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p53 Codon 72 Genetic Polymorphism in Asthmatic Children: Evidence of Interaction With Acid Phosphatase Locus 1

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Several lines of evidence are implicating an increased persistence of apoptotic cells in patients with asthma. This is largely due to a combination of inhibition, or defects in the apoptotic process and/or impaired apoptotic cell removal mechanisms. Among apoptosis-inducing genes, an important role is played by p53. In the present study, we have investigated the possible relationship between p53 codon 72 polymorphism and asthma and the interaction with *ACP1*, a genetic polymorphism involved in the susceptibility to allergic asthma. We studied 125 asthmatic children and 123 healthy subjects from the Caucasian population of Central Italy. p53 codon 72 and *ACP1* polymorphisms were evaluated using a restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) method. There is a statistically significant association between p53 codon 72 polymorphism and allergic asthma: Arg/Arg genotype is more represented in asthmatic patients than in controls ($P=0.018$). This association, however, is present in subjects with low *ACP1* activity A/A and A/B only ($P=0.023$). The proportion of children with A/A and A/B genotype carrying Arg/Arg genotype is significantly high in asthmatic children than in controls (OR, 1.941; 95% CI, 1.042-3.628). Our finding could have important clinical implications since the subjects with A/A and A/B genotypes of *ACP1* carrying Arg/Arg genotype are more susceptible to allergic asthma than Pro/Pro genotype.

Key Words: p53; *ACP1*; genetic polymorphism; asthma; allergy; apoptosis

INTRODUCTION

Asthma is a chronic inflammation of airways with complex etiologies involving both genetic and environmental contributions; the disease is associated with the release of myriad pro-inflammatory substances, including lipid mediators, inflammatory peptides, chemokines, cytokines and growth factors. However, the mechanism leading to the persistent accumulation of inflammatory cells is not fully understood.[1] The data present in the literatures are controversial, and a straightforward association between asthma and the various factors investigated has not been unequivocally demonstrated.[1,2]

Several lines of evidence are implicating an increased persistence of apoptotic cells in patients with asthma: this is largely due to a combination of inhibition, or defects in the apoptotic process and/or impaired apoptotic cell removal mechanisms.[3] Other studies have shown that apoptosis of bronchial epithelial cells increases in adult asthma.[4-7] Zhou *et al.*[8] have provided the first evidence of apoptotic abnormality of the asthmatic bronchial epithelium in children, mirroring the significantly increased apoptosis seen in adult asthmatic epithelium.

Among apoptosis-inducing genes, an important role is played by p53 (also known as protein 53 or tumor protein 53), which is considered a guardian of the genome and is necessary for some forms of programmed cell death.[9] Two common polymorphic forms of p53 encoding either proline (CCC) or arginine (CGC) at position 72 (4th exon of the human p53 gene) are widely distributed throughout the human population; these 2 polymorphic variants are functionally distinct. The p53-Arg72 variant is significantly more efficient than the p53-Pro72 variant in inducing apoptosis; however, the p53-Pro72 variant appears to induce a higher level of G1 arrest than the p53-Arg72 variant. These differences demonstrate how codon 72 polymorphism affects the biological activity of p53.[10,11]

Acid phosphatase locus 1 (*ACP1*), a cytosolic low-molecular-weight phosphotyrosine phosphatase, is a highly polymorphic

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enzyme controlled by an autosomal locus showing 3 codominant alleles *ACP1* A, B, and C. By a combination of the 3 alleles can have 6 genotypes with a different enzymatic activity, in the order of A/A<A/B<B/B≤A/C<B/C<C/C, the genotype C/C is very rare.[12]

ACP1 specifically dephosphorylates the negative regulatory Tyr-292 of ZAP-70 (zeta-chain-associated protein kinase 70), thereby counteracting the inactivation of ZAP-70. The ZAP-70 protein-tyrosine kinase plays a central role in signaling from the T-cell antigen receptor. Thus these results indicate that *ACP1* strengthens T-cell receptor signaling.[13]

In the past studies from different populations (Italians, English, and Chinese) our group has found that the proportion of allergic subjects is higher among genotypes with low enzymatic activity than among genotypes with high activity of *ACP1* suggesting that carriers of *ACP1* genotypes associated with low enzyme activity are more susceptible to allergic disorders.[14-17] In the present study we investigated the relationship between p53 codon 72 polymorphism and asthma and the possible interaction with *ACP1*.

MATERIALS AND METHODS

Subjects

We studied 125 asthmatic children admitted consecutively to Pediatric Unit of the Gabriele D'Annunzio, University of Chieti and 123 healthy subjects from the same Caucasian population of Central Italy. All children's parents gave informed consent for participation in this study, which was approved by the IRB. Blood was obtained by forearm venipuncture, and genomic DNA was extracted using the MagNA Pure LC Instrument (Roche, Mannheim, Germany).

p53 genetic polymorphism

p53 codon 72 polymorphisms was evaluated using RFLP-PCR as described by de La Calle-Martín *et al.*[18] with a few modifications. The following primer sequences corresponding to the 4th exon of the human p53 gene were used: sense oligo 5'-AATGGATGATTTGATGCTGTCCC-3' and antisense oligo 5'-CGTGCAAGTCACAGACTTGGC-3'. PCRs were carried out in a total volume of 25 mL containing the following: 100 ng of genomic DNA, 0.4 pmol of each primer, 2 mM $MgCl_2$, 200 mM dNTPs, 1×Buffer and 2 U of Taq polymerase. Amplification was performed for 35 cycles with an annealing temperature of 62°C. The amplified DNA was digested for 3 hours with 3 U of Acc II restriction enzyme. DNA fragments were resolved by electrophoresis through a 3% agarose gel.

ACP1 genetic polymorphism

The three *ACP1* alleles show single base substitutions located at 3 specific sites: *ACP1* A and B alleles differ for 2 base substitutions, a silent C-T transition at codon 41 (exon 3), and an A-G transition at codon 105 (exon 6). The *ACP1* C allele differs from A and B alleles for C-T transition at codon 43 (exon 4).[19] All polymerase chain reactions (PCRs) were set up in 30 μL, 0.2 μmol/L of both primers, 0.1 mmol/L dNTPs, 1.5 mmol/L $MgCl_2$, 0.5 U of Taq polymerase (AmpliTaq, Applied Biosystem, Mannheim, Germany) 1×AmpliTaq buffer (PE), and 50 ng of DNA template. The amplification conditions consisted of an initial denaturation at 94°C for 2 minutes, followed by 35 cycles of 94°C for 45 seconds, 54°C for 45 seconds, 72°C for 45 seconds, and final extension at 72°C for 5 minutes. The annealing temperature, extension time, and primer concentration for the 2-Kb amplification product were 57°C, 120 seconds, and 0.1 μmol/L, respectively. Oligonucleotide primers used for PCR amplification are reported in Table 1. The C-T transition at codon 43 and A-G transition at codon 105 generate, respectively, a Cfo I and a Taq I restriction site that, together, were used for PCR-based genotyping.[19] A 341 bp segment spanning the entire exons 3 and 4 was amplified using primers #263 to #264 (Table 1). A 299 bp segment including exon 6 was amplified using primers #267 and #268. Ten microliters of the 341 bp exon 3 amplicon was fully cleaved by Cfo I at 37°C for 1 hour according to the manufacturer's instructions and then electrophoresed on 1.8% agarose gels. The digestion created 2 fragments of 255 and 86 bp for the *ACP1* A and *ACP1* B alleles, while the *ACP1* C allele was not cut. Similarly, the 299 bp PCR product was digested by Taq I at 65°C for 1 hour according to the manufacturer's instructions, generating 2 fragments of 100 and 199 bp for the *ACP1* A allele, but not for the B and C alleles (modified from Lazaruk).

Table 1. Primers used for ACP1 SNPs analysis

Primer number	Target amplification	Nucleotide sequence 5'-3'
#263	Exon 3 forward	GTCGATCACCCATTGCAGAAGC
#264	Exon 3 reverse	CACACAACTTCAAGTCCAAGGACG
#267	Exon 6 forward	GGATGTTTCAGAAGACCCTAGCAG
#268	Exon 6 reverse	GCTCCCAAGTAGTTCAATTTTAGC

SNPs, single-nucleotide polymorphisms.

Statistical analyses

Genotype and allele frequency was compared between cases and controls, and the statistical significance of the associations was tested using the chi-square test. Odds ratios with 95% confidence intervals were also calculated to estimate the strength of the association between p53 and *ACP1* genotypes and asthma and its statistical significance.

RESULTS

Table 2 shows the demographic characteristics of the asthmatic subjects. In control group, the mean age was 13 years, and males accounted for 61.8%.

Table 3 shows the distribution of p53 codon 72 genotypes in

children with allergic asthma and in controls in relation to *ACP1* genotype. There is a statistically significant association between p53 codon 72 polymorphism and allergic asthma: Arg/Arg genotype is more represented in asthmatic patients than in controls (P=0.018). This association, however, is present in subjects with low *ACP1* activity A/A and A/B only (P=0.023).

The proportion of subjects with A/A and A/B genotype carrying the Arg/Arg genotype is significantly high in asthmatic children (32%) than in controls (19.5%) (OR, 1.941; 95% CI, 1.042-3.628). We also performed a log linear analysis to search for an interaction between p53 and *ACP1* concerning their effects on asthma. No significant epistatic interaction was detected, suggesting an additive effect of the 2 systems on the susceptibility to the disease (data not shown).

DISCUSSION

Airway inflammation and remodeling are well established features of pathologic changes in adult asthma, also epithelial loss has been described as a typical pathologic feature of asthma in adults and children.[8,20-26] This fact may suggest that the epithelial shedding-repair process plays an important pathogenic role in initiating and maintaining the airway inflammation and remodeling in asthma. To accomplish cell removal, the current focus of investigators is almost exclusively on the pharmacology of apoptosis.[27] Apoptosis is considered to be a non-inflammatory mode of cell death because apoptotic cells are immediately and silently eliminated through phagocytosis. Efficient clearance of cells undergoing apoptotic death is crucial for normal tissue homeostasis and for the modulation of immune responses. Engulfment of apoptotic cells is finely regulated by a highly redundant system of receptors and bridging molecules on phagocytic cells that detect molecules specific for dying cells.[28]

For the past 2 decades several types of genetic and genome approaches, including linkage analysis, candidate gene single nucleotide polymorphism studies and whole genome-wide association studies have been applied. The data present in the literatures suggest that there are many genetic and environmental factors that contribute to etiology of allergic asthma.[29] In this study we investigated the association between Arg 72 Pro SNP in codon 72 of the p53 tumor suppressor gene, that it is a well known factor regulation of apoptosis in a wide variety of cells, and asthma.

Our results are in agreement with Zhou *et al.*[8]: the increased susceptibility of carrier of the p53-Arg72 variant, that is significantly more efficient than the p53-Pro72 variant in inducing apoptosis, may explain the increase of apoptosis and loss of bronchial epithelial cells in allergic asthma. This is supported by the fact that a significant subgroup of asthmatic patients responded poorly or not at all to high-dose inhaled or systemic glucocorticoids treatment.[3] The subjects with Arg/Arg genotype are more susceptible to allergic asthma than Pro/Pro genotype. Moreover, in this study, we found that the association between asthma and p53 is dependent on *ACP1* suggesting an additive effect of Arg/Arg genotype and *ACP1* genotypes with low enzymatic activity (A/A and A/B) concerning their effects on susceptibility to asthma.

In multifactorial disorders such as asthma the analysis of a single genetic factor could be misleading. Epistatic and additive interactions with other systems and environmental factors should be considered in order to clarify the pathogenic mecha-

Table 2. Demographic characteristics of the asthmatic subjects

Number of subjects	125
Age (year)	9.12
Sex (% males)	65.9
Family history of allergic disease (%)	26.3
Passive smoking (%)	34.3
Atopy* (%)	64.6
Rhinitis (%)	43.4
Bronchodilators (%)	33.3
Corticosteroids (%)	72.5
FEV1 (% predicted)	93.33 (72-127)
FVC (% predicted)	95.85 (72-152)

*Atopy=subjects positive to 2 or more allergens.
FEV1, forced expiratory volume at first second; FVC, forced vital capacity.

Table 3. Distribution of p53 genotypes in children with allergic asthma. The effect of ACP1 genotype

	All ACP1 genotypes			A/A and A/B genotypes			Other ACP1 genotypes		
	Proportion of Arg/Arg genotype		Total n°	Proportion of Arg/Arg genotype		Total n°	Proportion of Arg/Arg genotype		Total n°
Asthmatic children	58.40%		125	67.80%		59	50.00%		66
Controls	43.10%		123	46.20%		52	40.80%		71
Chi square test of independence	χ^2	df	P	χ^2	df	P	χ^2	df	P
	5.822	1	0.018	5.333	1	0.023	1.130	1	0.310
Odds ratio analysis	OR, 1.85 95% CI, 1.086-3.169			OR, 2.46 95% CI, 1.059-5.734			OR, 1.45 95% CI, 0.697-3.015		

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nisms and the role of genetic and environmental factors in susceptibility and clinical course of disease.

In our finding Arg/Arg genotype of p53 and A/A and A/B genotypes of *ACP1* may influence, each with different mechanisms, but with additive interaction, the susceptibility to asthma. Recent studies suggest that p53 may be involved in autoimmune inflammation regulating STAT1 and pro-inflammatory cytokines.[30,31] On the other hand *ACP1*, through regulation of ZAP-70 could have an important role in immunological functions.[13]

Our finding could have important clinical implications since the subjects with A/A and A/B genotypes of *ACP1* carrying Arg/Arg genotype are more susceptible to allergic asthma than Pro/Pro genotype. The study has 2 limitations: a small sample size and findings are not replicated. Further studies are, however, necessary to elucidate the mechanism of the observed statistical association.

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