Evidence of Interaction between *PTPN22* and *p53 codon 72* Polymorphisms on Susceptibility to Immune Related Diseases

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FGB, EB, AM and GR designed the study, wrote the protocol and the first draft of the manuscript. Authors PS, NR, AN and LC managed the control of laboratory methods, collection of data, genotype determination, preparation of data files and statistical analysis. Authors FGB, AM, PS and AN managed the literature search. All authors read and approved the final manuscript.

Research Article

ABSTRACT

Background: *PTPN22* codifies for a protein-tyrosine-phosphatase (Lyp) involved in T cell receptor signaling regulation. *p53* is involved in immune related inflammation regulating *STAT 1* and pro-inflammatory cytokines. Possible interaction between the two systems concerning the susceptibility to immune related disorders are therefore biologically plausible. In the present note we have searched for such interaction in type 1 diabetes mellitus and reviewed previous data from our laboratory.
Methods: We have studied 287 children with type 1 diabetes, 129 non diabetic adult subjects admitted to the Hospital for Coronary Artery Disease, 130 women with endometriosis and 256 healthy blood donors. *PTPN22* and *p53 codon 72* genotypes were determined by DNA analysis.
Results: In all diseases the proportion of *PTPN22* *T allele is higher in *p53* *Pro allele carriers than in *p53*Arg/*Arg genotype. In *Arg/*Arg patients the proportion of *T allele

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carriers does not differ significantly from controls while in subjects carrying the *Pro allele is higher in patients than in controls. A significant increase of Odds Ratio is observed only in presence of both *T and *Pro alleles suggesting a cooperative interaction.

Conclusion: It has been suggested that the susceptibility to autoimmune disorders in the presence of *T allele could be related to failure to delete auto reactive T cell during intrathymic selection. *Pro allele variant with its strong transcriptional activity could enhance the multiplication of such auto reactive T cell escaping intrathymic thus explaining a significant increase of Odds Ratio in the presence of both factors .The present observation could have relevance to identify individuals at high risk of clinical manifestations.

1. INTRODUCTION

PTPN22 codifies for a protein-tyrosin-phosphatase (LYP) involved in the regulation of T cell receptor signaling (TCR). The gene shows a single nucleotide polymorphism C/T at +1858 resulting in the W620 variant that is associated with autoimmune disorders [1].

Codon 72 in exon 4 of *p53* gene shows a polymorphism due to the substitution of G with C that determines the change of arginine to proline in the protein. The amino acid change affects biochemical and functional properties of *p53*: the proline variant (*Pro allele) is a stronger transcriptional activator, whereas the arginine variant (*Arg allele) is a stronger apoptosis inducer [2]. Activation of *p53* gene has been associated with immune related disorders [3,4] and anti *p53* autoantibodies have been found in the serum of patients with these disorders [5].Recent studies suggest that p53 may be involved in immune related inflammation regulating *STAT1* and proinflammatory cytokines [6,7]. Possible interactions between the two systems concerning the susceptibility to autoimmune disorders are therefore biologically plausible.

In the population of Rome *T allele of *PTPN22* and *Pro allele of p53 codon 72 polymorphisms have been found more frequent in type 1 diabetes (T1D) than in controls [8-10], therefore, we have searched for possible interaction between the two systems on susceptibility to T1D. We have previously observed an interaction between *PTPN22* and *p53 codon 72* in endometriosis [11] and in Coronary Artery Disease (CAD) [12] in which immunological factors couldy have a significant role: thus, in the present note we have also reviewed our data on these diseases. The pattern of interaction is concordant in these disorders suggesting a significant effect on susceptibility in presence of both *Pro allele of *p53 codon 72* and of *T allele of *PTPN22*.

2. MATERIALS AND METHODS

We have studied 287 subjects with type 1 diabetes (T1D), 129 non diabetic subjects admitted consecutively to Valmontone Hospital (Italy) for CAD (Coronary Artery Disease), 130 women with endometriosis and 256 healthy blood donors. All subjects were from the White Caucasian population of Rome. Written informed consent was obtained from each subject. The study protocol conforms to the Ethical Guidelines of the 1975 declaration of Helsinki and was approved by the Ethical Committee of Valmontone Hospital. All subjects were collected consecutively and separately for each sample in order to perform a case-control study.

The *PTPN22* polymorphism (chromosome 1) has two alleles, *C1858 (encoding the R620 variant, here simply called *C) and *T1858 (encoding the W620 variant, here simply called *T), and has three genotypes, *C/*C, *C/*T and *T/*T. The *T/*T genotype is very rare. Patients were genotyped as previously described [13].

The *p53 codon 72* polymorphism (chromosome 17) has three genotypes: *Arg/*Arg, *Arg/*Pro and *Pro/*Pro and was evaluated using the restriction fragment length polymorphism polymerase chain reaction method described by de la Calle-Martin et al. [2] as previously described [11].

Chi-square test of independence and odds ratio analyses were performed by the SPSS programs [14]. Three way contingency table analysis by a log linear model was carried out according to Sokal and Rohlf [15].

3. RESULTS

Clinical data of the patients and controls are shown in Table 1. Table 2 shows the proportion of *T allele carriers in *Arg/*Arg genotype and in *Pro allele carriers of *p53* codon 72 polymorphism in healthy controls and in subjects with immune related diseases. In all these disorders but not in controls the proportion of *T allele is higher in carriers of *Pro allele than in *Arg/*Arg genotype. The proportion of *T allele carriers in *Arg/*Arg subjects with immune related disorders does not differ significantly from controls but in carriers of *Pro allele is significantly higher in patients than in controls (p=0.0005).

Table 3 shows the proportion of subjects carrying both *T allele of *PTPN22* and *Pro allele of *p53 codon 72* in diseases and in controls. No significant difference is observed among the three diseases. The proportion of subjects carrying both *T and *Pro alleles is significantly higher in the diseases than in controls (Odds Ratio=3.736; 95%C.I. 1.506-9.853; p=0.002).

A three way contingency table analysis by a log linear model performed on 2x2 contingency table reported in sections (A) and (D) of Table 2 has shown a lack of a significant three way interaction and a significant additive effect of *PTPN22* and *p53 codon 72* on susceptibility to diseases considered (p=0.003,data not shown). These results appear against the hypothesis of epistasis and in favor of cooperative interaction.

Odds ratio analysis is also in favour of an additive effect of the two alleles on susceptibility to the diseases. Fig. 1 shows the Odds Ratio in relation to presence of one risk factor only (*Pro allele or *T allele) and to presence of both risk factors (*Pro and *T alleles). A very high Odds Ratio is observed in presence of both factors.

In T1D the pattern of associations described in Tables 2 and 3 does not depend neither on sex nor on age at onset. In CAD the pattern is not influenced by age. In endometriosis diffusion of lesion, parity and duration of pharmacological treatment do not influence significantly the pattern of associations shown in Tables 2 and 3. Significant differences have been observed concerning the socio-economic status with a higher proportion of the high risk joint genotype in the low as compared to the high status ($p<0.05$).

Table 1. Clinical data on the patients studied

Type 1 diabetes	
Proportion of males	52%
Mean age at onset (years)	8.5
Familiarity for autoimmune diseases	13.9%
Association with autoimmune diseases	27.6%
Endometriosis	
Mean age (years)	34.7
Proportion of patients with at least one newborn	28.6%
Minimal-mild lesion	15.5%
Moderate-severe lesion	84.5%
Patients with CAD	
Mean age (years)	66.7
Mean Body Mass Index	27.4
Proportion of males	52.6%
Infarction	40.5%
Major coronary lesions	83.2%
Bypass	35.2%
Angioplastic	27.1%
Smoking habit	65.4%
Controls	
Mean age (years)	37.3
Proportion of males	81.3%

Table 2. Proportion of *T allele carriers of *PTPN22* polymorphism in *Arg/*Arg genotype and in *Pro allele carriers of *p53 codon 72* polymorphism. Comparison between immune related diseases and healthy blood donors

Subjects	p53	PTPN22	
(A) Blood donors		*C/*C	*T allele carriers
	*Arg/*Arg	129	10(7.2%)
	*Pro allele carriers	111	6(5.1%)
(B) T1D			
	*Arg/*Arg	148	17(10.3%)
	*Pro allele carriers	102	20(16.4%)
Comparison with controls	χ^2 df p		
*Arg/*Arg	0.257 1 0.612		
*Pro allele carriers	8.459 **1** 0.003		
(C) Endometriosis			
	*Arg/*Arg	53	6(10.2%)
	*Arg/*Arg	53	6(10.2%)
	*Pro allele carriers	55	16(22.5%)
Comparison with controls	χ^2 df p		
*Arg/*Arg	0.057 1 0.811		
*Pro allele carriers	14.201 1 0.0002		
(D)CAD non diabetic			
	*Arg/*Arg	61	5(7.6%)
	*Pro allele carriers	52	11(17.5%)
Comparison with controls	χ^2 df p		
*Arg/*Arg	0.044 1 0.834		
*Pro allele carriers	7.113 1 0.009		
(E) All diseases			
	*Arg/*Arg	262	28(9.7%)
	*Pro allele carriers	209	47(18.4%)
Comparison with controls	χ^2 df p		
*Arg/*Arg	0.157 1 0.691		
*Pro allele carriers	12.238 1 0.0005		

Table 3. Proportion of subjects carrying both *T allele of *PTPN22* and *Pro allele of *p53 codon 72*

	Per cent proportion	Total nº
T1D (A)	6.96%	287
Non Diabetic patients with CAD (B)	8.53%	129
Endometriosis (C)	12.3%	130
Healthy Adults (Blood Donors) (D)	2.52%	256

Chi square test of independence

	χ^2	df	p
A vs B vs C	3.243	2	0.197
(A + B + C) vs D	9.229	1	0.002

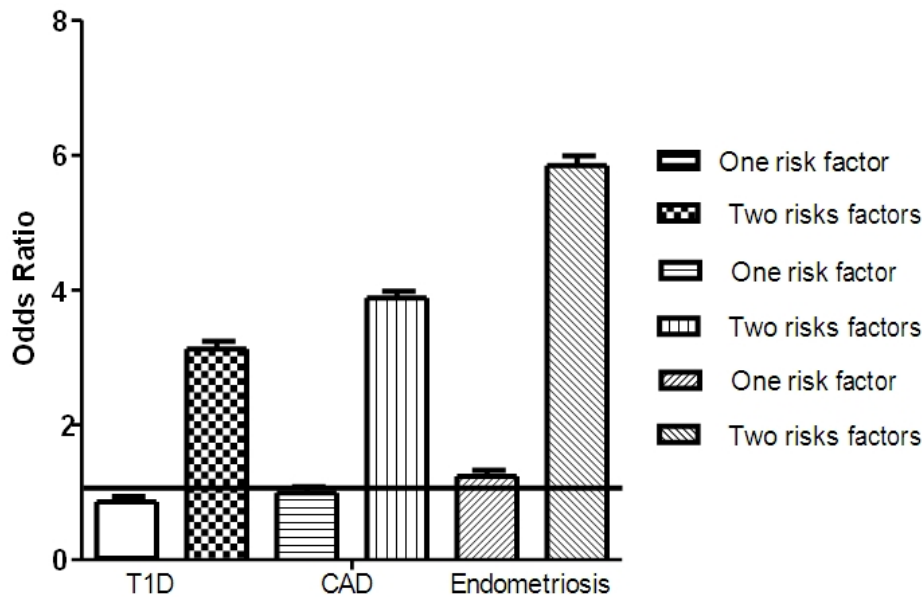OR=3.924   95% C.I. 1.505-9.853

Fig. 1. Odds ratio for the diseases considered: comparison with controls. The horizontal line indicates neutrality. A cumulative analysis shows a significant increase of risk (O.R. = 3.924; C.I. =1.506-9.853; p=0.002) in presence of two risk factors (*Pro and *T alleles)

4. DISCUSSION

Our observations suggest that the susceptibility to the diseases studied is increased in presence of both *Pro and *T alleles. It has been suggested that the susceptibility to autoimmune disorders in presence of *T allele of *PTPN22* could be related to failure to delete auto reactive T cell during intratymic selection [16]. *Pro allele variant of *p53 codon 72* with its strong transcriptional activity could enhance the multiplication of auto reactive T cell escaping intratymic selection contributing to emergence of clinical manifestation. This could explain the high risk in presence of both factors.

The limitation of the present study is represented by the relatively small number of subjects examined. Therefore our observations need to be confirmed in an independent clinical setting. However the concordance of the pattern observed in the three diseases makes unlikely the possibility of a mere chance sampling artifact. It would be interesting to search for such interaction also in other diseases involving the immune system.

Appropriate studies on immunological parameter in presence of only one and in presence of both risk factors could contribute to elucidate the mechanism of interaction. From a practical point of view the observation could have relevance to identify individuals at high risk of clinical manifestations. It would be also interesting to elucidate the possible role of these polymorphic genes of the clinical evolution of the diseases: unfortunately we have no reliable information of this important aspect of the problem.

5. CONCLUSION

The present data suggest a cooperative interaction between PTPN22 and P53 codon 72 polymorphisms conserning their effects on susceptibility to immune related diseases.

Genetic analysis of multifactorial disorders represents an important problem for medical genetics: the study of single genetic factors in a mendelian perspective is reductionist and cannot solve the problem. It is likely that simultaneous analysis of multiple genes related to a specific function will provide a more productive approach to clarify the etiology of multifactorial diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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