GENETICS

Gene-Gene Interactions between Glutathione-S Transferase M1 and Matrix Metalloproteinase 9 in the Formation of Hereditary Predisposition to Chronic Obstructive Pulmonary Disease

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The incidence of allele variants of glutathione-S transferase M1 xenobiotic detoxification gene and matrix metalloproteinase 9 gene was analyzed in patients with chronic obstructive pulmonary disease. A strict gene-gene interaction between these two genes in the formation of hereditary predisposition to this disease was first demonstrated. The combination of glutathione-S transferase M1 genotype 0/0 and matrix metalloproteinase 9 mutant allele (-15621) is a risk factor for chronic obstructive pulmonary disease (OR-7.7).

Key Words: *chronic obstructive pulmonary disease; allele variants of metalloproteinase 9; glutathione-S transferase M1*

Chronic obstructive pulmonary disease (COPD) is a highly prevalent pathology that ranks 4th in the list of nosological entities responsible for lethal outcomes. Genetic and exogenous factors, first of all tobacco smoking, contribute to the formation of COPD [5]. Investigation of the molecular genetic basis of hereditary predisposition to COPD is a pressing problem of pulmonology. Tobacco smoking leads to increased load of the detoxification system with proven imbalance in the protease-antiprotease system [3]. From this viewpoint, investigation of gene polymorphism of the detoxification system and proteolytic enzymes is a promising approach [5].

Glutathione-S transferase M1 (GSTM1; 1p13.3) gene is a candidate gene responsible for the development of COPD [2]. Deletion of 2 alleles of GSTM1 gene (~10,000 b. p. fragment) leads to complete absence of the enzyme, and this genotype (GSTM1 0/0) is highly prevalent in the Russian population [1]. Matrix metalloproteinases are a family of structurally related proteinases, playing an important role in remodeling of the main substance of pulmonary connective tissue [6]. Structural polymorphism (-1562C/T) in the promotor area of matrix metalloproteinase 9 (MMP9) gene at the -1562 position (C-T substitution) is responsible for high expression of the gene with accumulation of the corresponding protein in human organs and tissues. We found no published data on the molecular genetics of this determinant in COPD.

Here we studied the incidence of allele variants of GSTM1 (xenobiotic detoxification system) and MMP9 (proteolysis system) genes and their possible interactions in COPD.

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MATERIALS AND METHODS

Seventy-two patients with COPD were included in the study. The reference group consisted of 38 patients with chronic non-obstructive bronchitis (CNB) and the control group consisted of 39 tobacco smokers without chronic pulmonary diseases and severe concomitant diseases. The study was carried out only in men none of whom were blood-related, with similar working conditions and socioeconomic status. Groups of CNB patients and tobacco smokers were matched for age and smoking history. Clinical laboratory studies and regular prophylactic examinations were carried out at Institute of Pulmonology, I. P. Pavlov Medical University.

The allele variants of GSTM1 and MMP9 genes were identified by PCR with subsequent restriction analysis as described previously [8,9]. The following primers were used for amplification of GSTM1 gene fragment: P1 - 5”CGC CAT CTT GTG CTA CAT TGG CCG 3'; P2 - 5”ATC TTC TCC TCT TCT GTC TC 3'; and P3 - 5”TTC TGG ATT GTA GCA GAT CA 3' [9]. The P1/P2 pair (230 b. p. fragment) was specific for normal GSTM1 gene, P1/P3 pair (157 b. p. fragment) served for amplification control. The following primers were used for MMP9 gene fragment amplification: A - 5”GCC TGG CAC ATA GTA GGC CC 3' and B - 5”CTT CCT AGC CAG CCG GCA TC 3' [8]. After amplification the 435-b.p. fragment was cleaved by Sph I restrictase (Sibenzim) into 2 fragments (247 and 188 b. p.) in carriers of MMP9 (-1562T) allele carriers.

The results were statistically processed using STATISTICA 5.0 software. The genotype association with COPD was evaluated by the valua of relative risk (OR) [4].

RESULTS

The GSTM1 (0/0) genotype was significantly more incident in COPD patients than in control tobacco smokers (χ^2=3.90, p=0.045; Table 1). The presence of GSTM1 (0/0) genotype increased the risk of COPD 2.5 times. No significant differences between the distribution of GSTM1 genotypes in COPD and CNB patients and in CNB patients and control tobacco smokers were detected (χ^2=1.26, p=0.27 and χ^2=0.57, p=0.45). None of the examinee carried homozygous MMP9 (-1562T) mutation. The studied groups virtually did not differ by the distribution of MMP9 genotypes (Table 1).

All examinees were divided into 4 groups depending on the genotype combination: with two mutant genotypes (GSTM1 (0/0/MMP9 (CT), with GSTM1 homozygotic by gene deletion and normal MMP9 (GSTM1 (0/0)/MMP9 (CC), with normal/heterozygotic by GSTM1 deletion and heterozygotic by MMP9 gene mutation (GSTM1(1/-)/MMP9 (CT), and with two normal genotypes (GSTM1 (1/-)/MMP9 (CC); Table 2).

The incidence of carriers of 2 mutant genotypes among patients with COPD was significantly higher than in healthy tobacco smokers (χ^2=3.63, p=0.051); a similar tendency was observed in the group of patients with CNB (χ^2=3.50, p=0.055). Combination of 2 normal genotypes in COPD patients was significantly more rare than in the control group (43 and 62% respectively, χ^2=4.65, p=0.031). The risk of COPD in tobacco smokers with 2 mutant genotypes was almost 8-fold higher than in those with 2 normal genotypes, while the risk of COPD in subjects with a combination of 2 normal genotypes was lower.

These results attest to an important role of associative effect of detoxification and proteolytic enzymes in the development of COPD in tobacco smokers. GSTM1 0/0 genotype is relevant to the formation of COPD in tobacco smokers and almost 3-fold increases the risk of this pathology. The effect of MMP9 factor (CT) is weaker, and OR does not reach the level of statistical significance, but is potentiating for the carriers of GSTM1 0/0, i.e. the risk of COPD in tobacco smokers with GSTM1 (0/0)/MMP9 (CT) genotype combination increases 7.7 times in comparison with carriers of 2 normal genotypes.

Hence, a relationship between polymorphism of xenobiotic transformation enzyme GSTM1 gene and predisposition to COPD was demonstrated and it was

TABLE 1. Distribution of GSTM1 and MMP9 Genotypes in Patients with COPD, CNB, and Controls

Genotype		Group, *n*			OR; CI	
		COPD	CNB	control	COPD/control	*p*
GSTM1	0/0	28; 38.89%	12; 31.58%	8; 20.51%	2.47 (1.10-5.54)	0.045
	1/-	44; 61.11%	26; 68.42%	31; 79.49%		
MMP9	CT	23; 31.94%	9; 23.68%	8; 20.51 %	1.82 (0.68-4.90)	0.200
	CC	49; 68.06%	29; 76.32	31; 79.49%		

Note. Here and in Table 2: 95% confidence interval (C1) is shown in parentheses.

TABLE 2. Distribution of Combinations of Studied Allele Variants of GSTM1 and MMP9 Genes in Patients with COPD and CNB and Controls

Combined genotype		Group, *n*			OR; C1	
GSTM1	MMP9	COPD	CNB	control	COPD/control	*p*
0/0	CT	10; 13.89%	1; 2.63%	1; 2.56%	6.13 (0.81-46.64)	0.056
					7.74 (1.12-53.34)*	0.029*
0/0	CC	18; 25.00%	11; 28.95%	7; 17.95%	1.52 (0.32-7.31)	0.396
1/—	CT	13; 18.06%	8; 21.05%	7; 17.95%	1.01 (0.99-1.03)	0.989
1/—	CC	31; 43.05%	18; 47.37%	24; 61.54%	0.47 (0.96-0.23)	0.053

Note. *Only carriers of 2 mutant and 2 normal genotypes were compared.

shown for the first time that the combination of homozygotic genotype by GSTM1 gene deletion and mutant allele MMP9 (-1562) is a factor of COPD risk.

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