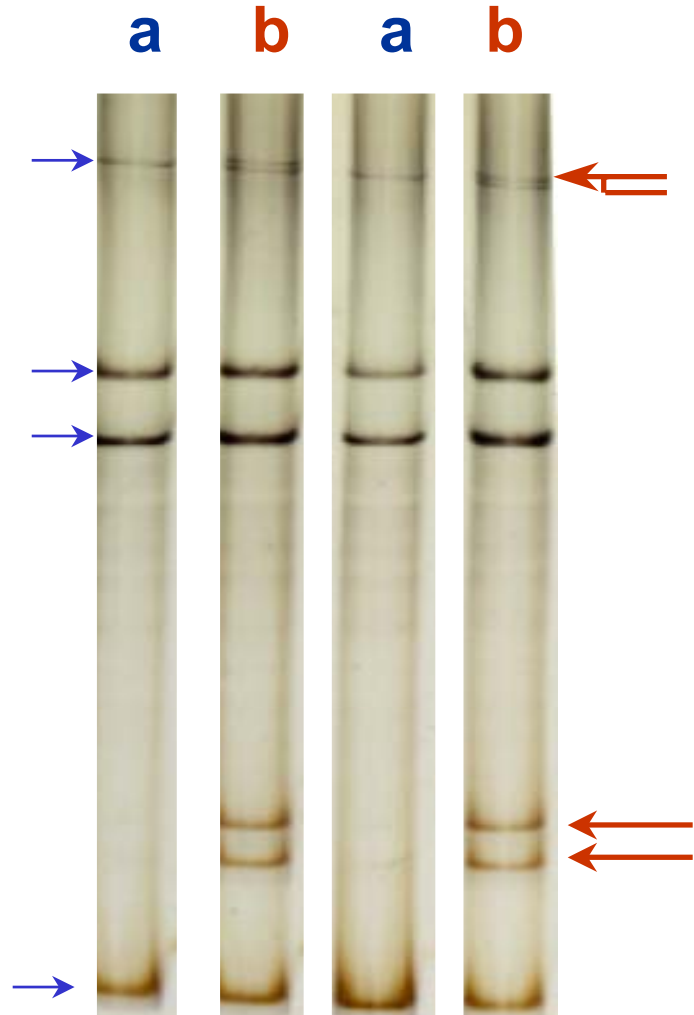
Translation of Exon 1



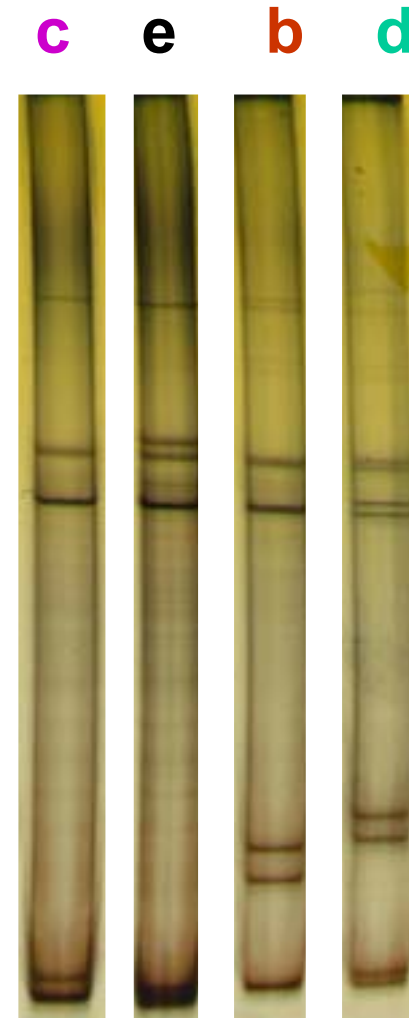
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CD28 5'UTR GENOTYPING BY SSCP

Common patterns



Uncommon patterns

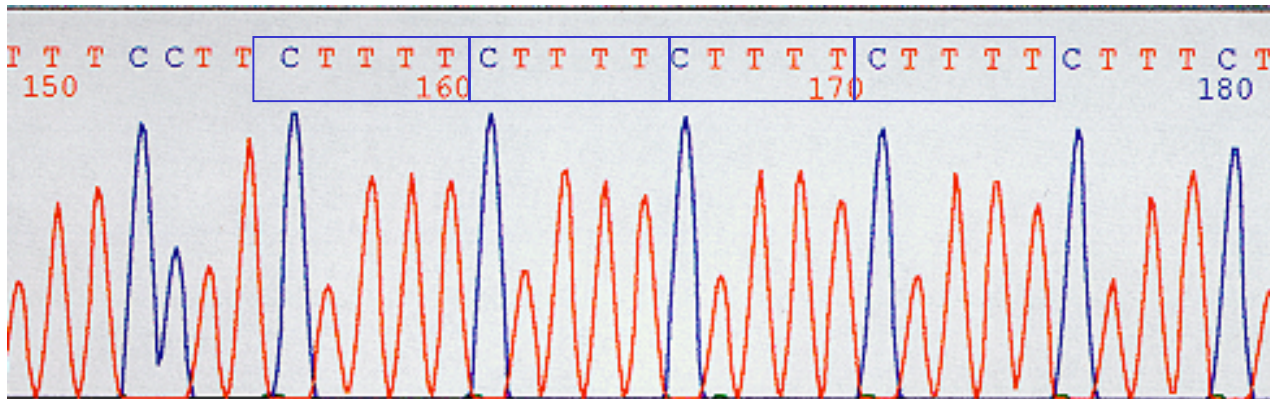


6

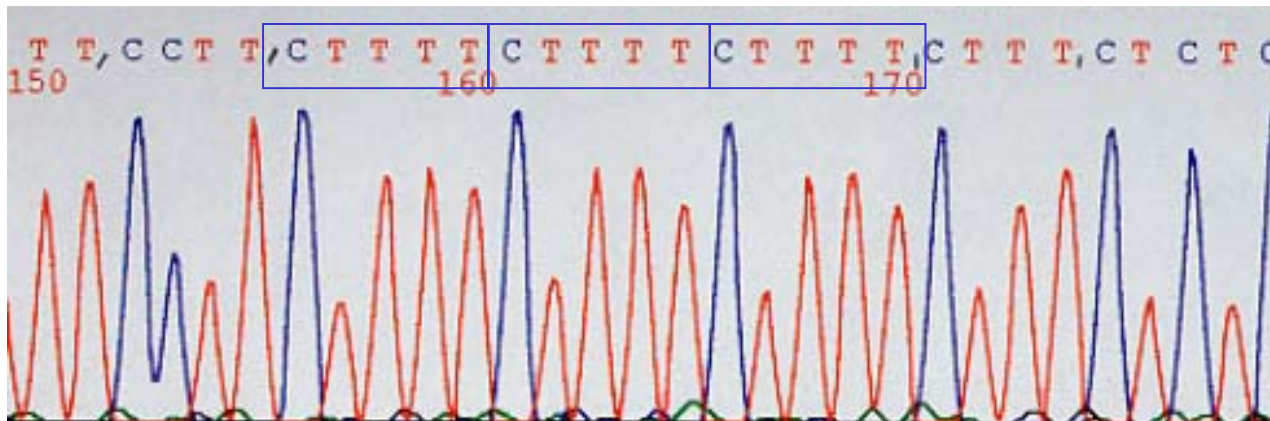
Identification of SSCP patterns a and b

SSCP PATTERN a: Homozigous combination of the wild type (wt) allele

SSCP PATTERN b: Heterozigous combination of the wt allele with a new allele resulting from the deletion of a 5bp repeat (CTTTT)



**WILD TYPE (WT)
ALLELE**



DELETED ALLELE

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DELETED ALLELE

5bp REPEAT (ctttt) DELETED

Amplified product

1 ccctttcctt ttttctctct ccctttcctt ccttcttttc t~~ctttt~~ctt ttcttttctt

61 **tt**cttttctct ctttctttct gtctttcttt **tctcattctg** **ttgccctggc** **tggagtgcag**

121 **tggcatgadc** **tcggctcata** **gcagcctcca** **cctcctgggt** **tcaagcgatt** **ctcctgcctt**

181 **agccctccct** **agtagctgga** **ttacaggtac** **ccaccatgat** **gcctggctaa** **ttttttgtat**

241 **tttcaatgga** **gacggggttt** **caccatgttg** **gccaggctcg** **tcttgacctc** **ctggcctcaa**

301 **atgatccacc** **cactttggcc** **tcccaaattg** **ctggcattac** **aggcgtgagc** **cactgcaccc**

361 ggctgtttcc ttcttaagaa cactttgtct ccctttaat ctctgctgga tttcaagcac

421 cccttttaca caactcttga tatccatcaa taaagaataa ttcccataag cccatcatgt

481 agtgaccgac tatttttcag **tgacaaaa** aaagtcttta aaaatagaag taaaagtct**ta**

541 **aagtcacaa** **aacaacgtta** **tatcctgtgt** **gaaatgctgc** **agtcaggatg** **ccttgtggtt**

601 tgagtgcctt gatcatgtgc cctaagggga tgggtggcggg ggtggtggcc gtggatgacg

661 gagactctca ggcttggca ggtgcgtctt tcagttcccc tcacacttgc ggttcctcgg

721 ggaggagggg ctggaaccct agcccatcgt caggacaaag **atgctcaggg** **tgctcttggc**

781 **tctcaactta** **ttcccttcaa** **ttcaagtaac** **ag**gtaaaca tgттаатgтc tttctttctg

841 taaatatttt ttgaggtctt ccaattggct tagtttattt taaatttcta acaatgtgtg

901 aaatttgaac atttgaagtg tagttttgct gtaatagggc aatgtgttat tttgaaaatc

961 attgattctc agactacata taga

Primers

8 CD28 5'UTR polymorphism distribution

GROUPS	(wt/wt)	(wt/ Δ 5bp)	
HC (n:77)	53 (69 %)	24 (31 %)	p: 0.008
HIV-1+ (n:160)	114 (71.25 %)	46 (28.75 %)	
LTNP (n:82)	60 (76 %)	22 (24 %)	p:0.009
PROG (n:78)	54 (71.5 %)	24 (28,5 %)	
AS (n:15)	9 (60 %)	6 (40 %)	
EU (n:57)	51 (89.5 %)	6 (10.5 %)	
Sex (n:32)	28 (88.5 %)	4 (12.5 %)	
Hem. (n:25)	23 (92 %)	2 (8%)	

HC, Healthy controls. LTNP, Lon-term non progressors. PROG, Progressors. AS, Advanced patients

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RESULTS AND CONCLUSIONS (i)

- In the amplified product, which encompassed the first known 559 bp of the CD28 5' UTR , (no. 4), several SSCP patterns were identified, the most common being those designated (a) and (b) (no.5).
- Sequencing and cloning disclosed that the pattern (a) corresponds to a homozygous state of the wild type (wt) allele, whereas pattern (b) is a heterozygous combination of the wt allele with a new allele resulting from a 5 bp repeat (CTTTT) deletion of the four CTTTT repeats found in the wt allele, 28 bp upstream the Alu family sequence present in the CD28 5' UTR (no.6 and7).
- There were no significant differences in the frequency of heterozygotes among healthy controls (31%), whole HIV+ individuals (28.75%) , LTNP (24 %) and Progressors (28.5%), although there was a trend to an increased frequency in the the most advanced patients (40%).

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RESULTS AND CONCLUSIONS (ii)

☛ In contrast, the frequency of heterozygotes among EU (exposed but uninfected) individuals was significantly decreased as compared with HC ($p=0.0008$) and HIV+ groups (0.0009).

☛ Data demonstrate the existence of a non previously reported genetic variant of CD28 gene consisting of a 5bp repeat deletion (CTTTT) at the 5' UTR . It is significantly underrepresented among highly exposed but uninfected individuals compared with healthy controls and HIV+ individuals, indicating its association with susceptibility to HIV-1 infection.

☛ The functional consequences of this deletion and its role for favoring HIV-1 infection remain to be determined.