Association of MCP1-2518A/G and CCR2 –V64I Polymorphisms and Vaso-occlusive Crisis among Sickle Cell Anemia Tunisian Patients

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Abstract

Objective: To further define the genetic basis of clinical variability in sickle cell anemia (SCA), we focus on the known functional polymorphisms of MCP-1 and CCR2 and we also discuss their associations with complications of SCA including vaso-occlusive crisis (VOC) and infection.

Methods: 100 SCA patients were enrolled in this investigation. The sample of patients was divided into two groups according to the presence or the absence of each complication. Polymorphisms studied namely: MCP-1-2518 A/G and CCR2 –V64I were genotyped for all subjects using PCR/RFLP. To test for trait association

polymorphisms (SNPs) identified in exon3 of which the major functional polymorphism V64I and its underlying G to A non-synonymous mutation at position 190, is the most common in the Caucasian population [18]. Furthermore, V64I polymorphism appears to result in a reduced binding of MCP-1 [19].

In this paper, we intend to study the impact of MCP-1-2518 A/G and CCR2 –V64I on the occurrence of VOC and infection for the first time among SCA Tunisian children. SCA is the second sickle cell hemoglobinopathy after b-thalassemia in Tunisia, representing a real public health problem. The average frequency of the trait in our country is 1.89% [20].

Polymorphisms	Primers (5'-3')	Product length	Cycling conditioned for 25 µl
-2518 A/G of MCP-1	F : GCTCCGGGCCCAGTATCT R : GGCCATCTCACCTCATCTTCC	689pb	94°C 10 mn 35x(94°C 1 mn 62°C 1 mn,72°C 1 mn) 72°C10 mn
V64I of CCR2	F : TTGTGGGCAACATGATGG R : TGAAGAAGATTCCGCCAAAA	222pb	95°C 10 mn 38x(95°C 30s 57°C 30s,72°C 1 mn)72°C 10 mn

Table 2: PCR conditions of studied polymorphisms

Detection of polymorphism MCP1-2518A/G was performed by PCR/RFLP. The PCR products were digested by PvuII (New England Biolabs, U.K.) which yields 507 pb and 182 pb when G is at position -2518. The products were separated on polyacrylamide gel, stained with ethidium bromide.

CCR2 –V64I was analyzed by PCR/RFLP .The PCR products were digested by BsabI (New England Biolabs, U.K.) which yields 204pb and 18pb when mutant allele A is found. The products were separated on polyacrylamide gel, stained with ethidium bromide.

Statistical analysis: The sample of patients was divided into two groups according to the presence or absence of each complication. The demographic and hematologic data were normally distributed, so we calculated means and standard deviations using SPSS (18.0). We compared demographic and hematological and clinical data between the two groups of patients applying the t test. All SNPs were tested for deviation from the Hardy-Weinberg equilibrium using the software package Arlequin (version 3.01). Chi Square test or fisher test was used to determine genetic differences between patients using compare 2 (version 1.02).

Results

The exploration of hemoglobin profile showed that the patients have homozygous β-S mutation and they haven't α thalassemia. The sample of patients was divided into two groups according to the presence or absence of each complication.

The two groups of patients stratified accordingly to the occurrence of VOC and infection were compared for age, sex ratio and hematological data including HbF. No significant association was found (p>0.05) (Table 1).

For each polymorphism the samples were found to be in Hardy-Weinberg equilibrium (p>0.05). The genotyping of MCP-1 -2518A/G polymorphism shows the presence of three genotypes namely: AA, AG and GG (Table 3). Our findings show that patients with VOC presented 69.04% of genotype AA, 27.38% of genotype AG and 3.57% of genotype GG. Moreover, patients with infection presented 61.76% of genotype AA, 38.23% of genotype AG and no one with genotype GG. The genotypic and allelic distribution between patients according to the presence or not of each complication using Fisher's exact test and Chi square test showed a significant association between genotype AG and genotype GG and VOC among SCA patients (Table 3). Interestingly, the latter genotype appears to present a protective factor to the occurrence of VOC among SCA patients (Table 3). These results showed that the -2518 MCP-1 polymorphism is related to the susceptibility of VOC. Individuals with the AA genotype were at higher risk of this complication than subjects with the AG and the GG genotype. The genotyping of CCR2-V64I polymorphism show the presence of three genotypes namely: GG, GA and AA (Table 3). Our findings showed that patients with VOC presented 65.47% of genotype GG, 30.95% of genotype GA and 3.57% of genotype AA. Moreover, patients with infection presented 85.29% of genotype GG, 11.76% of genotype GA and 2.94% of genotype AA. Statistical analysis showed a significant association between genotype AA and allele A with VOC. Genotype AA and allele a present a risk factor for VOC (Table 3).

	VOC		**P** **RR (CI95%)**	**Infection**		**p** **RR (CI95%)**
	Presence N=84	Absence N=16	Presence N=34	Absence N=66		
MCP12518A/G						
AA	58	3	1*	21	44	1*
AG	23	10	1.210-3 0.12 (0.020-0.530)	13	17	0.417
GG	3	3	7.510-3 0.05 (0.05-0.603)	0	5	0.313

A	0.82	0.50	1*	0.81	0.794	1*
G	0.17	0.50	2.910-4 (0.087-0.506)	0.18	0.206	0.300
CCR2-V64I						
GG	55	6	1*	29	52	1*
GA	26	6	0.372	4	10	0.538
AA	3	4	7.110-3 12.32 (1.551-98.474)	1	4	0.659
G	0.80	0.56	1*	0.92	0.86	1*
A	0.19	0.43	4.910-3 1.269(1.04-1.55)	0.07	0.14	0.425

P: index of significance; RR: Relative-Risk; 1*: reference group; VOC: vaso-occlusive crisis.

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