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***GPX1*Pro198Leu* and *GPX3* rs2070593 as genetic risk markers for Italian asthmatic patients.**

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Running title: *GPX1* and *GPX3* in asthma onset

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Abstract

Asthma is a chronic disease and several studies indicated that oxidative stress impairs pulmonary function. However, cellular antioxidant mechanisms prevent tissue damage and Glutathione peroxidases (GPXs) are the main enzymes in the antioxidant defense systems.

The aim of this case-control study was to verify the putative correlation between two polymorphisms of GPX genes and the onset of asthma.

The case-control sample was recruited from the “San Giovanni Calibita” Fatebenefratelli Hospital, Rome. The *GPX1***Pro198Leu* (rs1050450) polymorphism and a *GPX3* non-coding variant (rs2070593) were screened.

Significant results were found for the *GPX1* and *GPX3* polymorphisms. This study provided some interesting and novel insight into the interactions between genetic predisposition, exposure to environmental risk factor, and oxidative stress during the onset of asthma.

Keywords: atopy, coding variant, complex disease, enzyme activity, Glutathione peroxidases, respiratory disease, single nucleotide polymorphism, 3'-UTR variant.

Dear Editor,

our previous studies underlined a significant association between several detoxifying enzymes and asthma onset (1-4). Accordingly, the novel findings reported in this letter may be useful to understand the role of other antioxidant enzymes, Glutathione peroxidases (GPXs), in the pathways related to the development of asthma.

The onset of asthma is characterized by significant environmental and genetic factors. However there is no clear definition of the clinical phenotype of this complex disease. This allergic respiratory disorder is unquestionably a global concern, considering that about 300 million individuals are affected by it, with a prevalence ranging from 1-18% across populations (5, 6).

Asthma is an illness defined by its pathological, physiological, clinical and etiological features. The primary clinical feature of asthma is a sporadic airway obstruction that causes a decrease in respiratory flow, while the main pathological feature is an inflammation of the airways, sometimes associated with structural changes of the bronchoalveolar epithelium (7).

In recent years, a variety of genetic association studies have identified genes associated with the onset of asthma, mainly genes involved with the immune system and the regulation of the

serum IgE levels (8-11). In addition to the functions of immune system factors, some studies have shown that alterations in cellular detoxification and antioxidant systems may increase susceptibility to the onset of asthma (12, 13). For this reason, the study of genes involved in antioxidant defense could contribute to the body of knowledge concerning the onset and development of asthma and open new scenarios on the complex clinical spectrum of this disease.

GPXs are the main enzymes involved in cellular antioxidant defense systems (14). According to their tissue localization, six classes of GPXs have been identified (15) and some studies have shown that a decrease of GPX enzyme activity may increase the risk of developing pathological conditions (14, 16-18). Among GPX genes, *GPX1* and *GPX3* are the most frequently investigated and different genetic variants were identified in their coding and non-coding regions (18).

To date, in the literature few genetic association studies have focused on the involvement of GPXs at the onset of asthma. However, a biochemical study suggested that in asthmatic patients the GPX plasma levels are significantly lower than those of healthy subjects (19). Moreover, a different study confirmed a significant decrease of the GPX activity in asthmatics, and highlighted that these cases exhibited a high lipid peroxidation levels (20).

The case-control sample analyzed in this study was recruited from 'San Giovanni Calibita' Fatebenefratelli Hospital in Rome, Italy. It consists of 345 subjects (151 asthmatic patients and 194 healthy controls). The diagnosis of asthma was assessed according to the "Global Initiative on Asthma criteria" (4) and this study was approved by the Fatebenefratelli Hospital's Ethics Committee.

The *GPX1*Pro198Leu* (rs1050450) polymorphism was analyzed via the RFLP-PCR technique, as reported previously in literature (21). Conversely, the *GPX3* rs2070593 variant was detected by direct Sanger sequencing.

The power analysis was carried out using the Power for Genetic Association ver. 2.0 software (22), while the minimum detectable effect with odds ratios (ORs) was calculated with a co-dominant genetic model, based on an alpha of 0.05 and an asthma prevalence of 18% in the general population. With this sample size, we had an 90% power to detect an OR of 1.49 for asthma, even if the risk allele frequency was higher than 40%. ORs and confidence intervals (95% CIs) were calculated to estimate the association between GPX gene polymorphisms and asthma. Moreover, to ensure that the confounding factors did not alter the results, we applied a logistic regression to estimate an adjusted OR. We considered different genetic models to estimate the ORs, including: "dominant" (one copy of the allele is sufficient to increase the

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disease risk), “recessive” (two copies of the allele are necessary to increase the disease risk), “codominant” (each genotype has a different disease risk), “overdominant” (the heterozygosity state may confer risk or protection) and “log-additive” (there is an r -fold increased risk for one copy of the allele; there is an r^2 increased risk for two copies of the allele). We used the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) to evaluate the best genetic model.

Both *GPX1* and *GPX3* genotype frequencies were in H-W equilibrium for the case and control groups ($p > 0.05$). Only for the “active smoking” variable we did observe significant differences in frequency between asthmatic patients and controls ($p = 0.016$) (Table 1).

Both the studied polymorphisms showed a significant association with disease risk. In particular, regarding *GPX1*Pro198Leu*, the codominant model achieved the best scores for AIC and BIC [CT genotype: OR (CI95%) = 1.54 (1.12 – 2.44), $p = 0.008$] (Table 2). Our results shows that the presence of a Leu198 mutant allele in the heterozygous condition seems to increase individual susceptibility to the onset of asthma.

Several studies that focused on *GPX1* have shown that this gene is featured by different polymorphisms, including *GPX1*Pro198Leu*, which seems to modulate enzymatic activity (23).

In a study focusing on the *GPX1*Pro198Leu* polymorphism in a group of women with breast cancer, the authors showed that the GPX1 activity was significantly lower in women with the Leu198 allele than in those with the Pro198 allele (24).

In addition to the GPX1 antioxidant deficiency caused by the Leu198 allele, a recent study proposed a novel mechanism that involves GPX1 in the onset of asthma (25). The authors demonstrated that GPX1 prevents airway inflammation and alveolar destruction by increasing the activity of the PTP1B protein and, subsequently, of the PP2A protein in the lung. GPX1 activates PTP1B, which interacts with PP2A, a key modulator of the inflammatory response of asthma, because it contrasts with the release of pro-inflammatory agents. Since the activity of PTP1B is subject to oxidative inactivation, and PTP1B strongly affects PP2A activity, the authors hypothesized that GPX1 protects against inflammation induced by tobacco smoking (25).

Regarding *GPX3* rs2070593, the recessive model achieved the best scores for AIC and BIC [AA genotype: OR (CI95%) = 0.42 (0.22 - 0.81), $p = 0.0089$] (Table 2). Our results suggested that two mutated alleles are necessary to confer protection against the onset of asthma.

In literature no case-control studies have focused on the correlation between this *GPX3* polymorphism and asthma. However, some investigations show a significant association between *GPX3* rs2070593 and other complex diseases (26-28). A recent study suggested that the presence of at least one mutated allele in the 3'-UTR of the *GPX3* gene confers protection to the gastric cancer (29). Therefore, given the localization of this variant in the 3'-UTR and the lack of data in the literature, we hypothesize that the mutated allele of this SNP could be associated with an increased expression of the gene, that could determine an increase in *GPX3* antioxidant activity.

In conclusion, our investigation confirms the pivotal role *GPX1* plays in asthma risk, permitting us to hypothesize that *GPX3* rs2070593 is a protection locus for this allergic respiratory disease. The results of this genetic association study have allowed us to open new lines of inquiry into the implication of antioxidant enzymes during the onset of asthma. Moreover, the investigated SNPs possibly act contrariwise and further studies focusing on the gene expression could contribute to support our hypothesis about the onset of this respiratory disease.

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References

1. Piacentini S, Polimanti R, Moscatelli B, Re MA, Fuciarelli R, Manfellotto D, Fuciarelli M. Glutathione S-transferase gene polymorphisms and air pollution as interactive risk factors for asthma in a multicentre Italian field study: A preliminary study. *Ann Hum Biol* 2010; 37: 427-39.

2. Piacentini S, Polimanti R, Simonelli I, Donno S, Pasqualetti P, Manfellotto D, Fuciarelli M. Glutathione S-transferase polymorphisms, asthma susceptibility and confounding variables: a meta-analysis. *Mol Biol Rep* 2013; 40: 3299-313.
3. Piacentini S, Verrotti A, Polimanti R, Giannini C, Saccucci P, Manfellotto D, Fuciarelli M. Functional polymorphisms of GSTA1 and GSTO2 genes associated with asthma in Italian children. *Clin Chem Lab Med* 2012; 50: 311-5.
4. Piacentini S, Polimanti R, Iorio A, Cortesi M, Papa F, Rongioletti M, Liumbruno GM, Manfellotto D, Fuciarelli M. GSTA1*-69C/T and GSTO2*N142D as asthma- and allergy-related risk factors in Italian adult patients. *Clin Exp Pharmacol Physiol* 2014; 41: 180-4.
5. Masoli M, Fabian D, Holt S, Beasley R. The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy* 2004; 59: 469-78.
6. (GINA). Global Strategy for Asthma Management and Prevention. In: Available from: <http://www.ginasthma.org/> (accessed 25 March 2012). 2011.
7. Xu W, Cardenes N, Corey C, Erzurum SC, Shiva S. Platelets from Asthmatic Individuals Show Less Reliance on Glycolysis. *PLoS One* 2015; 10: e0132007.
8. Raeiszadeh Jahromi S, Mahesh PA, Jayaraj BS, Holla AD, Vishweswaraiah S, Ramachandra NB. IL-10 and IL-17F Promoter Single Nucleotide Polymorphism and Asthma: A Case-Control Study in South India. *Lung* 2015.
9. Shirzade H, Meshkat R, Ganjalikhani-Hakemi M, Mosayebian A, Ghasemi R, Deress F, Parchami Barjui S, Sadri M, Salehi R. Association analysis of -416 G>C polymorphism of T-cell immunoglobulin and mucin domain-1 gene with asthma in Iran. *Int J Immunogenet* 2015.
10. Butsch Kovacic M, Martin LJ, Biagini Myers JM, He H, Lindsey M, Mersha TB, Khurana Hershey GK. Genetic approach identifies distinct asthma pathways in overweight vs normal weight children. *Allergy* 2015.
11. Sordillo JE, Kelly R, Bunyavanich S, McGeachie M, Qiu W, Croteau-Chonka DC, Soto-Quiros M, Avila L, Celedon JC, Brehm JM, Weiss ST, Gold DR, Litonjua AA. Genome-wide expression profiles identify potential targets for gene-environment interactions in asthma severity. *J Allergy Clin Immunol* 2015.
12. Al-Harbi NO, Nadeem A, Al-Harbi MM, Imam F, Al-Shabanah OA, Ahmad SF, Sayed-Ahmed MM, Bahashwan SA. Oxidative airway inflammation leads to systemic and vascular oxidative stress in a murine model of allergic asthma. *Int Immunopharmacol* 2015; 26: 237-45.

13. Gorska K, Krenke R, Domagala-Kulawik J, Korczynski P, Nejman-Gryz P, Kosciuch J, Hildebrand K, Chazan R. Comparison of cellular and biochemical markers of airway inflammation in patients with mild-to-moderate asthma and chronic obstructive pulmonary disease: an induced sputum and bronchoalveolar lavage fluid study. *J Physiol Pharmacol* 2008; 59 Suppl 6: 271-83.
14. Lubos E, Loscalzo J, Handy DE. Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. *Antioxid Redox Signal* 2011; 15: 1957-97.
15. Schriever SC, Barnes KM, Evenson JK, Raines AM, Sunde RA. Selenium requirements are higher for glutathione peroxidase-1 mRNA than gpx1 activity in rat testis. *Exp Biol Med (Maywood)* 2009; 234: 513-21.
16. Lubos E, Loscalzo J, Handy DE. Homocysteine and glutathione peroxidase-1. *Antioxid Redox Signal* 2007; 9: 1923-40.
17. Yuzhalin AE, Kutikhin AG. Inherited variations in the SOD and GPX gene families and cancer risk. *Free Radic Res* 2012; 46: 581-99.
18. Polimanti R, Fuciarelli M, Destro-Bisol G, Battaggia C. Functional diversity of the glutathione peroxidase gene family among human populations: implications for genetic predisposition to disease and drug response. *Pharmacogenomics* 2013; 14: 1037-45.
19. Al-Afaleg NO, Al-Senaïdy A, El-Ansary A. Oxidative stress and antioxidant status in Saudi asthmatic patients. *Clin Biochem* 2011; 44: 612-7.
20. Ahmad A, Shameem M, Husain Q. Relation of oxidant-antioxidant imbalance with disease progression in patients with asthma. *Ann Thorac Med* 2012; 7: 226-32.
21. Miller JC, Thomson CD, Williams SN, van Havre N, Wilkins GT, Morison IM, Ludgate JL, and Skeaff CM. Influence of the glutathione peroxidase 1 Pro200Leu polymorphisms on the response of glutathione peroxidase activity to selenium supplementation: a randomized controlled trial. *Am J Clin Nutri* 2012; 96: 923-31.
22. Menashe I, Rosenberg PS, Chen BE. PGA: power calculator for case-control genetic association analyses. *BMC Genet* 2008; 9: 36.
23. Banescu C, Trifa AP, Voidazan S, Moldovan VG, Macarie I, Benedek Lazar E, Dima D, Duicu C, Dobreanu M. CAT, GPX1, MnSOD, GSTM1, GSTT1, and GSTP1 genetic polymorphisms in chronic myeloid leukemia: a case-control study. *Oxid Med Cell Longev* 2014; 2014: 875861.
24. Ravn-Haren G, Olsen A, Tjønneland A, Dragsted LO, Nexø BA, Wallin H, Overvad K, Raaschou-Nielsen O, Vogel U. Associations between GPX1 Pro198Leu polymorphism,

erythrocyte GPX activity, alcohol consumption and breast cancer risk in a prospective cohort study. *Carcinogenesis* 2006; 27: 820-5.

25. Geraghty P, Hardigan AA, Wallace AM, Mirochnitchenko O, Thankachen J, Arellanos L, Thompson V, D'Armiento JM, Foronjy RF. The glutathione peroxidase 1-protein tyrosine phosphatase 1B-protein phosphatase 2A axis. A key determinant of airway inflammation and alveolar destruction. *Am J Respir Cell Mol Biol* 2013; 49: 721-30.

26. Zhou JD, Wen XM, Zhang YY, Yang L, Ma YJ, Ma JC, Yang J, Guo H, Yao DM, Lin J, Qian J. Down-regulation of GPX3 is associated with favorable/intermediate karyotypes in de novo acute myeloid leukemia. *Int J Clin Exp Pathol* 2015; 8: 2384-91.

27. Zhao H, Li J, Li X, Han C, Zhang Y, Zheng L, Guo M. Silencing GPX3 Expression Promotes Tumor Metastasis in Human Thyroid Cancer. *Curr Protein Pept Sci* 2015; 16: 316-21.

28. Cao S, Yan B, Lu Y, Zhang G, Li J, Zhai W, Guo W, Zhang S. Methylation of promoter and expression silencing of GPX3 gene in hepatocellular carcinoma tissue. *Clin Res Hepatol Gastroenterol* 2015; 39: 198-204.

29. Wang JY, Yang IP, Wu DC, Huang SW, Wu JY, Juo SH. Functional glutathione peroxidase 3 polymorphisms associated with increased risk of Taiwanese patients with gastric cancer. *Clin Chim Acta* 2010; 411: 1432-6.

Table 1: Description of the case-control group.

	ASTHMATICS	CONTROLS	Test	P value
SEX				
<i>M n, (%)</i>	62 (41)	89 (46)	$\chi^2 = 0.80$	0.371
<i>F n, (%)</i>	89 (59)	105 (54)		
AGE mean $\pm$ SD	54.06 $\pm$ 16.53	57.07 $\pm$ 14.18	t = 1.81	0.069
BMI mean $\pm$ SD	25.09 $\pm$ 4.48	24.98 $\pm$ 3.41	t = 0.25	0.796
ACTIVE SMOKING				
<i>smokers n, (%)</i>	31 (20)	41 (21)	$\chi^2 = 10.25$	0.016*
<i>non-smokers n, (%)</i>	85 (57)	92 (47)		
<i>ex-smokers n, (%)</i>	31 (20)	61 (32)		
<i>NA n, (%)</i>	4 (3)	0 (0)		
PASSIVE SMOKING				
<i>passive smokers n, (%)</i>	54 (36)	54 (28)	$\chi^2 = 5.87$	0.053
<i>non-passive smokers n, (%)</i>	81 (54)	128 (66)		
<i>NA n, (%)</i>	16 (10)	12 (6)		
OCCUPATIONAL RISK				
<i>(+) n, (%)</i>	50 (33)	47 (24)	$\chi^2 = 3.32$	0.068
<i>(-) n, (%)</i>	101 (67)	147 (76)		
TOTAL	151	194		

* p < 0.05

Table 2: Allele and genotype distribution for *GPX1* and *GPX3* and OR values with 95% CIs, after adjustment for various confounders in overall individuals. Akaike information criterion (AIC) and Bayesian information criterion (BIC) are reported.

<i>GPX1*Pro198Leu</i>	ASTHMATICS n, (%)	CONTROLS n, (%)	OR (95% CI)	P value	AIC	BIC
C (Pro198)	202 (67)	261 (67)	<i>Dominant</i> : CT-TT = 1.26 (0.81 - 1.94)	0.3	468	483.4
T (Leu198)	100 (33)	127 (33)	<i>Recessive</i> : TT = 0.39 (0.18 - 0.83)	0.012*	462.8	478.2
C/C (Pro198/Pro198)	72 (48)	79 (41)	<i>Codominant</i> : CT = 1.54 (1.12 - 2.44)	0.008*	461.4	478
C/T (Pro198/Leu198)	58 (38)	103 (53)	TT = 0.49 (0.22 - 1.00)			
T/T (Leu198/Leu198)	21 (14)	12 (6)	<i>Overdominant</i> : CT = 1.75 (1.13 - 2.72)	0.012*	462.7	478.1
TOTAL	151	194	<i>Log-additive</i> : 0.94 (0.67 - 1.31)	0.72	469	484.3
<i>GPX3 rs2070593</i>						
G	186 (62)	283 (73)	<i>Dominant</i> : GA-AA = 0.62 (0.40 - 0.96)	0.032	464.5	479.8
A	116 (38)	105 (27)	<i>Recessive</i> : AA = 0.42 (0.22 - 0.81)	0.0089*	462.3	477.6
G/G	28 (19)	16 (8)	<i>Codominant</i> : GA = 0.74 (0.46 - 1.18)	0.015*	462.7	481.8
G/A	60 (40)	73 (38)	AA = 0.37 (0.18 - 0.74)			
A/A	63 (41)	105 (54)	<i>Overdominant</i> : GA = 0.91 (0.59 - 1.42)	0.69	468.9	484.3
TOTAL	151	194	<i>Log-additive</i> : 0.64 (0.47 - 0.98)	0.045	464.6	479.9

* p < 0.025, according to Bonferroni adjustment