Human Multidrug Resistance 1 gene polymorphisms and Idiopathic Pulmonary Fibrosis

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Background: For the first time we tested an association between the human multidrug resistance gene 1 (MDR1) polymorphisms (SNPs) and idiopathic pulmonary fibrosis (IPF). Several MDR1 polymorphisms are associated with pathologies in which they modify the drug susceptibility and pharmacokinetics. **Materials and Methods:** We genotyped three MDR1 polymorphisms of 48 IPF patients and 100 control subjects with Italian origins. **Results:** No evidence of association was detected. **Conclusion:** There are 50 known MDR1 SNPs, and their role is explored in terms of the effectiveness of drug therapy. We consider our small-scale preliminary study as a starting point for further research.

Key words: Idiopathic pulmonary fibrosis, multidrug resistance 1 gene, polymorphism

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INTRODUCTION

Anti-inflammatory, anti-fibrosis, immunosuppressive treatments (azathioprine, corticosteroids, cyclophosphamide) or antioxidant strategies and other therapeutic agents targeting fibrogenesis (interferons, antagonists of cytokines, growth factors or their receptors and angiogenic agents) are useless for idiopathic pulmonary fibrosis (IPF).[1] No studies have considered a possible relationship between the human multidrug resistance 1 gene (MDR1) polymorphisms and the therapy outcome in IPF.

Multidrug resistance 1 gene is expressed in epithelial cells of a certain number of organs, including the lung.[2] The MDR1 phenotype is acquired after administration of drugs[3] and when expressed, it extrudes chemotherapeutic substances from cells causing the ineffectiveness of the treatment.[2]

The MDR1 encodes for a plasma membrane MDR protein, the P-glycoprotein (P-gp)[2] that functions as an energy-dependent membrane efflux pump to maintain cytoplasmic concentrations steady.[3] P-gp drags out of the cell a number of substances with diverse chemical structures. It is not clear how P-gp recognizes and transports and hence various range of compounds, but it is not based on simply chemical structure.[4]

Several drugs have not been evaluated as P-gp substrates, so additional unidentified substances could be transported by the P-gp across cell membrane.

Furthermore, variations in P-gp expression and function are associated with the pathology susceptibility[5] and have important consequences in therapeutic outcome and disease progression.[6] By now, 50 single nucleotide and 3 insertion/deletion polymorphisms have been mapped in the MDRl gene.[7] To evaluate MDR1 as possible risk factor for IPF, we analyzed the three most commonly studied MDR1 polymorphisms in our case record.

MATERIALS AND METHODS

Between 2008 and 2013, 48 consecutive Italian patients with IPF (31 males and 17 females) were enrolled for this study at the Respiratory Division, Sant'Orsola Hospital, University of Bologna. Diagnosis of IPF was made according to ATS/ERS guidelines of 2011.

A total of 100 healthy volunteers (50 males and 50 females), were enrolled as controls. The study was approved by the Ethical Committee of Sant'Orsola-Malpighi General Hospital and complied with the ethical principles for medical research involving human subjects of the Helsinki declaration. Written informed consent was obtained from all patients and healthy controls

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beforehand. Peripheral blood samples were drawn from each individual and DNA extraction was performed using the GenElute Blood Genomic DNA kit (Sigma, Milan, Italy).

Three single-nucleotide polymorphisms (SNPs) mapping respectively on exon 12 (1236C>T; rs1128503), 21 (2677G>T/A; rs2032582), and 26 (3435C>T; rs1045642) of MDR1 gene were selected. Polymorphism selection was based on data available from published investigations. Among all known gene variation, we selected three SNPs that were previously found associated with other pathologies, thus having higher chances to directly alter gene function or to be linked with susceptibility variation.

Each polymorphism was amplified by polymerase chain reaction using flanking primers [Table 2] and products incubated with a restriction endonuclease, BsuRI, GsaI and DpnII (CABRU, Milan, Italy), respectively. Fragments were separated by 10% polyacrylamide gel electrophoresis, subsequently stained with ethidium bromide [Figure 1]. To assess the accuracy of the genotyping outputs, one-third of randomly selected samples were blindly tested by a second operator.

The distribution of genotypes in both probands and control groups was tested for deviations from the Hardy-Weinberg equilibrium using Pearson's Chi-square test. Association analyses were performed with the Unphased software v3.1.5 in a Windows Vista operative system.[8] This included a tests for both allele and genotype association, as well as odds ratio calculation, which was shown in relation to a single reference allele or genotype.

Two and three SNPs combinations were considered for haplotype analysis. Since the likelihood ratio statistics may be sensitive to rare haplotypes, a frequency threshold of 0.02 was adopted. An overall association analysis, that tested whether any haplotypes are associated (global *P* value), as well as a specific test for each haplotype, was carried out.

RESULTS

Genotype frequency in cases and controls were distributed accordingly with the Hardy-Weinberg equilibrium law.

Allele frequency was similar in case and control groups at all the tested loci. No evidence of the association between IPF and MDR1 genotypes was detected [Table 1].

Multilocus approach could be more sensitive than single locus analysis. For this reason, two and three locus haplotypes were tested for association with IPF. Both the global and the single haplotype tests for association rejected the hypothesis of the association between MDR1 polymorphisms and IPF (data not shown).

DISCUSSION

C1236T, G2677T/A and C3435T are the most commonly studied among the 50 known MDR1 gene SNPs in disease conditions and different populations. We did not find any differences about the frequencies of these three MDR1 polymorphisms between IPF patients and control subjects in our Italian case study. The relatively low number of patients examined could be the cause of false negative results, especially if MDR1 had minor effects on risk to develop IPF. Moreover, additional untyped polymorphisms could have an effect on IPF risk. This is only the start point to evaluate the role of the MDR1 SNPs in IPF considering the large

Table 1: Case-control association analysis between MDR1 SNPs and IPF

SNP ID	Allele/ genotype	IPF case	Control	IPF-frequency	Co-frequency	Chi-square	*P*	OR (CI)
rs1128503	C	58	111	0.60	0.56	0.64	0.42	Reference
	T	38	89	0.40	0.44			0.82 (0.50-1.34)
	CC	18	33	0.38	0.33	0.67	0.72	Reference
	CT	22	45	0.46	0.45			0.90 (0.42-1.93)
	TT	8	22	0.17	0.22			0.67 (0.25-1.80)
rs2032582	T	57	108	0.59	0.54	0.76	0.38	Reference
	A	39	92	0.41	0.46			0.80 (0.49-1.32)
	TT	17	32	0.35	0.32	1.07	0.59	Reference
	TA	23	44	0.48	0.44			0.98 (0.45-2.1)
	AA	8	24	0.17	0.24			0.63 (0.23-1.69)
rs1045642	C	52	100	0.54	0.50	0.45	0.50	Reference
	T	44	100	0.46	0.50			0.85 (0.52-1.38)
	CC	14	29	0.29	0.29	1.31	0.52	Reference
	CT	24	42	0.50	0.42			1.18 (0.53-2.67)
	TT	10	29	0.21	0.29			0.71 (0.27-1.87)

IPF = Idiopathic pulmonary fibrosis; OR = Odds ratio; CI = Confidence interval; MDR1 = Multidrug resistance 1 gene; SNPs = Single-nucleotide polymorphisms

Table 2: Characteristics of polymorphisms

SNP ID	Primer sequences 5'-3'	Nucleotide change[a]	Location	Ta[b]	Restriction enzyme
rs1128503	For TTGAATGAAGAGTTTCTGATGTTTT	1236C>T	Exon 12	45°C	*BsuR*I
	Rev CTCTGCATCAGCTGGACTGT				
rs2032582	For TGTTGTCTGGACAAGCACTGA	2677G>T	Exon 21	55°C	*Gsa*I
	Rev TGTGAAGACAATGGCCTGAA				
rs1045642	For GGCTCCGAGCACACCTGGGCA	3435C>T	Exon 26	59°C	*Dpn*II
	Rev GGCCAGAGAGGCTGCCACATGCT				

[a]Major allele/minor; [b]Annealing temperature; Conditions of amplification for 35 cycles were 30 s at 94°C, 30 s at specific Ta[b], 30 s at 72°C and final extension for 8 min at 72°C; SNP: Single-nucleotide polymorphism

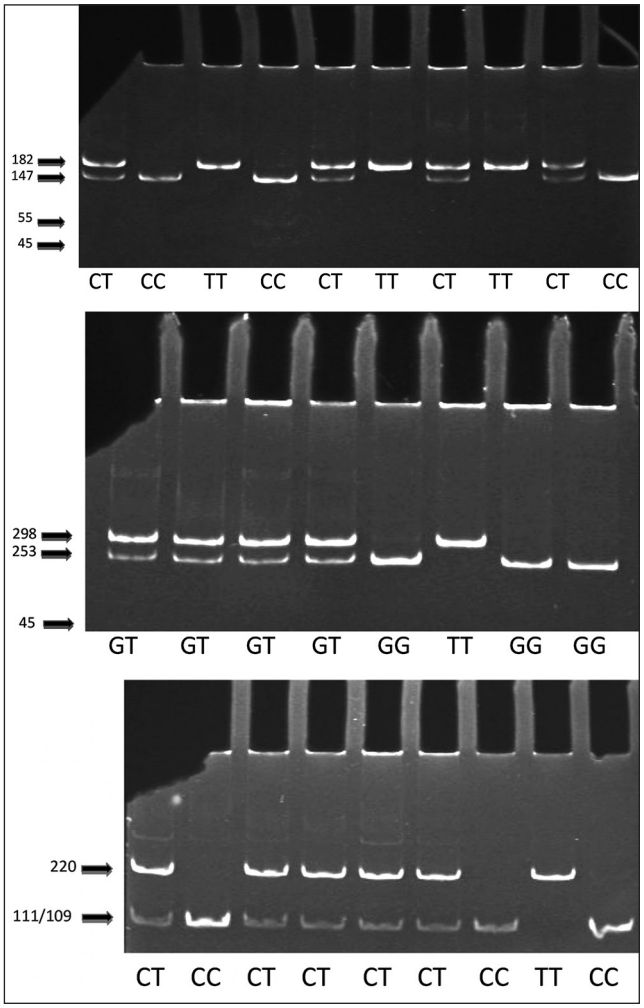

Figure 1: Electrophoretic pattern on 10% acrylamide gel of multidrug

amount of SNPs to analyze and the differences determined by the variety of ethnic populations.[9] In fact, significant differences in the frequencies of MDR1 haplotypes have been found among Caucasian, African-American, and Asian-American population.[6] We believe that further understanding of the correlation between the MDR1 genetic variations and the physiology and biochemistry of P-gp may be critically important for the development of new treatment modalities and personalized pharmacotherapy for patients with IPF.

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AUTHOR'S CONTRIBUTION

MM contributed in the conception of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. LS contributed in the conception of the work, was responsible for the data analysis and interpretation, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. AMGP contributed in the conception of the work, conducting the study, particularly was responsible for the sample collection; revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. PC contributed in the conception of the work, conducting the study, particularly was responsible for the sample collection; revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. AG contributed conducting the study, particularly was responsible for the experimental operating; revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. GM contributed conducting the study, particularly was responsible for the experimental operating; revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. MTR contributed conducting the study, particularly was responsible for the experimental operating; revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. RS contributed in the conception of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

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