Cardiol Young 2007; 17: 295–300

ISSN 1047-9511
doi: 10.1017/S1047951107000534

Original Article

Polymorphism C242T of the gene of the p22phox subunit for nicotinamide adenine dinucleotide phosphate oxidase, and erythrocytic antioxidant enzymes, in patients with tetralogy of Fallot

António Guerra,[1] Carla Rego,[1] Constança Coelho,[2] Nuno Guimarães,[2] Catarina Thiran,[2] Álvaro Aguiar,[1] José Carlos Areias,[1] Manuel Bicho[2]

[1]*Department of Paediatrics, Hospital S. João/Faculty of Medicine – University of Porto, Portugal;* [2]*Genetics Laboratory, Faculty of Medicine – University of Lisbon, Portugal*

Abstract *Background:* Nicotinamide adenine dinucleotide phosphate oxidase of the vascular cell membrane is an important source of reactive oxygen species. The aim of our study was to evaluate the possible influence of the p22phox C242T gene polymorphism on blood pressure and some markers of oxidative stress in children with tetralogy of Fallot. *Methods:* After surgical repair in early life, we recruited 38 children, aged 11.7 plus or minus 3.2 years, including 185 healthy individuals as controls for the purposes of establishing frequencies of alleles and genotypes. From this latter group, we matched a sub-sample of 53 healthy caucasian children, aged 11.0 plus or minus 1.0 years, in order to compare enzymic activities. *Results:* The children with tetralogy of Fallot showed significantly lower values of low-molecular-weight protein tyrosine phosphatase, particularly in carriers of CC genotype for the p22phox gene, with values of 145.2 plus or minus 77.4 μmol/g Hb/h, compared to controls, at 344.4 plus or minus 100.4 μmol/g Hb/h (p less than 0.001). Methemoglobin reductase activity in the patients with tetralogy was also lower in those with the CC genotype, at 9.8 plus or minus 3.2 μmol/g Hb^{-1} min^{-1} compared to 24.2 plus or minus 11.8 μmol/g Hb^{-1} min^{-1} as measured in the controls (p less than 0.01). Lower systolic (p less than 0.05) and diastolic (p less than 0.01) blood pressures were also observed in the patients with tetralogy of Fallot. *Conclusions:* Patients with tetralogy of Fallot having the CC genotype may be at a higher state of oxidative stress than T allele carriers, a finding which could have prognostic implications. Long term follow-up of these patients, however, may be necessary in order to draw definite conclusions.

Keywords: Cyanotic congenital heart disease; NAD(P)H oxidase; oxidative stress

The chronic hypoxia of cyanotic cardiac disease that occurs in the setting of tetralogy of Fallot leads to a down regulation of the antioxidant defences. Chronically cyanotic myocardium may be less well protected than the normally perfused myocardium against oxygen-mediated free radical injury, which potentially represents a higher risk for cardiovascular surgery, due to the higher vulnerability of cells to oxidative damage resulting from the sudden increase in oxygen concentration at the time of surgical repair.[1–3]

The myocardial antioxidant enzyme activities, such as glutathione peroxidase, are reduced in patients with tetralogy of Fallot.[4] Reperfusion of acutely ischaemic myocardium is associated with various distinctive pathophysiologic derangements, which are collectively referred to as reperfusion injury. Among these, arrhythmias, transient mechanical dysfunction, and cell death have been attributed to reactive oxygen species.[5] Increased production of vascular reactive

Correspondence to: Manuel Bicho, MD, PhD, Laboratório de Genética, Faculdade de Medicina de Lisboa, Av. Prof. Egas Moniz – 1699 Lisboa, Portugal. Tel: +351 21 799 94 52; Fax: +351 21 799 94 51; E-mail: manuelbicho@fm.ul.pt

Accepted for publication 21 July 2006

oxygen species, especially superoxide anion, contributes significantly to functional and structural alterations that occur in hypertension.[6,7] The nicotinamide adenine dinucleotide phosphate oxidase of the vascular cell membrane is an important source of reactive oxygen species, particularly superoxide anion, altering the redox state in the vasculature, namely in vascular smooth muscular and endothelial cells.[8] This enzyme is involved in both hypertrophy of vascular smooth muscle, and in some forms of impaired endothelial-dependent relaxation. Some studies suggest a possible association between polymorphisms of the CYBA gene, which encodes the p22phox subunit of nicotinamide adenine dinucleotide phosphate oxidase, with hypertension and atherosclerosis. Of the several identified polymorphisms of CYBA, the C242T allele, which results in the substitution of tyrosine for histidine at codon 72 of p22phox, seems to have a functional effect in the production of superoxide by the vascular wall of patients with atherosclerosis.[9] Since it is presently accepted that oxidative stress plays a role in the pathogenesis of hypertension and atherosclerosis, the enzymes that generate reactive oxygen species may be important determinants of the course of vascular disease.[10]

The downstream effects of production of reactive oxygen species are the oxidation of proteins, which may be reversible or irreversible. Redox-sensitive proteins, which include the low-molecular-weight protein tyrosine phosphatase, are the targets for specific oxidation by various oxidants, including hydrogen peroxide. This modification can be reversed by intracellular reducing agents, such as glutathione and thioredoxin.[11,12] Low-molecular-weight protein tyrosine phosphatase is involved in the dephospholylation of phosphorilated tyrosines in proteins, which result from protein tyrosine kinase activities at the membrane and cytoplasm, thus controlling the rate of glycolysis and several signal transduction pathways of growth factors and cytokines.[12,13]

Nicotinamide adenine dinucleotide dependent-methemoglobin reductase, or nicotinamide adenine dinucleotide cytochrome b5 reductase, are responsible for the conversion of methaemoglobin to deoxyhaemoglobin. This represents an important mechanism that allows the maintenance of levels of deoxyhaemoglobin, since in conditions of oxidative stress there is a rise of methaemoglobin, which is known to be incapable of reversible binding of oxygen. Methaemoglobin reductase thus maintains the iron of haemoglobin in the ferrous state. It has been shown that its activity increases after production of hydrogen peroxide and nitric oxide by inflammatory and endothelial cells, as a consequence of the activation of nicotinamide adenine dinucleotide phosphate oxidase, and nitric oxide synthase, respectively.[14]

The aim of our study was to evaluate the possible influence of the p22phox C242T gene polymorphism on blood pressure and the low-molecular-weight proteins tyrosine phosphatase and methemoglobin reductase as markers of oxidative stress in children with tetralogy of Fallot.

Materials and methods

Subjects

We selected randomly 38 caucasian children with tetralogy of Fallot of both genders from a larger cohort of patients being followed in our unit for paediatric cardiology. The median age at the time of surgical repair was 1.5 years, with the 25th centile at 0.9 years and the 75th at 3.4 years. We also included 185 healthy individuals as controls so as to establish frequencies of alleles and genotypes. From this group, we matched a sub-sample of 53 healthy caucasian children for age and participation in a longitudinal study on cardiovascular health risks in order to compare enzymic activities. All data were obtained at a mean age of 11.7 plus or minus 3.2 years for the 38 patients with tetralogy, and 11.0 plus or minus 1.0 years for the 53 controls.

Methods

Recommended methods and techniques were used to evaluate the anthropometric parameters of weight and height.[15,16] Body mass index was calculated.[17] Charts of growth prepared by Centers for Disease Control and Prevention were used for reference.[18] Blood pressure was evaluated by oscillometry with a Dinamap Critikon® monitor attached to cuffs of an appropriate size for each child, as recommended[19] using the values provided by the 1987 Task Force on Blood Pressure values as the reference.[20]

Activity of the low-molecular-weight protein tyrosine phosphatase, in μmol/gHb/h, was evaluated using the method of the Dissing et al.[21] Activity of methaemoglobin reductase, in μmol/gHb/min, was determined using the method of Board et al.[22] Levels of erythropoietin in the plasma were assessed by a commercially available enzyme-linked immunosorbent assay kit, and expressed in mlU/ml. The p22phox C242T gene polymorphism was determined by polymerase chain reaction restriction fragment length polymorphism.[23] We used the Statistical Package for the Social Sciences for statistical analysis. Differences between groups were tested with the Chi-square test, Student's t test, and one-way analysis of variance. Results were regarded as statistical significant for p less than 0.05. The study was approved by the ethical committee of our Hospital, and informed parental consent was obtained.

Results

Clinical data, including anthropometric parameters, body mass index and blood pressure of individuals from the two groups are summarized in Table 1. There were no significant differences between groups concerning nutritional state. Both systolic and diastolic levels for blood pressures, however, were lower in the patients with tetralogy of Fallot.

Frequencies of the p22phox C242T polymorphism gene and its alleles are shown in Table 2. Genotype frequencies in both groups are in Hardy-Weinberg equilibrium. CC, CT and TT genotypic frequencies were 63.1%, 31.6% and 5.3% in those with tetralogy of Fallot, and 34.1%, 52.4%, and 13.5% in the controls. Frequencies of the C and T alleles were 79.0% and 21.0% for those with tetralogy of Fallot, and 60.3% and 39.7% in the controls. The frequency of the T allele was significantly higher in the controls than in those with tetralogy of Fallot (p less than 0.01).

Activities of the low-molecular-weight protein tyrosine phosphatase, and methaemoglobin reductase, according to the p22phox C242T genotypes, are shown in Table 3. The patients with tetralogy of Fallot showed significantly lower values of both proteins, particularly the carriers of the p22phox C242T CC genotype when compared to controls.

There was a correlation between the values of low-molecular-weight it protein tyrosine phosphatase and methaemoglobin reductase in the patients with tetralogy of Fallot (r equal to 0.48; p equal to 0.007), that was not found in the age-matched controls (r equal to 0.02; p equal to 0.91). Levels of erythropoietin in the plasma from patients with tetralogy of Fallot, at 17.76 plus or minus 8.01 mlU/ml, were significantly higher than in controls, in whom levels were measured at 9.35 plus or minus 2.99 mlU/ml (p less than 0.001).

Discussion

The oxidative stress caused by an imbalance of the production of reactive oxygen species and the protection by several antioxidant systems can lead to electrophysiological, biochemical, and mechanical dysfunction, dramatically impairing the ability of the heart to recover from an initial ischaemic insult. A functional effect has been described for p22phox

Table 1. Descriptive analysis: age, anthropometric parameters, body mass index and blood pressure (means and sd) in the patients with tetralogy of Fallot and their controls.

	Tetralogy of Fallot (38 patients)	Control group (53 subjects)
Age (years)	11.7 (3.2)	11.0 (1.0)
Weight (Zsc)	−0.535 (0.99)	−0.102 (0.941)
Height (Zsc)	−0.799 (0.97)	−0.525 (0.98)
BMI (Zsc)	−0.312 (0.87)	0.075 (0.884)
SBP (50th percentile)	96.9 (11.6)[a]	101.6 (7.7)[a]
DBP (50th percentile)	81.3 (14.3)[b]	91.22 (14.7)[b]

Abbreviations: SBP: systolic blood pressure; DBP: diastolic blood pressure; Zsc: Z-scores
[a]p less than 0.05; [b]p less than 0.01

Table 2. Frequency distribution of alleles and genotypes of the C242T p22phox polymorphism in the patients with tetralogy of Fallot and their controls.

Allele frequency (%)[a]	Tetralogy of Fallot (38 patients)	Control group (185 subjects)
C	79.0	60.3
T	21.0	39.7
Genotype distribution – n (%)[a]		
CC	24 (63.1)	63 (34.1)
CT	12 (31.6)	97 (52.4)
TT	2 (5.3)	25 (13.5)

[a]p less than 0.01 (same allele and same genotypes between groups)

Table 3. Erythrocytic activities of low-molecular-weight protein tyrosine phosphatase and methaemoglobin reductase (means and sd) according to C242T p22phox genotypes in the patients with tetralogy of Fallot and their controls.

	Tetralogy of Fallot (38 patients)			Control group (53 subjects)		
Enzymic Activities	CC (n = 24)	CT + TT (n = 14)	Total (n = 38)	CC (n = 23)	CT + TT (n = 30)	Total (n = 53)
LMW PTP (μmol/gHb/h)	145.2 (77.4)[a]	158.7 (93.4)[b]	150.1 (82.7)[f]	344.4 (100.4)[a,e]	256.5 (93.4) [b,e]	289.9 (113.1)[f]
MHbR (mmol/gHb/min)	9.8 (3.2)[c]	12.3 (4.6)[d]	10.8 (3.9)[f]	24.2 (11.8)[c]	21.2 (9.4)[d]	22.1 (10.6)[f]

Abbreviations: LMW PTP: low-molecular-weight protein tyrosine phosphatase; MHbR: methaemoglobin reductase
[a]and [b]p less than 0.001 (same genotypes between groups) [c]and [d]p less than 0.01 (same genotypes between groups)
[e]p less than 0.05 (same group between genotypes)
[f]p less than 0.001 (total sample between groups)

https://doi.org/10.1017/S1047951107000534 Published online by Cambridge University Press

C242T polymorphism on the nicotinamide adenine dinucleotide phosphate oxidase-driven production of superoxide anion in the vascular wall of patients with atherosclerosis.[9] It is possible that such polymorphisms might regulate the production of superoxide anions driven by nicotinamide adenine dinucleotide phosphate oxidase in hypertensive patients, particularly in cells vulnerable to oxidative damage.[9] This could be particularly relevant in individuals born with cyanotic cardiac malformations, and hence more vulnerable to damage to the vascular wall.

There are several reasons why children with both cyanotic and acyanotic congenital cardiac malformations show significant retardation of growth, including decreased intake of energy, malabsorption, and increased requirements for energy caused by increased metabolism.[24] Surgical correction, however, results in catch-up growth for most individuals.[25] Pulmonary hypertension appears to be the most important factor, and cyanotic patients with pulmonary hypertension are the most severely affected.[26] In this study, we found that, subsequent to operative correction, children with tetralogy of Fallot showed normal growth, with similar nutritional states compared to their controls. The early timing of the surgical repair probably explains the observed normal growth.

The prevalence of the CT and TT genotypes of the p22phox C242T polymorphism was more frequent in our control subjects, being found in two-thirds, than in those with tetralogy of Fallot, making up only one-third of the latter group. To our knowledge, there are no other studies showing the distribution of this polymorphism in the setting of tetralogy of Fallot. Inoue et al[23] found lower frequencies of the CT and TT genotypes in patients with coronary arterial disease, in whom oxidative stress is strongly implicated, suggesting that the p22phox C242T polymorphism could be a genetic marker for coronary arterial risk.

Since individuals carrying the C allele have greater activity of nicotinamide adenine dinucleotide phosphate oxidase, an important source of reactive oxygen species, this particular polymorphism may be particularly relevant in individuals more vulnerable to oxidative damage.[9] In our study, a higher frequency of the C allele was found in the patients with tetralogy (79.0%) compared to their controls (60.3%), with a level of significance of less than 0.01. The functional significance of these findings is not yet well explained, and can be ascribed to the influence of prenatal selection. It is possible that the observed distribution of genes may represent a bias towards survival, related to the fact that carriers of the CC variant, which in prenatal life means a greater production of superoxide anions, probably have a greater chance of survival due to the activation of vascularization elicited by growth factors via production of the superoxide anions.[27] It would be of interest to assess the distribution of the alleles in a cohort of patients with tetralogy of Fallot identified at birth.

In our study, the frequency of the T allele was similar to that found in other studies of healthy caucasians individuals.[28–30] A pro-oxidative marker, namely low-molecular-weight protein tyrosine phosphatise, and an anti-oxidant enzyme, specifically methaemoglobin reductase, were assessed in order to evaluate oxidative stress. These enzymic activities are currently accepted as intermediate phenotypes associated with essential hypertension, thus being relevant to follow up from childhood or adolescence in individuals at risk. The activity of low-molecular-weight protein tyrosine phosphatase is known to be modulated by oxidative stress, and also by a genetic polymorphism.[21,31] In our study, we found significantly lower activities for both low-molecular-weight protein tyrosine phosphatase and methaemoglobin reductase in the patients with tetralogy of Fallot when compared to their controls (Table 3). Although with no significant difference, the lowest values for both enzymic activities were observed in individuals with the CC genotype for p22phox C242T compared to carriers of the T allele. The opposite was found in the controls, with carriers of the CC genotype showing higher values for both enzymes compared to those observed in carriers of the T allele. According to previous studies, individuals with the CC genotype exhibit stronger activities for nicotinamide adenine dinucleotide phosphate oxidase, an important source of reactive oxygen species.[9] This enzyme is induced by hypoxia, producing reactive oxygen species, which lower the levels of low-molecular-weight protein tyrosine phosphatase. This does not happen in normoxic individuals, as demonstrated in controls.[32] We found no differences in the children with tetralogy of Fallot, probably due to the reduced enzymic activities in all individuals. We speculate that a sustained oxidative stress since birth could modulate the expression, and thus the activity, of these enzymes.

These patients have different degrees of hypoxaemia, particularly before surgical correction. There is also evidence that a certain degree of hypoxaemia persists after surgical correction, which is suggested by the raised levels of erythropoietin, even after adjusting for levels of haemoglobin. Pulmonary hypoxia stimulates the activity of hypoxia inducing factor-alpha, and consequently the angiotensin-converting enzyme and angiotensin receptor type 1 in adventitial fibroblasts involved in the proliferation and remodelling of the pulmonary vasculature.[33] This increases the arterial pulmonary pressure, and consequently reduces the left ventricular filling and cardiac output. The mitogenic effect did not happen with human systemic aorta or mesenteric arterial fibroblasts.[33]

These mechanisms might explain why these individuals do not have systemic hypertension.

Our results also suggest that decreased enzymic activities in the patients with tetralogy of Fallot, particularly methaemoglobin reductase, and a possible imbalance between the enhanced generation of superoxide anions and the increased antioxidant activity, could be in part mediated by the p22phox C242T polymorphism. These results could also have prognostic implications for these patients with regard to the occurrence and development of vascular damage.

The induction of anti-oxidant enzymes by reactive oxygen species is well known.[2] In our patients with tetralogy of Fallot, the values for erythropoietin were significantly higher compared to controls, with a level of significance of less than 0.001. This might be explained by higher levels of deoxyhaemoglobin in the previously cyanotic patients. This lower oxygenation of the tissues can be responsible for the lower levels of anti-oxidant enzymes, such as methaemoglobin reductase and low-molecular-weight protein tyrosine phosphatase. Levels of deoxyhaemoglobin in these patients might stimulate the production of erythropoietin, which activates endothelial nitric oxide synthase.[34] It has recently been demonstrated that deoxyhaemoglobin promotes the conversion of intra-erythrocyte nitrites into nitric oxide, a powerful vasodilator. This mechanism could account for the lower blood pressures observed in patients with tetralogy of Fallot.[35] Other mechanisms might also be involved, such as reduced cardiac contractility and a lower cardiac output, already reported, which were not evaluated. Individuals in our group with tetralogy of Fallot, however, did not present any clinical symptoms or any limitation to their normal daily activities.

The p22phox C242T polymorphism seems to be important, particularly in people with increased susceptibility to oxidative stress in early life. Patients with tetralogy having the p22phox C242T CC genotype may be at a higher state of oxidative stress than the carriers of the T allele, which could also have prognostic implications, especially concerning the occurrence and development of vascular damage. Longer term follow-up of these patients will be necessary in order to draw definite conclusions.

References

1. Chaturvedi RR, Shore DF, Lincoln C, et al. Acute right ventricular restrictive physiology after repair of tetralogy of Fallot: association with myocardial injury and oxidative stress. Circulation 1999; 100: 1540–1547.
2. Li RK, Mickle DA, Weisel RD, et al. Effect of oxygen tension on the anti-oxidant enzyme activities of tetralogy of Fallot ventricular myocytes. J Mol Cell Cardiol 1989; 21: 567–575.
3. Morita K, Ihnken K, Buckberg GD, Sherman MP, Young HH, Ignarro LJ. Role of controlled cardiac reoxygenation in reducing nitric oxide production and cardiac oxidant damage in cyanotic infantile hearts. J Clin Invest 1994; 93: 2658–2666.
4. Teoh KH, Mickle DA, Weisel RD, et al. Effect of oxygen tension and cardiovascular operations on the myocardial antioxidant enzyme activities in patients with tetralogy of Fallot and aorta-coronary bypass. J Thorac Cardiovasc Surg 1992; 104: 159–164.
5. Bolli R. Oxygen-derived free radicals and myocardial reperfusion injury: an overview. Cardiovasc Drugs Ther 1991; 5 Suppl 2: 249–268.
6. Zalba G, San José G, Moreno MU, et al. Oxidative stress in arterial hypertension: role of NAD(P)H oxidase. Hypertension 2001; 38: 1395–1399.
7. Lacy F, O'Connor DT, Schmid-Schonbein GW. Plasma hydrogen peroxide production in hypertensives and normotensive subjects at genetic risk of hypertension. J Hypertens 1998; 16: 291–303.
8. Ushio-Fukai M, Zafari AM, Fukui T, Ishizaka N, Griedling KK. p22phox is a critical component of the superoxide-generating NADH/NADPH oxidase system and regulates angiotensin II-induced hypertrophy in vascular smooth muscle cells. J Biol Chem 1996; 271: 23317–23321.
9. Guzik TJ, West NE, Black E, et al. Functional effect of the C242T polymorphism in the NAD(P)H oxidase p22phox gene on vascular superoxide production in atherosclerosis. Circulation 2000; 102: 1744–1747.
10. Griendling K, Ushio-Fukai M. NADH/NADPH oxidase and vascular function. Trends Cardiovasc Med 1997; 7: 301–306.
11. Xu D, Rovira II, Finkel T. Oxidants painting the cysteine chapel: redox regulation of PTPs. Dev Cell 2002; 2: 251–252.
12. Raugei G, Ramponi G, Chiarugi P. Low molecular weight protein tyrosine phosphatases: small, but smart. Cell Mol Life Sci 2002; 59: 941–949.
13. Lukyanenko LM, Kozlova NM, Slobozhanina EI. Activity of membrane-bound NADH-methemoglobin reductase and physical state of lipids in erythrocyte membranes. Bioelectrochemistry 2004; 62: 191–193.
14. Inal ME, Eguz AM. The effects of isosorbide dinitrate on methemoglobin reductase enzyme activity and antioxidant states. Cell Biochem Funct 2004; 22: 129–133.
15. Fomon SJ. Nutritional disorders of children: prevention, screening and follow-up. Washington, DC: Dept. Health, Education and Welfare; 1977. Publication (HSA) 77–5104.
16. Cameron N. The methods of auxological anthropometry. In: Falkner F, Tanner FM (eds.). Human growth: A comprehensive treatise. 2nd edition. Plenum Press, New York, 1986; pp. 13–45.
17. Lee J, Kolonel LN, Hinds W. Relative merits of the weight-corrected for height indices. Am J Clin Nutr 1981; 34: 2521–2529.
18. Ogden CL, Kuczmarski RJ, Flegal KM, et al. Centers for Disease Control and Prevention 2000 growth charts for the United States: improvements to the 1977 National Center for Health Statistics version. Pediatrics 2002; 109: 45–60.
19. Frohlich ED, Grim C, Labarthe DR, Maxwell MH, Perloff D, Weidman WH. Recomendations for human blood pressure determination by sphygmomanometers: report of a special task force appointed by the steering committee. American Heart Association. Circulation 1988; 77: A501–A514.
20. Report of the second Task Force on blood pressure control in children. Pediatrics 1987; 79: 1–25.
21. Dissing J, Dahl O, Svensmark O. Phosphonic and arsonic acids as inhibitors of human red cell acid phosphatase and their use in affinity chromatography. Biochim Biophys Acta 1979; 569: 159–176.
22. Board PG, Pidcock ME. Methahemoglobinaemia resulting from heterozygosity for two NADH-Methaemoglobin redutase variants: characterization as NADH-Ferricyanide redutase. Br J Haematology 1981; 47: 361–370.
23. Inoue N, Kawashima S, Kanazawa K, Yamada S, Akita H, Yokoyama M. Polymorphism of the NADH/NADPH oxidase

p22phox gene in patients with coronary artery disease. Circulation 1998; 97: 135–137.
24. Jacobs EG, Leung MP, Karlberg JP Postnatal growth in southern Chinese children with symptomatic congenital heart disease. J Pediatr Endocrinol Metab 2000; 13: 387–401.
25. Schuurmans FM, Pulles-Heintzberger CF, Gerver WJ, Kester AD, Forget PP. Long-term growth of children with congenital heart disease: a retrospective study. Acta Paediatr 1998; 87: 1250–1255.
26. Varan B, Tokel K, Yilmaz G. Malnutrition and growth failure in cyanotic and acyanotic congenital heart disease with and without pulmonary hypertension. Arch Dis Child 1999; 81: 49–52.
27. Chiarugi P and Cirri P. Redox regulation of protein tyrosine phosphatases during receptor tyrosine kinase signal transduction. Trends Biocheml Sci 2003; 28: 509–514.
28. Cai H, Duarte N, Wilcken DE, Wang XL. NADH/NADPH oxidase p22 phox C242T polymorphism and coronary artery disease in the Australian population. Eur J Clin Invest 1999; 29: 744–748.
29. Wolf G, Panzer U, Harendza S, Wenzel U, Stahl RAK. No association between a genetic variant of the p22phox component of NAD(P)H oxidase and the incidence and progression of IgA nephropathy. Dial Transplant 2002; 17: 1509–1512.
30. Mata-Balaguer T, de la Herran R, Ruiz-Rejon C, Ruiz-Rejon M, Garrido-Ramos MA, Ruiz-Rejon F. Angiotensin-converting enzyme and p22(phox) polymorphisms and the risk of coronary heart disease in a low-risk Spanish population. Int J Cardiol 2004; 95: 145–151.
31. Hecht D, Zick Y. Selective inhibition of protein tyrosine phosphatase activities by H_2O_2 and vanadate in vitro. Bioch and Biophys Res Communications 1992; 188: 773–779.
32. Goyal P, Weissmann N, Grimminger F, et al. Upregulation of NAD(P)H oxidase 1 in hypoxia activates hypoxia-inducible factor 1 via increase in reactive oxygen species. Free Radic Biol Med 2004; 36: 1279–1288.
33. Krick S, Hanze J, Eul B, et al. Hypoxia-driven proliferation of human pulmonary artery fibroblasts: cross-talk between HIF-1alpha and an autocrine angiotensin system. FASEB J 2005; 19: 857–883.
34. Beleslin-Cokic BB, Cokic VP, Yu X, Weksler BB, Schechter AN, Noguchi CT. Erythropoietin and hypoxia stimulate erythropoietin receptor and nitric oxide production by endothelial cells. Blood 2004; 104: 2073–2080.
35. Huang Z, Shiva S, Kim-Shapiro DB, et al. Enzymatic function of hemoglobin as a nitrite reductase that produces NO under allosteric control. J Clin Invest 2005; 115: 2099–2107.