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VDR gene polymorphisms impact on anemia at 2 week of anti-HCV therapy: a possible mechanism for early RBV-induced anemia.

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BACKGROUND

Vitamin D receptors (VDR) binds the active form of calcitriol and modulates several physiological systems, through genomic and non genomic pathways. Calcitriol stimulates store-operated channels Ca2+ influx by translocation of the caveolar VDR to the plasma membrane. An increase in intracellular Ca2+ concentration can deregulate erythrocytes membrane composition, cell volume, glycolytic enzymes regulation, redox state and cell clearance[1].

OBJECTIVES

Our aim was to retrospectively evaluate the role of individual single nucleotide polymorphisms (SNPs) in *ITPA*, *CYP27B1*, *CYP24A1* and *VDR* genes in the prediction of ribavirin (RBV) and pegylated-interferon-alpha (pegIFN-α) therapy-induced anemia in a cohort of HCV mixed genotypes monoinfected patients at 2 and 4 weeks of treatment.

MATERIALS AND METHODS

Allelic discrimination for *ITPA rs7270101 A>C, rs6051702 A>C* and *rs1127354 C>A ,CYP27B1 rs4646536 (+2838) C>T* and *rs10877012 (-1260) G>T, CYP24A1 rs2248359 T>C, rs2585428 A>G* and *rs927650 C>T, VDR rs7975232 (ApaI) C>A, rs731236 T>C (TaqI), rs1544410 G>A (BsmI), rs10735810 T>C (FokI)* and *rs11568820 A>G (Cdx2)* SNPs was performed by real-time PCR.

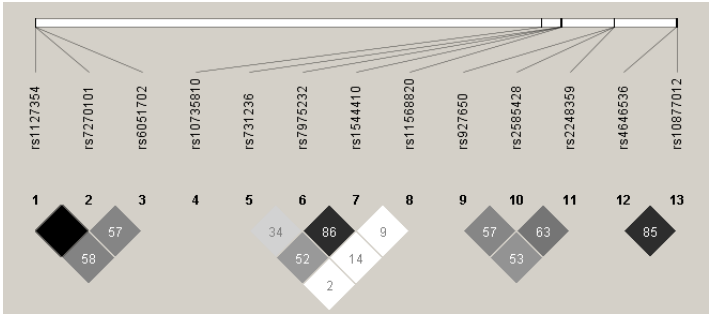

Figure. Pairwise linkage disequilibrium calculated with HaploView using the D' statistic for *ITPA*, *VDR*, *CYP24A1* and *CYP27B1* gene SNPs. Intensity of shading indicates the degree of confidence in the D' value and numbers in blocks denote D' values. Dark filled squares indicate a D' value of 1 with LOD (logarithm of odds) < 2.0. D' was calculated as follows: D'= (D) divided by the theoretical maximum for the observed allele frequencies. LOD was defined as $\log_{10}$(L1/L0), where L1= likelihood of the data under linkage disequilibrium, and L0= likelihood of the data under linkage equilibrium. The physical position of each SNP is presented in the top diagram.

RESULTS

Two hundred and twenty five patients were included in the study. The linkage disequilibrium (LD) analysis was shown in figure 1. At week 2, *CYP24A1 rs927650* (*p*=0.025), *ApaI* (*p*=0.042) and *BsmI* (*p*=0.004) SNPs were associated with anemia. The univariate analysis identified the following factors: gender, body mass index (BMI) at baseline > 30 Kg/m^2, ALT at baseline > 37 IU/L, steatosis, *ITPA rs6051702* AC/CC, *CYP24A1 rs927650* TT, *CYP27B1 +2838* TT, *ApaI* CC and *BsmI* AA profiles. All the patients who developed early anemia at week 2 have CC genotype for *ITPA rs1127354* SNP. In multivariate analysis only BMI at baseline > 30 Kg/m^2 (*p*= 0.013), ALT at baseline > 37 IU/L (*p*= 0.020) and *BsmI* AA profile (*p*= 0.003) were statistically significant (table 1).

At week 4, *ITPA rs6051702* (*p*=0.002) and *rs1127354* (*p*=0.003) SNPs were associated with anemia. In the univariate analysis age > 48 years, diabetes, insulin resistance, peg-type, *FokI* TC/CC and *ITPA rs6051702* AC/CC profiles remained in the model. Also in this case, all the anemic patients were CC for *ITPA rs1127354* SNP. In the multivariate analysis *ITPA rs6051702* AC/CC genotype (*p*= 0.001) was the only retained factor (table 2).

CONCLUSIONS

BsmI AA genotype is a predictive factor of anemia at 2 weeks and could be related to an enhanced activity of the VDR, thus an increased calcium influx, resulting in the deregulation of the Ca^{2+}-dependent signaling. These results indicate for the first time the strong, significant and ITPA-independent role of VDR in the early development of RBV-induced anemia and confirm the *ITPA* function in the prediction of anemia at week 4[2,3].

Tables . Factors, in univariate and multivariate logistic regression analyses, able to predict RBV/PEG-IFNα treatment response at weeks 2 (**table 1**) and 4 (**table 1**) of therapy. * False Discovery Rate (Benjamini-Hocheberg) corrected p-value for univariate analysis. OR: odd ratio. IC: interval of confidence. NC: all the cases belong to a single group.

	WEEK 2		
	UNIVARIATE		MULTIVARIATE
	p; OR (IC 95%)	*q**	*p*; OR (IC 95%)
Age> 48 Years	0.265; 1.69 (0.67-4.25)	0,38333333	
Gender	**0.085; 2.69 (0.87-8.27)**	0,29513889	0.085; 3.41 (0.85-13.76)
BMI at baseline>30 Kg/m^2	**0.069; 3.73 (0.90-15.38)**	0,29930556	**0.013; 10.95 (1.66-72.41)**
HCV baseline viral load>800.000 IU/mL	0.355; 1.53 (0.62-3.77)	0,3625	
ALT at baseline>37	**0.012; 0.29 (0.11-0.76)**	0,10416667	**0.020; 0.26 (0.09-0.81)**
Cryoglobulins	0.603; 1.29 (0.50-3.36)	0,55069444	
Diabetes	0.320; 1.96 (0.52-7.36)	0,37013889	
Steatosis	**0.181; 2 (0.72-5.52)**	0,34930556	0.918; 0.93 (0.24-3.68)
Insulin Resistance	0.750; 1.16 (0.47-2.89)	0,56597222	
Metavir score (0=F0/F1/F2 and 1=F3/F4)	0.578; 1.31 (0.50-3.42)	0,55763889	
PEG type	0.320; 1.51 (0.67-3.43)	0,34722222	
RBV dose	0.208; 1.00 (0.99-1.01)	0,36111111	
PEG dose	0.638; 0.99 (0.98-1.00)	0,55416667	
ITPA rs6051702 AC/CC	**0.139; 0.32 (0.07-1.44)**	0,34444444	0.077; 0.22 (0.04-1.18)
ITPA rs1127354 CA	NC	/	NC
ITPA rs7072101 AC/CC	0.271; 0.43 (0.10-1.93)	0,36180556	
CYP27B1 +2838 TT	**0.166; 0.53 (0.21-1.30)**	0,36041667	0.234; 0.53 (0.19-1.51)
CYP27B1 -1260 GT/TT	0.284; 1,64 (0.67-4.03)	0,35208333	
CYP24A1 rs2248359 TC/CC	0.261; 0.59 (0.23-1.49)	0,41180556	
CYP24A1 rs2585428 AG/GG	0.673; 0.81 (0.31-2.12)	0,53125	
CYP24A1 rs927650 TT	**0.127; 0.21 (0.03-1.57)**	0,36736111	0.069; 0.10 (0.01-1.2)
VDR ApaI AA	**0.036; 2.64 (1.07-6.54)**	0,20833333	0.733; 1.26 (0.33-4.75)
VDR TaqI TC/CC	0.977; 0.99 (0.94-2.83)	24.425	
VDR BsmI AA	**0.006; 3.75 (1.47-9.59)**	0,10416667	**0.003; 5.09 (1.72-15.05)**
VDR FokI TC/CC	0.644; 0.76 (0.24-2.42)	0,53263889	
VDR Cdx2 AG/GG	0.852; 0.86 (0.19-4.03)	0,61666667	

	WEEK 4		
	UNIVARIATE		MULTIVARIATE
	p; OR (IC 95%)	*q**	*p*; OR (IC 95%)
Age> 48 Years	0.156; 1.52 (0.85-2.719	0,52013889	0.201; 1.49 (0.81-2.74)
Gender	**0.477; 1.25 (0.68-2.28)**	0,48680556	
BMI at baseline>30 Kg/m^2	**0.901; .0.91 (0.23-3.59)**	21624	
HCV baseline viral load>800.000 IU/mL	0.712; 0.89 (0.49-1.62)	0,62430556	
ALT at baseline>37	**0.870, 1.07 (0.50-2.309**	0,63055556	
Cryoglobulins	0.222; 0.66 (0.34-1.29)	0,4625	
Diabetes	0.187; 0.43 (0.12-1.51)	0,51944444	0.696; 0.76 (0.19-3.08)
Steatosis	NC	/	NC
Insulin Resistance	0.128; 0.63 (0.34-1.15)	0,53333333	0.326; 0.72 (0.97-1.39)
Metavir score (0=F0/F1/F2 and 1=F3/F4)	0.264; 1.43 (0.77-2.66)	0,36666667	
PEG type	0.055; 1.67 (0.99-2.83)	0,45833333	0.082; 1.61 (0.94-2.75)
RBV dose	0.263; 1.00 (0.99-1.00)	0,39861111	
PEG dose	0.336; 0.99 (0.98-1.00)	0,37361111	
ITPA rs6051702 AC/CC	**0.001; 0.195 (0.07-0.52)**	0.024	**0.001; 0.19 (0.07-0.51)**
ITPA rs1127354 CA	NC	/	NC
ITPA rs7072101 AC/CC	0.215; 0.60 (0.27-1.34)	0,51180556	
CYP27B1 +2838 TT	**0.712; 1.12 (0.62-2.03)**	0,65902778	
CYP27B1 -1260 GT/TT	0.330; 0.75 (0.41-1.35)	0,42291667	
CYP24A1 rs2248359 TC/CC	0.790; 0.92 (0.49-1.74)	0,65833333	
CYP24A1 rs2585428 AG/GG	0.243; 0.69 (0.38-1.28)	0,45	
CYP24A1 rs927650 TT	**0.254; 0.63 (0.28-1.40)**	0,42361111	
VDR ApaI AA	**0.712; 1.12 (0.61-2.07)**	1005	
VDR TaqI TC/CC	0.850; 1.07 (0.54-2.11)	1	
VDR BsmI AA	**0.332; 0.68 (0.31-1.48)**	0,39513889	
VDR FokI TC/CC	0.091; 2.23 (0.88-5.66)	0,50555556	0.112; 2.17 (0.84-5.64)
VDR Cdx2 AG/GG	0.822; 0.89 (0.32-2.45)	0,65208333	

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