Distribution of IL28b and CCR5 gene polymorphisms in a cohort of patients with hemophilia and HIV/HCV coinfecion

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A substantial number of patients with hemophilia are infected with both human immunodeficiency virus (HIV) and hepatitis C virus (HCV). They had acquired HCV and HIV infections through contaminated clotting-factor concentrates before effective virus-inactivation procedures. HIV has been shown to accelerate the course of HCV chronic liver disease and there is evidence that HCV infection may worsen the prognosis of HIV.

Several studies have shown that genetic polymorphisms near the gene for Interleukin 28b (IL28b) that encodes IFN-λ3 (type III), are associated with viral eradication (VE) and the favorable response to therapy in individuals infected with HCV. It has been observed that the C/T polymorphism at position rs12979860, is the main predictor of sustained virological response (SVR). The favorable genotype (CC) is strongly associated with SVR and spontaneous VE, even in people coinfected with HIV. Although it was also found to be associated to more rapid progression to HCV related fibrosis.

The CCR5 gene encodes an integral membrane protein, member of the beta chemokine receptors family and an HIV coreceptor. A 32 base pair deletion (Δ*32* del) in one or both alleles of the CCR5 gene, has been related to decreased expression of this receptor, with consequent impact on HIV-1 replication and delay in the progression to AIDS. The presence of this deletion is considered a disadvantage in HCV infection and patients who have it have higher HCV viral loads.

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Objectives

❖ Analyze the genotypes distribution of IL28b rs12979860 polymorphism and the presence of the CCR5 Δ32 del in a cohort of patients with hemophilia, coinfected with HCV and HIV.

❖ Determine their association with clinical, virological and immunological parameters.

Methods

The samples analyzed belong to 38 patients with hemophilia and HIV/HCV coinfection. Genomic DNA was obtained from samples of peripheral blood mononuclear cells. The IL28b genotypes rs12979860 and CCR5 were determinated using conventional PCR techniques. The CCR5 fragment size correlates directly with the genotype, but for IL28b was required digestion with a restriction enzyme, BStU-I.

The IL28b (rs12979860) and CCR5 allele polymorphisms distribution were evaluated in parallel with clinical, immunological and virological markers.

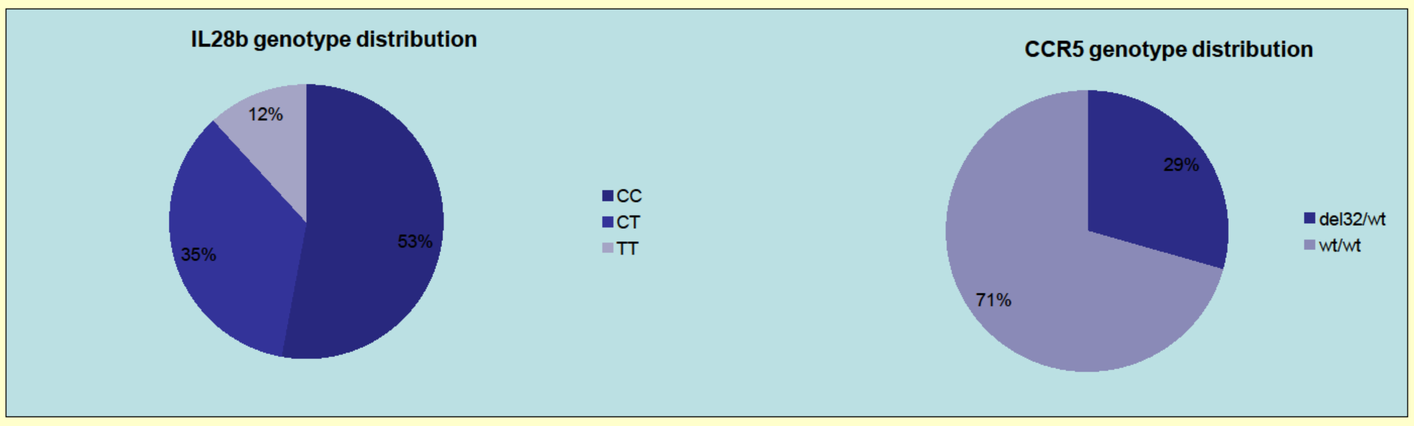
To compare groups, the results were analyzed with Student t or Mann Whitney tests depending on their distribution. Chi square or Fisher tests were used for qualitative analysis (GraphPad Prism, 5.3-2009 version).

Age, BMI (Body Mass Index), HIV and HCV viral loads, hepatic enzymes: (aspartate aminotransferase (AST) , alanine aminotransferase (ALT), gamma glutamyltransferase (γ-GT), alkaline phosphatase (ALP), $CD4^+$ and $CD8^+$ T cells counts, platelet counts, APRI (AST -to-platelet ratio index) and FORNS indexes were evaluated as surrogate clinical progression markers.

Results

The IL28b genotypes were distributed as follows (n=38): 53% CC, 34% CT and 13% TT, similar to our control population (n=23). None of the parameters showed statistically significant differences between groups. Altered values were similarly distributed among the different groups. Seventy three percent of the patients showed wild type allelic variants for CCR5 while only 27% demonstrated the presence of Δ32 del in one allele. Among the group with non detectable HCV viral load as a result of therapy or spontaneous clearance, 44% showed CC genotype and 25% showed the Δ32 del. Also 44% of the patients with non detectable HIV loads were CC genotype and 15% of them showed Δ32 del in CCR5.

Patient Profiles n=38	Mean	Median	Range
Age	40,25	39	24-69
BMI	26,19	25,8	21-32,20
Platelet count 10^3/ml (NV: 150-400)	196,1	198	80-292
AST level UI/L (NV: to 38)	49,53	43	17-174
ALT level UI/L (NV: to 41)	58,81	49	14-168
Cholesterol level mg/dl (NV: 150-220)	169,8	170	98-238
γ-GT UI/L (NV: 11-50)	100,8	62,75	22-315
ALP level UI/L (NV: 65-300)	280,4	269,5	167-519
$CD4^+$ count (cells/ul)	452,5	399	47-1384
$CD8^+$ count (cells/ul)	856	812	293-2257
HIV VL log UI/ml	2,49	1,69	1,69-5,32
HCV VL log UI/ml	4,12	5,75	0-6,9
APRI Score	0,81	0,54	0,16-4,72
FORNS Score	5,1	4,7	1,94-8,23
FIB-4	1,54	1,01	0.62-4.98



BMI: Body Mass Index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; γ-GT: gamma Glutamyltransferase; ALP: alkaline phosphatase; APRI: AST-to- platelet ratio index; Chol T: total cholesterol; NV: Normal Value; VL: Viral Load.

CC	age (years)	$CD4^+$ (cells/ml)	AST (IU/l)	ALT (IU/l)	Platelets (10^3/ul)	APRIscore	Chol T (mg/dl)	γGT (IU/l)	ALP (IU/l)	FORNS score	FIB-4 score	BMI	CV HIV	CV HCV
Mean	41,94	385,1	43,83	53,78	200,6	0,7111	169,7	86,92	304,4	5,096	1,566	26,85	2,397	3,905
Median	39	371	43	46,5	198	0,5232	171	55	289,5	4,934	1,01	26,5	1,92	5,56
Range	27-69	135-755	17-92	14-160	80-290	0.16-2.05	98-238	25-276	169-490	1.94-8.23	0.62-4.8	22.8-31.6	1.69-4.5	0-6.84

CT+TT	age (years)	$CD4^+$ (cells/ml)	AST (IU/l)	ALT (IU/l)	Platelets (10^3/ul)	APRIscore	Chol T (mg/dl)	γGT (IU/l)	ALP (IU/l)	FORNS score	FIB-4 score	BMI	CV HIV	CV HCV
Mean	38,56	516,1	55,22	63,83	191,6	0,9196	169,9	114,6	256,3	5,109	1,517	25,92	2,576	4,344
Median	36,5	442,5	42,5	51	196,5	0,5787	169,5	78	222	4,696	1,045	25,65	1,69	5,945
Range	24-52	47-1384	22-174	23-168	97-292	0.30-4.72	107-237	22-315	167-519	3.45-7.84	0.73-4.98	21-32.2	1.69-5.32	0-6.9

Conclusions

Although the number of patients is still small, no combination of polymorphism presence in IL28b and CCR5 seem to be associated to a better or worse disease progression in our cohort.

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