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Research paper

Common haplotypes in *CD209* promoter and susceptibility to HIV-1 infection in intravenous drug users

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ABSTRACT

Introduction: CD209 is a receptor expressed in the dendritic cells involved in recognition of oligosaccharides present in several pathogens with a relevant impact on human health. SNPs located in the promoter region have been associated with HIV-1 susceptibility, although this finding has not been replicated in other populations. The objective of this study is to evaluate the association of *CD209* promoter haplotypes with risk of HIV-1 infection in a cohort of Spanish male intravenous drug users (IDU) infected with hepatitis C virus (HCV) and to characterize the phenotypic effects of the associated variants.
Methods: We genotyped 4 SNPs of *CD209* promoter in 295 HCV males exposed to HIV-1 infection by IDU, 165 HIV-1-infected and 130 exposed uninfected (EUI) and 142 healthy controls (HC). We have cloned the promoter variants in a reporter vector and evaluated the promoter activities in a cell culture model. *CD209* mRNAs were measured in PBMC.
Results: Single-marker analysis revealed no significant allelic association with the risk of HIV-1 infection by parenteral route. Nevertheless, one haplotype was significantly overrepresented in EUI compared with HIV-1 positive patients and was associated with HIV-1 status ($P = 0.0008$; OR: 0.43). Functional experiments suggested that the protective haplotype displayed lower transcriptional activity in vitro ($P < 0.05$) and this was correlated with lower CD209 mRNA expression in PBMC ($P = 0.014$).
Conclusions: This study suggests that the promoter haplotypes of *CD209* influence the risk of HIV-1 acquisition in IDU and that this association is correlated with the mRNA expression level.

1. Introduction

DC-SIGN or CD209 is an adhesion receptor expressed in dendritic cells (DCs) that mediates the interaction between DCs and resting T cells through ICAM-2/3 (Geijtenbeek et al., 2000) (van Kooyk and Geijtenbeek, 2002). This protein is also involved in recognizing mannose N-linked oligosaccharides (Pomerantz et al., 1990), which are evolutionary selected structures present in several families of viruses with an important impact on human health, as Retroviruses, Flavivirus, Filovirus, and Herpesvirus (Khoo et al., 2008). Alternative splicing of *CD209* results in multiple variants, some of them probably secreted or intracellular isoforms (Bleiber et al., 2005; Hladik et al., 2005). Two SNPs in the *CD209* promoter (−336G and −871A) have been associated with tuberculosis susceptibility (Barreiro et al., 2006). The −336G allele has been found to confer protection against HIV-1 in Caucasians (Martin et al., 2004), while other studies observed the opposite effect in Indians (Selvaraj et al., 2009) and no effect in Thais or Africans (Martin et al., 2004; Wichukchinda et al., 2007).

Because of multiple exposures to blood, as a consequence of needle sharing, most injection drug users (IDU) in Spain become co-infected with HCV and HIV-1. Nevertheless, a few of them remain HIV-1 seronegative, despite showing markers of HCV infection. These patients are considered to be exposed uninfected (EUI). *CCR5* genotype is associated with HIV-1 serostatus in IDU in our area (de la Torre et al., 2008; Real et al., 2015). However the majority of EUI individuals are homozygous for the wild type CCR5 allele. This fact suggests that other causes

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may have an influence on the susceptibility to HIV-1 infection in this group.

In the present work, we have examined the contribution of 4 polymorphisms located in the promoter region of CD209 gene to the susceptibility to HIV-1 infection. We have identified differences in haplotype prevalence between 2 extreme populations, HIV-1 EUI and HIV-1–infected subjects. On the basis of this finding, we infered that *CD209* haplotypes might influence the risk of HIV-1 acquisition. Functional experiments revealed that *CD209* promoter harboring the protective haplotype displays lower transcriptional activity in a cell culture model, and lower expression in PBMCs, all of this suggesting that reduced expression of this receptor can increase the resistance to HIV-1 infection in IDUs.

2. Materials and methods

2.1. Patients and controls

We recruited 295 white males exposed to HIV-1 infection by injection drug use (IDU), both infected (165 subjects) and EUI (130 subjects). These patients had been enrolled in prospective cohort studies in Spain (Arnau de Vilanova Hospital, Lleida; Valme Hospital, Sevilla and Reina Sofía Hospital, Córdoba). All of them had shared injection equipment for longer than 3 months (Table 1). The main epidemiological and clinical characteristics of the cohorts have been previously reported (de la Torre et al., 2008) (Herrero et al., 2015; Real et al., 2015). Serum hepatitis C virus (HCV) antibodies, were present in 100% of EUI and HIV-1 positive subjects. In addition, a group of 142 healthy white males, recruited among the anonymous blood donors from the City of Jaen Hospital, who tested negative for HIV-1 and HCV, was used as healthy controls (HC). HIV-1 and HCV diagnostics were performed as previously described (de la Torre et al., 2008). The main epidemiological characteristics of the cohorts are shown in the Table 1.

2.2. Ethical approval

The study was designed and performed according to the Helsinki declaration and was approved by the Ethics Committee of the three participating hospitals. All patients provided written informed consent to participate in this study.

2.3. Polymorphism identification and genotyping

The SNPs in the *CD209* promoter were selected according to the following criteria: genotypes from Hapmap-CEU population were directly downloaded from the Hapmap database and the Tag SNPs were identified using Haploview's V4.1 software (Barrett et al., 2005) tagger option. Second, SNPs list were complemented with potentially functional polymorphisms previously associated with infectious disease susceptibility and SNPs associated with eQTL in the GTEX database (GTEx Consortium, 2013).

Table 1
Epidemiological characteristics of the cohorts.

Population	Blood donors	HIV-1 infected	Exposed Uninfected
No. subjects (% male)	142 (100)	165 (100)	130 (100)
Age, years	36 (174–61)	38 (23–54)	31 (18–47)
Time of injection drug use, months	–	32 (1–195)	36 (3–240)
Period of recruitment	2000–2001	1998–2005	1997–2005
Positive HCV serology n (%)	142 (0)	165 (100)	130 (100)
Positive HIV serology n (%)	142 (0)	165 (100)	130 (0)

DNA was extracted from frozen whole blood using the Quick Pure Blood DNA extraction Kit (Macherey-Nagel). The polymorphic markers located in the *CD209* promoter region: rs2287886 (A/G), rs4804803 (A/G), rs735239 (A/G), rs735240 (A/G) (positions −139, −336, −871 and −939 respectively) were genotyped using commercial Taqman assay (Applied Biosystems), according to the manufacturer's instructions, in a MX3005 thermocycler (Stratagene). All subjects were also genotyped for the rs333 (Δ32) polymorphism in the *CCR5* gene. Three EUI, who were homozygous for *CCR5* Δ32 polymorphism, were not included in this study. *CCR5* Δ32 polymorphism was determined as previously described (Real et al., 2015).

2.4. Biostatistics and bioinformatics

Hardy-Weinberg equilibrium and pairwise linkage disequilibrium (D′) were calculated using Haploview V4.1 software. Block structure was considered for marker pairs showing $D' > 0.8$, following the solid-spine block definition. Allelic, genotypic and haplotype frequencies were estimated and compared using the Fisher's exact test implemented in the PLINK software (Purcell et al., 2007). Single-marker and haplotype association *P* values were corrected for multiple testing following the SNP spectral decomposition approach (Nyholt, 2004). A *P* value <0.012 was considered statistically significant. The paired *t*-test and the *Mann-Whitney U test* were used for comparing continuous variables. The estimation of power to detect alleles related to HIV-infection risk was performed by the Episheet software, available at http://members.aol.com/krothman. All presented *P* values are two tailed.

2.5. CD209 promoter variants cloning and in vitro reporter assays

A 1.4 kb fragment of *CD209* promoter and 5′UTR was amplified by nested PCR using the Long Expand PCR Kit (Roche) from genomic DNAs of two patients harboring the XAGA or the XGAG haplotypes. The primers used were: DC-forward1: 5′AG GTCATTGCCATGGCATCGG T3′; DC-reverse1: 5′AGGAGTCCAGGAGTCTCTCGTC3′, DC-forward2: 5′TACCTCATTACCATGTGAAATG3′, DC-reverse2: 5′GGCTGGCCATCCC TTCC CCCTT3′. The PCR products were cloned in the plasmid pSC-A-amp/kan using the Strataclone PCR cloning kit (Stratagene). The fidelity of PCR amplifications and subcloning were confirmed by Sanger DNA sequencing. The Gaussia luciferase reporter plasmids were constructed by digestion of the pSC-A-AGA and pSC-A-GAG plasmids with the restriction enzymes Not-I and Kpn-I and the products ligated to the promoterless pGLUC-basic plasmid (New England Biolabs).

2.6. Cells, transient transfection, and luciferase assays

Two cell lines expressing *CD209* were used for transfection experiments: LC5 (embryonic lung) and THP1 (human monocytic leukemia). Both cell lines were propagated and transfected as previously described (García-Moruja et al., 2005). The CD209 promoter plasmids were cotransfected with a plasmid coding for the firefly luciferase under the control of the CMV promoter. The luciferase activities were measured using BioLux® Gaussia Luciferase Assay Kit (New England Biolabs). In parallel, the cells were washed with PBS and processed for Firefly luciferase using the Luciferase Assay System (Promega) and Bradford Protein Assay (Bio—Rad). The transfection efficiencies were calculated after normalization by firefly luciferase and μg of protein. The experiments were repeated four (THP1 cells) and five (LC5 cells) times in independent days. All the measurements were done in triplicate. The *CD209* promoter expression plasmids names were codified and the researchers responsible for functional experiments were blinded to the genotypes of the plasmids.