

Letters

Genetic variants of vitamin D receptor and antioxidant enzyme genes in bronchial asthma

Epistatic interactions

Although the environmental factors that trigger and exacerbate bronchial asthma (BA) are well known, it is not possible to define a clear genetic pattern of BA. Because genome-wide association studies assume only the independent influence of single genetic variants on a certain phenotype, taking into consideration their additive effects, they can explain only a part of the genetic information.¹ Thus, the detection of epistatic interactions was introduced as an important approach to reveal the genetic background of complex diseases by finding interactions among 2 or more loci and their effect on phenotype in addition to evaluating their nonadditive effects (synergistic or antagonistic).²

Increased oxidative stress, involved in the BA pathogenesis, augments inflammation and stimulates bronchial hyperreactivity, mucus production, and bronchospasm.³ It is caused by the decreased activity of antioxidant enzymes (eg, mitochondrial superoxide dismutase [SOD2] and catalase), which may also be influenced by the single nucleotide polymorphisms (SNPs).⁴ In addition, vitamin D may control the activity of these enzymes through its receptor (vitamin D receptor [VDR]).⁵

In this study, we aimed to elucidate the epistatic interactions between the genetic variants of VDR (FokI, BsmI, ApaI, and TaqI) and genes of antioxidant enzymes (catalase, CAT-21 A/T and CAT-262 C/T; and SOD2 Ala16Val) in Serbian patients with BA.

Single nucleotide polymorphisms were determined using the polymerase chain reaction–restriction fragment length polymorphism method^{6,7} in 79 patients with BA (men/women, 32/47; mean age, 47.48 ± 15.81 years) recruited from the Clinic for Pulmonary Diseases and Tuberculosis, University of Niš, Niš, Serbia; and 85 unrelated healthy patients (men/women, 41/44; mean age, 46 ± 16.59 years) with no personal and family history of BA or any chronic inflammatory or autoimmune disease.

The multifactor dimensionality reduction method⁸ was used to exhaustively evaluate all possible 2- to 5-loci interactions among the studied genetic variants. A 10-fold cross-validation was performed to estimate training and test accuracies. The models reaching cross-validation consistency of 9 of 10 or 10 of 10 and the highest test accuracy were selected as the best models.⁸ For the best-selected models, low- and high-risk genotype combinations were determined using the threshold value of 1.0. The information gain was used to evaluate the relationship among studied genetic variants and to create an interaction graph. The statistical significance of the model was evaluated using 1000-fold permutation testing.⁸

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Analysis of every single genetic variant indicated a significant difference in the distribution of SOD2 and VDR FokI genotypes ($P < .001$ and $P = .006$, respectively) and alleles ($P < .001$ and $P = .002$, respectively) in BA compared with controls (eTable 1). Haplotype analysis revealed no linkage disequilibrium (LD) among the studied VDR variants (eTable 1).

Among all predicted models of epistatic interactions, 2- (SOD2/FokI) and 5-loci (CAT-21/CAT-262/SOD2/ApaI/TaqI) models were selected as the best ones (eTable 1). However, the 5-loci model was excluded from further evaluation because of the substantial discrepancy between training and test accuracy values (eTable 1), indicating overfitting that occurs when the model fits too closely with the data used for training but fails to generalize to the previously unseen data.

The genotypes containing SOD2 Val allele in combination with CT or TT FokI genotypes are indicated as high-risk genotypes for BA, whereas genotype combinations containing AlaAla and CC or CT in addition to AlaVal and CC are indicated as low-risk genotypes for BA (Fig 1A). Further analysis indicated that carriers of Val/C combination of alleles had 2.2-fold higher risk for BA in comparison with Ala/C carriers ($P = .004$; odds ratio = 2.231; 95% CI = 1.283–3.381), whereas Val/T carriers have 3.7-fold higher risk in comparison with Ala/C carriers ($P < .001$; odds ratio = 3.706; 95% CI = 1.913–7.181).

Additional evaluation of relationships among the studied SNPs based on information gain found positive information gain between ApaI and catalase genetic variants indicating a synergistic effect of the interactions, whereas the negative values between SOD2 and VDR genetic variants indicate redundancy (Fig 1B). Moreover, a weak synergistic effect was obtained between CAT-21 and CAT-262, which is not influenced by LD ($D' = 0.309$, $r^2 = 0.018$), and between CAT-262 and TaqI or BsmI (2.18% and 1.57%, respectively) (Fig 1B).

These results are supported by previous research that found a decreased SOD2 activity in Val allele carriers and a lower affinity for VDR protein synthesized in the presence of the FokI T allele.^{4,9,10} Decreased activity of SOD2 in the presence of rs4880 Val allele is a consequence of a protein structural change. The β -sheet structure, which is formed in the presence of the Val allele, instead of the α -helix formed in the presence of Ala allele, slows down the translocation of the protein through the mitochondrial membrane, retaining it within the TIM23 inner membrane import complex, and leading to its partial degradation in the proteasome with a consequently lower activity of the enzyme.^{4,9} Furthermore, VDR protein synthesized in the presence of the CC genotype indicates increased transcriptional activity and higher affinity for vitamin D binding in comparison with the protein synthesized in the presence of the TT genotype.¹⁰ Moreover, vitamin D, acting through its receptor, may

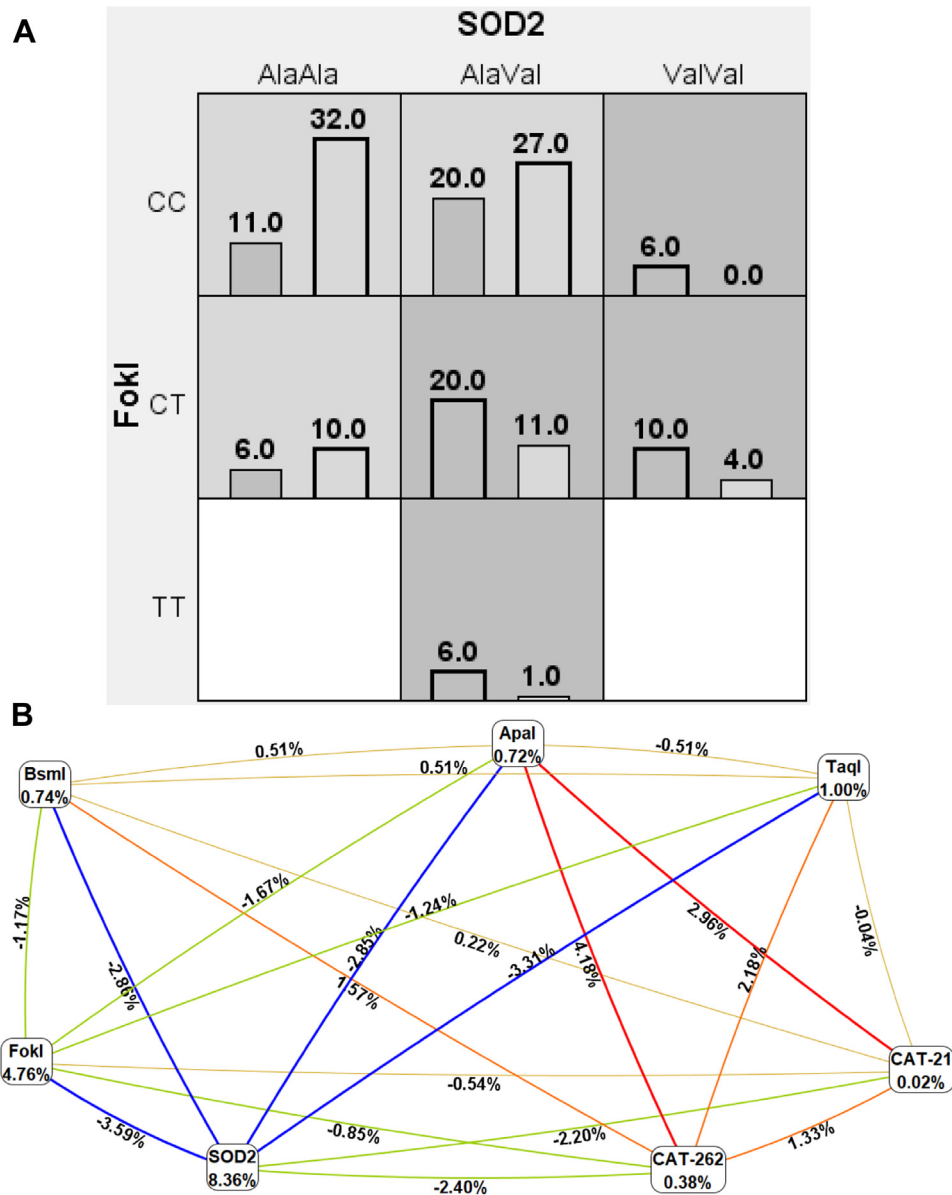


Figure 1. Distribution of high- and low-risk genotype combinations in the 2-locus interaction model (A) and interaction graph for all studied genetic variants (B). In the 2-locus interaction model, dark gray cells refer to high-risk, whereas light gray cells refer to low-risk; white cells indicate that genotype combination has not been detected. The left bar on each cell refer to the number of patients with BA, whereas the right bar refers to the number of controls. In the interaction graph for all studied genetic variants, the percentage below each SNP refers to the information gain of the single SNP; the percentages on the line are the information gain of the interaction between 2 SNPs. Type of interactions include the following: red indicates strong, orange indicates mild, green indicates weak, blue indicates no interaction or redundancy, and brown indicates independence. BA, bronchial asthma; SNP, single nucleotide polymorphism.

control the activity of antioxidant enzymes, including SOD2, by regulating the expression and transcriptional activity of Nrf2 and Klotho.⁵ This further suggests that low vitamin D levels and the presence of T allele might be associated with increased oxidative stress because of the altered SOD2 activity in BA. Nevertheless, further studies are needed to elucidate this potential mechanism in BA pathogenesis.

The limitation of this study is a relatively small sample size, which might be one of the reasons for the overfitting of the 5-loci model. In addition, information gain indicated a potential synergistic effect between catalase (CAT-21 and CAT-262) and VDR Apal genetic variants. Apal is located in the intron 8 of VDR noncoding region, and

thus, does not influence VDR expression; however, its role remains unclear. Although it is usually examined in the LD studies together with BsmI and TaqI, our study did not reveal significant differences ($P > .05$) in the BsmI/Apal/TaqI haplotype distribution between patients and controls. Therefore, further functional studies are needed to explain the potential independent role of Apal in VDR signaling and its interaction with catalase genetic variants.

To the best of our knowledge, this is the first study that evaluated the epistatic interactions between genetic variants in VDR and antioxidant enzyme genes, suggesting an increased risk for BA in the carriers of the Val/T allele combination of SOD2 Ala16Val and VDR FokI genetic variants.

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Supplementary Data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jana.2020.07.020>.

Supplementary Data

eTable 1

The Distributions of Genotypes, Alleles and Haplotypes, and Epistatic Interaction Models in BA and Control Groups

Gene	Single nucleotide polymorphism (rs number)	Sample	Genotype n (%)				Allele n (%)				
Catalase	CAT A-21T (rs7943316)	BA	AA 5 (6.3)	AT 38 (48.1)	TT 36 (45.6)	<i>P</i> .98	A 48 (30.4)	T 110 (69.6)	<i>P</i> .97	Odds ratio (95%CI) 1.01 (0.631-1.617)	
		Control	6 (7.1)	40 (47.1)	39 (45.9)		52 (30.6)	118 (69.4)			
	CAT C-262T (rs1001179)	BA	CC 36 (45.6)	CT 38 (48.1)	TT 5 (6.3)	<i>P</i> .65	C 108 (69.2)	T 48 (30.8)	<i>P</i> .70	1.098 (0.682-1.765)	
		Control	43 (50.6)	35 (41.2)	7 (8.2)		121 (71.2)	49 (28.8)			
	SOD2	Ala16Val (rs4880)	BA	AlaAla 17 (21.5)	AlaVal 46 (58.2)	ValVal 16 (20.3)	< .001 ^b	Ala 80 (50.6)	Val 78 (49.4)	< .001 ^b	2.552 (1.613-4.036)
	Control	42 (49.4)	39 (45.9)	4 (4.7)	123 (72.4)	47 (27.6)					
VDR	FokI (rs2228570)	BA	CC 37 (46.8)	CT 36 (45.6)	TT 6 (7.6)	.006 ^b	C 110 (69.6)	T 48 (30.4)	.002 ^b	2.311 (1.356-3.938)	
		Control	59 (69.4)	25 (29.4)	1 (1.2)		143 (84.1)	27 (15.9)			
	BsmI (rs1544410)	BA	GG 28 (35.4)	AG 40 (50.6)	AA 11 (13.9)	.44	G 96 (60.8)	A 62 (39.2)	.50	0.858 (0.552-1.333)	
		Control	30 (35.3)	37 (43.5)	18 (21.2)		97 (57.1)	73 (42.9)			
	ApaI (rs7975232)	BA	CC 22 (27.8)	AC 41 (51.9)	AA 16 (20.3)	.45	C 85 (53.8)	A 73 (46.2)	.21	1.320 (0.851-2.047)	
		Control	31 (36.5)	41 (48.2)	13 (15.3)		103 (60.6)	67 (39.4)			
	TaqI (rs731236)	BA	CC 35 (44.3)	CT 34 (43.0)	TT 10 (12.7)	.19	C 104 (65.8)	T 54 (34.2)	.20	1.359 (0.849-2.174)	
		Control	42 (49.4)	39 (45.9)	4 (4.7)		123 (72.4)	47 (27.6)			
	BsmI/ApaI/TaqI	Haplotype frequency	GCC	AAT	GAC	AAC	GAT	ACC			
			BA	0.438	0.334	0.162	0.041	0.001	0.017		
			Control	0.37	0.375	0.158	0.04	0.032	0.014		
			χ ²	1.593	0.598	0.008	0.002	4.844	0.042		
			<i>P</i>	.21	.44	.93	.96	.03	.84		
			<i>P</i> ^a	0.68	0.95	> .99	> .99	0.11	> .99		
The best models of epistatic interaction analysis											
Model					Number of loci	Cross-validation consistency	Training accuracy	Test accuracy	<i>P</i> ^a		
SOD2/FokI (rs4880/rs2228570)					2	10/10	0.6717	0.6717	.01 ^b		
CAT-21/CAT-262/SOD2/ApaI/TaqI (rs7943316/rs1001179/rs4880/rs7975232/rs731236)					5	10/10	0.8423	0.6481	.01 ^b		

Abbreviations: BA, bronchial asthma; CI, confidence interval; n, number of subjects; SOD2, superoxide dismutase 2; VDR, vitamin D receptor.

^a*P* value based on permutation test.^b*P* ≤ .05 is considered statistically significant.