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Gene Polymorphisms of Tumor Necrosis Factor Alpha and Antioxidant Enzymes in Bronchial Asthma*

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Bronchial asthma is an inflammatory disease resulting from a combination of genetic and environmental factors. Single nucleotide polymorphisms in the regulatory regions of cytokine and antioxidant enzyme genes may affect cytokine production and enzyme activity, and thus play a contributory role in asthma pathogenesis.

Objectives. The aim of this study was to examine the association of manganese superoxide dismutase (MnSOD) Ala16Val, catalase (CAT) A-21T and tumor necrosis factor alpha (TNF-α) G-308A polymorphisms with bronchial asthma.

Material and Methods. A total of 79 patients with asthma and 95 healthy controls were screened for MnSOD Ala16Val, CAT A-21T and TNF-α G-308A polymorphisms using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Results. The results obtained showed significantly higher prevalence of the MnSOD ValVal genotype ($\chi^2 = 14.463$, df = 2, p = 0.001) and MnSOD 16Val allele ($\chi^2 = 12.862$, p = 0.026, OR = 0.451, 95% CI = 0.291–0.699) in patients with asthma compared to controls. The genotype and allele frequencies distribution of CAT A-21T and TNF-α G-308A gene polymorphisms did not show differences between patients and controls.

Conclusions. Our results show an association of MnSOD Ala16Val genetic polymorphism with asthma in a Serbian population and suggest a protective role of the MnSOD 16Ala allele (**Adv Clin Exp Med 2015, 24, 2, 251–256**).

Key words: bronchial asthma, gene polymorphism, tumor necrosis factor alpha, catalase, manganese superoxide dismutase.

Reactive oxygen species (ROS), superoxide ($O_2^{\cdot}-$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (·OH) are considered to have a role in asthma pathogenesis. The lungs function in a high-oxygen environment together with its large surface area and blood supply are susceptible to injury mediated by ROS [1]. Various inflammatory and structural cells in the airways have been reported to generate increased amounts of ROS [2]. The lungs have developed several endogenous antioxidant systems to deal with the production of free radicals [3]. Superoxide dismutase (SOD) catalyses the dismutation of $O_2^{\cdot}-$ to H_2O_2 and oxygen (O_2), while catalase (CAT) decomposes H_2O_2 to water and oxygen.

Single nucleotide polymorphisms (SNPs) of genes coding the antioxidant enzymes may alter enzyme structure, substrate specificity or activity and thus modify inter-individual variability in the defense capacity against oxidative stress [4]. The most extensively studied rs4880 (MnSOD Ala16Val) polymorphism of the manganese superoxide

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dismutase (MnSOD) gene is located at amino acid position 16 in the mitochondrial targeting sequence (MTS) resulting in the replacement of an alanine with a valine (Ala16Val). This polymorphism has been suggested to alter the conformation of the leader signal and thereby affect the import of MnSOD into the mitochondria [5]. Catalase is a homotetramer, encoded by a gene consisting of 13 exons separated from each other by 12 introns and located on 11p13 chromosome [6]. Polymorphisms A-21T, C-262T and C-844T, were identified in the 5'-untranslated region of the CAT gene [7]. The CAT A-21T (rs7943316) polymorphism, located inside the promoter region proximal to the start site, is less studied, especially in bronchial asthma [8]. Asthma occurrence is dependent on the relationship between antioxidant and inflammatory genes [9]. Higher levels of oxidative stress overwhelm antioxidant defenses and lead to the induction of many pro-inflammatory factors including tumor necrosis factor α (TNF-α) [10].

Tumor necrosis factor α is a multifunctional, proinflammatory cytokine which is produced in response to inflammation, infection and injury by mast cells, macrophages and other cell types (eosinophils, epithelial cells and neutrophils) implicated in asthma pathogenesis. In response to TNF-α, airway epithelial cells produce greater amount of intracellular ROS [1]. Single nucleotide polymorphism at the position G-308A (rs1800629) is among the most investigated in the promoter region of TNF-α. The presence of the TNF -308A allele is considered to be associated with higher TNF gene transcription and TNF-α overproduction and may amplify the intensity of the inflammatory response to oxidants [9, 11].

To the best knowledge of the authors, there were no reported attempts in the literature to investigate the mutual influence of TNF-α G-308A, CAT A-21T and MnSOD Ala16Val polymorphisms in asthma, as well as their individual associations with bronchial asthma in a Serbian population. Analysis of the mutual influence of TNF-α G-308A, CAT A-21T and MnSOD Ala16Val polymorphisms was performed in this paper in order to demonstrate the genetic influences that were not detected by the analysis of the SNPs.

Material and Methods

Subjects and Samples Collection

Seventy-nine patients with bronchial asthma (male/female: 32/47; mean age 47.48 ± 15.81) were involved in this study. Bronchial asthma was diagnosed according to the guidelines of the Global Initiative for Asthma (GINA). No patient had evidence of chronic obstructive pulmonary disease. Ninety-five unrelated, healthy subjects (male/female: 46/49; mean age 44.81 ± 16.78), with no previous history of asthma, atopy or acute and chronic inflammatory diseases were involved in the study as a control group. Informed consent was obtained from all participants. The study was approved by the Ethical Committee of the Faculty of Medicine, University of Nis, Serbia.

Blood samples were obtained from the median cubital vein and collected into EDTA vacutainer tubes. Two-hundred microliters of blood were used for DNA isolation.

DNA Analysis

Genomic DNA was isolated from the whole blood samples using a QIAamp DNA Blood Mini Kit (Quiagen GmbH, Hilden, Germany). The polymorphisms were determined using the polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) technique. Polymerase chain reaction was performed in a final volume of 25 μL containing 20 ng of DNA, 12.5 μL KAPA2G Fast HotStart ReadyMix (Kapa Biosystems, Inc, USA) and 20 pmol of each primer. The primer sequences used in this study are summarized in Table 1.

Table 1. Primer sequences, restriction enzymes and size of fragments generated by TNF-α G-308A, CAT A-21T, and MnSOD Ala16Val gene polymorphisms

Polymorphism	Primer sequence	Restriction enzyme	Allele determination
TNF-α G-308A	F 5'-AGGCAATAGGTTTTGAGGGCCAT-3'	NcoI	G 97 + 20 bp
	R 5'-ACACTCCCCATCCTCCCTGCT-3'		A 117 bp
CAT A-21T	F 5'-AATCAGAAGGCAGTCCTCCC-3'	HinfI	A 177 + 73 bp
	R 5'-TCGGGGAGCACAGAGTGTAC-3'		T 250 bp
MnSOD Ala16Val	F 5'-CCAGCAGGCAGCTGGCACCG -3'	BshTI(AgeI)	Ala 74 + 17 bp
	R 5'-TCCAGGGCGCCGTAGTCGTAGG -3'		Val 91 bp

The PCR conditions were as follows: initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 15 s, annealing at 60°C (TNF-α and CAT) or 66°C (MnSOD) for 15 s, and extension at 72°C for 15 s, ending with a final extension at 72°C for 1 min. The PCR products were identified by 2% agarose gel electrophoresis. Gels were stained with ethidium bromide and visualized under UV light. The amplification products were digested at 37°C overnight using NcoI, HinfI or AgeI restriction enzymes (Fermentas GmbH, St. Leon-Rot, Germany) and analyzed by 8% polyacrylamide gel electrophoresis (PAGE). The interpretation of the obtained results was performed according to Table 1.

Genotype analyses were performed by two independent researchers. After the polymorphic alleles were established to be homozygous, the PCR-RFLP was repeated in order to confirm the obtained results.

Statistical Analysis

Differences in genotype and allele frequencies between patients and controls were tested using a chi-square (χ^2) test or two-tailed Fisher's test when the number of expected cases was small. The differences were considered significant at $p < 0.05$. Genetic risks were assessed by calculating odds ratios (OR) with 95% confidence intervals (95% CI). Bonferroni corrections were used for multiple comparisons. Statistical analysis was performed using the SPSS version 13.0 statistical software package (SPSS Inc, Chicago, IL, USA).

Table 2. Demographic characteristics of the study subjects

	Asthma (n = 79)	**Control (n = 95)**
Gender male female	 32 (40.51%) 47 (59.49%)	 46 (48.42%) 49 (51.58%)
Age at entry to the study (years)*	47.48 ± 15.81	44.81 ± 16.78

* Data is presented as mean ± standard deviation; n – number of study subjects.

Results

Seventy-nine patients with asthma and 95 unrelated individuals were involved in the study. The demographic characteristics of the study population are summarized in Table 2.

Genotype frequencies for the SNPs in the study groups were in Hardy–Weinberg equilibrium ($p > 0.05$). The distributions of MnSOD Ala-16Val, CAT A-21T and TNF-α G-308A genotypes in patients with asthma and controls are shown in Table 3.

The obtained results showed a significantly higher prevalence of the MnSOD ValVal genotype in patients with asthma ($\chi^2 = 14.463$, df = 2, $p = 0.001$). Moreover, the MnSOD 16Val allele was more frequent in patients compared to controls ($p < 0.001$, Table 4).

The genotype distribution of the CAT A-21T gene polymorphism, as well as frequencies of CAT -21A and CAT-21T alleles did not show significant differences between patients and controls ($p > 0.05$).

As the TNF-α-308 AA genotype was present in only a small number of subjects (only one in the control group) it was analyzed together with subjects who were heterozygous for the TNF-α G-308A polymorphism. The observed genotype

Table 3. Distribution of MnSOD Ala16Val, CAT A-21T and TNF-α G-308A genotypes in patients with asthma and controls

Gene polymorphism	**Genotype**	**Asthma n = 79 (%)**	**Control n = 95 (%)**
MnSOD Ala16Val	AlaAla	17 (21.5)	44 (46.3)
	AlaVal	46 (58.2)	44 (46.3)
	ValVal	16 (20.3)	7 (7.4)
CAT A-21T	AA	5 (6.3)	6 (6.3)
	AT	38 (48.1)	44 (46.3)
	TT	36 (45.6)	45 (47.4)
TNF-α G-308A	GG	57 (72.2)	70 (73.7)
	GA	22 (27.8)	24 (25.3)
	AA	0	1 (1.0)

Table 4. Allele frequencies of MnSOD Ala16Val, CAT A-21T and TNF-α G-308A polymorphisms in patients with asthma and controls

Gene polymorphism	Allele	Asthma	Control	p-value	χ^2	OR	95% CI
		n (%)	n (%)				
MnSOD Ala16Val	Ala	80 (50.6)	132 (69.5)	< 0.001	12.862	0.451	0.291–0.699
	Val	78 (49.4)	58 (30.5)				
CAT A-21T	A	48 (30.4)	56 (29.5)	0.854	0.034	1.044	0.659–1.655
	T	110 (69.6)	134 (70.5)				
TNF-α G-308A	G	136 (86.1)	164 (86.3)	0.948	0.004	0.98	0.532–1.807
	A	22 (13.9)	26 (13.7)				

n – number of study subjects; OR – odds ratio; CI – confidence interval.

Table 5. Frequencies of simultaneous carriership of MnSOD 16Val, CAT-21T and TNF-α-308A alleles in patients with asthma and controls

TNF-α-308A	CAT-21T	MnSOD 16Val	Asthma n (%)	Control n (%)
+	+	+	13 (16.5)	13 (13.7)
+	+	–	8 (10.1)	9 (9.5)
+	–	+	0	1 (1.1)
+	–	–	1 (1.3)	2 (2.1)
–	+	+	45 (57)	38 (40)
–	+	–	8 (10.1)	29 (30.5)
–	–	+	4 (5.1)	1 (1.1)
–	–	–	0	2 (2.1)

"+" – carrier of the certain allele, "–" – non-carrier of the certain allele, n – number of study subjects.

distribution of the TNF-α G-308A polymorphism in patients with asthma did not show significant differences compared to controls ($\chi^2 = 0.051$, df = 1, p = 0.821, OR = 0.925, 95% CI = 0.473–1.811). Furthermore, no differences in the distribution of TNF-308G and TNF-308A alleles were observed between patients and controls (Table 3).

In order to investigate possible associations between specific alleles of MnSOD, CAT and TNF-α genes, individuals were classified depending on whether they were carriers (+) or non-carriers (–) of the less common allele of each polymorphic gene. The simultaneous carrying of CAT-21T and MnSOD 16Val alleles was present in 57% of asthmatics vs 40% of controls, while 30.5% of controls vs 10.1% of asthmatics were carriers of the CAT-21T allele only (Table 5). The presence of polymorphic CAT-21T and MnSOD 16Val, in the absence of the TNF-308A allele, is significantly higher in asthma patients than in controls ($\chi^2 = 4.974$, df = 1, p = 0.026, OR = 0.504, 95% CI = 0.275–0.923). However, after using a Bonferroni adjusted p-value of 0.0083 in order to account for multiple testing, this association does not remain significant anymore.

Discussion

Oxidative stress is caused by increased production of ROS and ineffective antioxidant defense. Genes involved in the regulation of antioxidant defense mechanisms are important for the cells' response to the increased amount of ROS. Since the lungs are highly exposed to oxygen, changes in antioxidant defense could be one of the important mechanisms in asthma pathogenesis.

This study examines for the first time TNF-α G-308A, CAT A-21T and MnSOD Ala16Val polymorphisms in Serbian patients with asthma. It shows an association of the MnSOD Ala16Val polymorphism with bronchial asthma in a Serbian population.

Superoxide is primarily a produced ROS and its dismutation is an important antioxidant defense mechanism in the ROS production cascade [12, 13]. The distribution of the MnSOD 16Ala allele varies from 11–30% in Japanese and Chinese populations to 41–62% in Caucasians [5]. Our results show that the distribution of the MnSOD 16Ala allele is 69.5% in a Serbian population. Some of the previous studies did not confirm the assumption that the MnSOD Ala16Val polymorphism is a predisposing factor for bronchial asthma [14–17]. Nevertheless, the possibility that, together with other defects in the antioxidant defense system, SOD genes may become significant in inflammatory airway diseases associated with oxidant stress, is not excluded. Our results show significant association of MnSOD Ala16Val with asthma in a Serbian population. In asthma patients, minor MnSOD 16Val allele is more frequent than in healthy controls. Calculated odds ratios suggest the protective effect of the MnSOD 16Ala variant in a Serbian population. Conformational changes in the protein structure, due to Val to Ala amino acid change at position 16 in the MTS of MnSOD in asthma patients, may result in a harder import of MnSOD through the inner mitochondrial membrane and subsequently decreased formation of the active form inside the mitochondrial matrix [18, 19].

Besides the SOD, CAT represents an important part of the enzymatic antioxidant system in the lungs. Only a few studies examining the association of CAT A-21T polymorphisms with asthma are available in the literature. Polonikov et al. reported a significant association of this polymorphism in the Russian population, suggesting that carriers of the polymorphic genotype are more susceptible to asthma development due to increased oxidative stress resulting from insufficient CAT activity [7]. However, in the current study, we did not find significant differences in the genotype and allele frequencies of CAT A-21T polymorphism in patients with asthma and controls.

In a white population, the TNF-α G-308A polymorphism is predominantly represented by the GG genotype, while the AA genotype appears in up to 3% of the population [20]. Available studies show contradictory results related to the association of the TNF-α G-308A polymorphism with bronchial asthma. Studies [21–25] confirm that the TNF-α G-308A polymorphism is associated with bronchial asthma and bronchial hyperreactivity. Our study failed to prove the association of TNF-α G-308A genotypes with asthma, which is in accordance with results obtained by Louis et al. and Buckova et al. [26, 27].

However, there are no available studies in the literature examining the mutual influence of TNF-α G-308A, CAT A-21T and MnSOD Ala16Val polymorphisms in asthma. Our results show that the polymorphic CAT-21T and MnSOD 16Val alleles, in the absence of the TNF-308A allele, are presented in 57% of asthmatics compared to 40% of healthy controls. However, this association does not reach statistically significant levels. The presence of the polymorphic TNF-308A allele is considered to be associated with increased TNF-α production [28]. Cell stimulation by TNF-α maintains increased ROS levels and increases MnSOD activity, while the presence of the MnSOD 16Val allele is associated with decreased MnSOD activity [29, 30]. In relation to these findings, in the absence of TNF-α-308A allele, the stimulatory effect of TNF-α on MnSOD activity in asthmatics could be intermitted and, together with the concurrent presence of polymorphic MnSOD 16Val and CAT-21T alleles, could lead to increased oxidative stress due to a lower defense capacity against ROS. Nevertheless, further studies are needed to explain the specific mechanisms of this relationship, as well as to explore the specific gene-gene and gene-environment interactions in their relationship and possible interindividual differences.

In conclusion, our results show an association of the MnSOD Ala16Val polymorphism with asthma in a Serbian population and suggest a protective role of the MnSOD 16Ala allele. Biallelic polymorphisms CAT A-21T and TNF-α G-308A are not associated with bronchial asthma.

References

[1] **Rahman I:** Oxidative stress, chromatin remodeling and gene transcription in inflammation and chronic lung diseases. J Biochem Mol Biol 2003, 36, 95–109.

[2] **Fujisawa T:** Role of oxygen radicals on bronchial asthma. Curr Drug Targets Inflamm Allergy 2005, 4, 505–509.

[3] **Mak JC, Ho SP, Yu WC, Choo KL, Chu CM, Yew WW, Lam WK, Chan-Yeung M:** Polymorphisms and functional activity in superoxide dismutase and catalase genes in smokers with COPD. Eur Respir J 2007, 30, 684–690.

[4] **Bohanec Grabar P, Logar D, Tomsic M, Rozman B, Dolzan V:** Genetic polymorphisms modifying oxidative stress are associated with disease activity in rheumatoid arthritis patients. Dis Markers 2009, 26, 41–48.

[5] **Mak JC, Leung HC, Ho SP, Ko FW, Cheung AH, Ip MS, Chan-Yeung MM:** Polymorphisms in manganese superoxide dismutase and catalase genes: functional study in Hong Kong Chinese asthma patients. Clin Exp Allergy 2006, 36, 440–447.

[6] **Galecki P, Szemraj J, Zboralski K, Florkowski A, Lewiński A:** Relation between functional polymorphism of catalase gene (–262C>T) and recurrent depressive disorder. Neuro Endocrinol Lett 2009, 30, 357–362.

[7] **Polonikov AV, Ivanov VP, Solodilova MA, Kozhuhov MA, Panfilov VI:** Tobacco Smoking, Fruit and Vegetable Intake Modify Association Between -21A>T Polymorphism of Catalase Gene and Risk of Bronchial Asthma. J Asthma 2009, 46, 217–224.
[8] **Nadif R, Mintz M, Jedlicka A, Bertrand JP, Kleeberger SR, Kauffmann F:** Association of CAT polymorphisms with catalase activity and exposure to environmental oxidative stimuli. Free Radic Res 2005, 39, 1345–1350.
[9] **Li YF, Gauderman WJ, Avol E, Dubeau L, Gilliland FD:** Associations of tumor necrosis factor G-308A with childhood asthma and wheezing. Am J Respir Crit Care Med 2006, 173, 970–976.
[10] **Dozor AJ:** The role of oxidative stress in the pathogenesis and treatment of asthma. Ann NY Acad Sci 2010, 1203, 133–137.
[11] **Abraham LJ, Kroeger KM:** Impact of the -308 TNF promoter polymorphism on the transcriptional regulation of the *TNF* gene: relevance to disease. J Leuk Biol 1999, 66, 562–566.
[12] **Sahiner U, Birben E, Erzurum S, Sackesen C, Kalayci O:** Oxidative stress in asthma. World Allergy Organ J 2011, 4, 151–158.
[13] **Yucesoy B, Johnson VJ, Lummus ZL, Kissling GE, Fluharty K, Gautrin D, Malo JL, Cartier A, Boulet LP, Sastre J, Quirce S, Germolec DR, Tarlo SM, Cruz MJ, Munoz X, Luster MI, Bernstein DI:** Genetic variants in antioxidant genes are associated with diisocyanate-induced asthma. Toxicol Sci 2012, 129, 166–173.
[14] **Gurel A, Tomac N, Yilmaz HR, Tekedereli I, Akyol O, Armutcu F, Yuce H, Akin H, Ozcelik N, Elyas H:** The Ala-9Val polymorphism in the mitochondrial targeting sequence (MTS) of the manganese superoxide dismutase gene is not associated with juvenile-onset asthma. Clin Biochem 2004, 37, 1117–1120.
[15] **Holla LI, Kankova K, Vasku A:** Functional polymorphism in the manganese superoxide dismutase (MnSOD) gene in patients with asthma. Clin Biochem 2006, 39, 299–302.
[16] **Yang LL, Huang MS, Huang CC, Wang TH, Lin MC, Wu CC, Wang CC, Lu SH, Yuan TY, Liao YH, Ko YC, Wang TN:** The association between adult asthma and superoxide dismutase and catalase gene activity. Int Arch Allergy Immunol 2011, 156, 373–380.
[17] **Kinnula VL, Lehtonen S, Koistinen P, Kakko S, Savolainen M, Kere J, Ollikainen V, Laitinen T:** Two functional variants of the superoxide dismutase genes in Finnish families with asthma. Thorax 2004, 59, 116–119.
[18] **Isbir S, Ergen A, Yilmaz H, Tekeli A, Arsan S:** Effect of Ala16Val genetic polymorphism of MnSOD on antioxidant capacity and inflammatory response in open heart surgery. *In Vivo* 2008, 22, 147–151.
[19] **Sutton A, Khoury H, Prip-Buus C, Cepanec C, Pessayre D, Degoul F:** The Ala16Val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. Pharmacogenetics 2003, 13, 145–157.
[20] **Jevtovic-Stoimenov T, Kocic G, Pavlovic D, Macukanovic-Golubovic L, Marjanovic G, Djordjevic V, Tosić N, Pavlović S:** Polymorphisms of tumor-necrosis factor-alpha -308 and lymphotoxin-alpha +250: possible modulation of susceptibility to apoptosis in chronic lymphocytic leukemia and non-Hodgkin lymphoma mononuclear cells. Leuk Lymphoma 2008, 49, 2163–2169.
[21] **Moffatt MF, Cookson WO:** Tumour necrosis factor haplotypes and asthma. Hum Mol Genet 1997, 6, 551–554.
[22] **Chagani T, Paré PD, Zhu S, Weir TD, Bai TR, Behbehani NA, Fitzgerald JM, Sandford AJ:** Prevalence of tumor necrosis factor-α and angiotensin converting enzyme polymorphisms in mild⁄moderate and fatal⁄near-fatal asthma. Am J Respir Crit Care Med 1999, 160, 278–282.
[23] **Witte JS, Palmer LJ, O'Connor RD, Hopkins PJ, Hall JM:** Relation between tumour necrosis factor polymorphism TNF alpha-308 and risk of asthma. Eur J Hum Genet 2002, 10, 82–85.
[24] **Kumar A, Gupta V, Changotra H, Sarin BC, Sehajpal PK:** Tumor necrosis factor – α and transforming growth factor – β1 polymorphisms in bronchial asthma. Indian J Med Sci 2008, 62, 323–330.
[25] **Li Kam Wa TC, Mansur AH, Britton J, Williams G, Pavord I, Richards K, Campbell DA, Morton N, Holgate ST, Morrison JF:** Association between TNF-α-308 tumour necrosis factor promoter polymorphism and bronchial hyperreactivity in asthma. Clin Exp Allergy 1999, 29, 1204–1208.
[26] **Louis R, Leyder E, Malaise M, Bartsch P, Louis E:** Lack of association between adult asthma and the tumour necrosis factor alpha-308 polymorphism gene. Eur Respir J 2000, 16, 604–608.
[27] **Bucková D, Hollá LI, Vasků A, Znojil V, Vácha J:** Lack of association between atopic asthma and the tumor necrosis factor alpha-308 gene polymorphism in a Czech population. J Investig Allergol Clin Immunol 2002, 12, 192–197.
[28] **Abraham LJ, Kroeger KM:** Impact of the -308 TNF promoter polymorphism on the transcriptional regulation of the TNF gene: relevance to disease. J Leuk Biol 1999, 66, 562–566.
[29] **Natarajan M, Gibbons CF, Mohan S, Moore S, Kadhim MA:** Oxidative stress signalling: a potential mediator of tumour necrosis factor alpha-induced genomic instability in primary vascular endothelial cells. Br J Radiol 2007, 80, S13–22.
[30] **Kinnula VL, Crapo JD:** Superoxide dismutases in the lung and human lung diseases. Am J Respir Crit Care Med 2003, 167, 1600–1619.

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