

Association study of *Glutathione S-transferase P1 (GSTP1)* with asthma and bronchial hyper-responsiveness in two German pediatric populations

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Glutathione S-Transferase P1 (GSTP1) is an important enzyme in the detoxification of products of oxidative stress. Several studies have shown an association of the amino acid variant Ile105Val with bronchial asthma and the reaction of the lung to inhalant pollutants. The aim of this study was to test the two known amino acid variants in *GSTP1* for association with bronchial asthma and airway hyper-responsiveness in two German pediatric populations. We genotyped Ile105Val and Ala114Val in the Multicenter Allergy Study cohort (85 children with asthma, 123 controls) and asthmatic children from Freiburg (n = 178). We did not find association of either polymorphisms with bronchial asthma or airway hyper-responsiveness. We conclude from our data that polymorphisms within *GSTP1* do not play a major role in the development of bronchial asthma in German children.

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Oxidative stress and the production of reactive oxygen species (ROS) play an important role in the pathogenesis of bronchial asthma. Enzymes detoxifying ROS and thereby protecting the lung before oxidative damage have been proposed as candidate genes for bronchial asthma.

The members of the super-gene family of Glutathione-S Transferases (GST) are enzymes, which can utilize a wide variety of ROS. GSTs are divided in several classes: α, μ, π (GSTPs), θ, σ and κ . *Glutathione S-Transferase P1 (GSTP1)* shows a predominant expression in the lungs. An amino acid exchange in *GSTP1*–Ile105Val – has been shown to be associated with asthma in several populations. Especially a protective role of the genotype Val105Val in the development of asthma and airway hyper-responsiveness has been demonstrated in two independent studies (1, 2). However, others failed to replicate these findings (3) or even found opposite effects of the polymorphism (4).

Recently, gene-environment interactions have been proposed for the variant: Homozygosity for 105Ile represents a risk factor for asthma in areas with high outdoor air pollution, but not in areas with low pollution (5). Furthermore 105Val confers protection against isocyanate induced asthma and airway hyper-responsiveness (6).

We evaluated the role of *GSTP1* Ile105Val and Ala114Val in pediatric asthma in two independent German populations.

Material and methods

The Multicenter Allergy Study (MAS) population

The MAS birth cohort has been described in detail before (7). Initially, 1314 children born in 1990 were recruited. These infants were followed from birth to 7 yr of age. Follow-up visits were performed each year including structured interviews focusing on relevant symptoms of asthma,

BHR and atopy, blood sampling (at up to eight timepoints) for determination of total IgE and specific IgE to common inhalant and food allergens (CAP-RAST FEIA, Pharmacia, Uppsala, Sweden). When the children were 7-year old, pulmonary function tests and histamine provocations were performed. For histamine challenges a validated reservoir method (Pari Provocation Test 2, Pari, Starnberg, Germany) was used as described before (8). Briefly, 0.5 mg/ml (in asthmatics 0.125 mg/ml) up to 8 mg/ml histamine were applied to children until a 20% fall in FEV₁ was observed (PC₂₀FEV₁). DNA has been collected from 888 children (85 asthmatics). Current asthma (at the age of 7 yr) was defined as one or more wheezing episode(s) during the previous 12 months. Other atopy related phenotypes were defined as described previously. As non-asthmatic, non-atopic controls, we selected MAS-children without symptoms of asthma or allergic rhinitis, no diagnosis of AD ever and IgE against any tested allergen <0.35 kU/l at the age of 7 yr.

The Freiburg asthmatic population

One hundred and seventy-eight children with doctor's diagnosed asthma aged between 5 and 18 yr were phenotyped and recruited at the Children's University Hospital in Freiburg. The clinical characterization included questionnaires on asthma symptoms and medication, determination of total and specific IgE antibodies to common food and inhalant allergens, pulmonary function tests, determination of exercise-induced fall in FEV₁, and bronchial histamine provocations (see also Ref. 9).

The collection of serum and the subsequent DNA material and the experimental procedures were approved by the local Ethical Committee. A statement of informed consent was signed by all participants or in the case of children signed by their parents.

Genotyping for GSTP1 variants

Genomic DNA was extracted from peripheral blood leukocytes by standard techniques. DNA samples were analyzed for *GSTP1* variants by PCR followed by melting curve analysis.

PCR primers. *GSTP1* Ile105Val: 5'-ACCCCAGGGCTCTATGGGAA-3' and 5'-TGAGGGCACAAGAAGCCCCT-3'; *GSTP1* Ala114Val: 5'-AAGCAGAGGAGAATCTGGGACTC-3' and 5'-GGCCAGATGCTCACCTGGTC-3'.

We performed duplex PCR using 0.75 U AmpliTaq Gold polymerase (Perkin Elmer,

Braunschweig, Germany), 400 μ m dNTPs, and 0.1 μ m of each primer in a total volume of 25 μ l. Cycle conditions were an initial denaturation for 12 min at 95°C, followed by 40 cycles of 20 s denaturing at 95°C, 40 s annealing at 52°C, 90 s primer extension at 72°C, followed by a final extension for 2 min at 72°C in an automated thermal cycler (Biometra, Germany). We performed melting curve analysis for the *GSTP1* SNPs using a pair of fluorescent resonance energy transfer (FRET) probes and a Light-Cycler (Roche Diagnostics, Germany). FRET probes were designed and synthesized by TIB MOLBIOL (Berlin, Germany). FRET probes. *GSTP1* Ile105Val: 5'-GCAAATACATCTCCCTCATCTACAC-FL and LC-Red-640-TGTGAGCATCTGCACCAGGGTTGGGC-ph; *GSTP1* Ala114Val 5'-GTCATCCTTGCCAC CACCTCCT-FL and 5'-LC-Red-705-CCAGACACCACATGTATCATCCTACTC-ph.

Statistical analysis

Pearsons chi-square tests and non-parametric Mann-Whitney *U*-tests were applied for the statistical analysis; *p* values below 0.05 were considered statistically significant.

Results

The allelic frequency of the polymorphisms and the genotype distribution within the three different populations – MAS asthmatics, MAS controls and Freiburg asthmatics – are given in Tables 1 and 2, respectively. The allelic frequency

Table 1. Allelic frequency of the Ile105Val polymorphism and genotype distribution in the three-pediatric populations

	n	Ile (%)	Val (%)	Ile105Ile, n (%)	Ile105Val, n (%)	Val105Val, n (%)
Asthmatics (MAS)	83	69.3	30.7	38 (0.46)	39 (0.47)	6 (0.07)
Controls (MAS)	122	68.9	31.1	54 (0.44)	60 (0.49)	8 (0.06)
Asthmatics (Freiburg)	178	68.0	32.0	80 (0.45)	82 (0.46)	16 (0.09)

MAS, Multicenter Allergy Study.

Table 2. Allelic frequency of the Ala114Val polymorphism and genotype distribution in the three-pediatric populations

	n	Ala (%)	Val (%)	Ala114Ala, n (%)	Ala114Val, n (%)	Val114Val, n (%)
Asthmatics (MAS)	85	92.9	7.1	73 (0.86)	12 (0.14)	0 (0.00)
Controls (MAS)	123	91.1	8.9	101 (0.82)	22 (0.18)	0 (0.00)
Asthmatics (Freiburg)	173	92.8	7.2	148 (0.85)	25 (0.14)	0 (0.00)

MAS, Multicenter Allergy Study.

of both polymorphisms was in accordance to previously published studies. There was no significant difference in between the populations.

Also, genotype distributions in individuals with allergic rhinitis, atopic dermatitis and allergic sensitization vs. non-atopic controls did not differ significantly in the MAS-cohort (data not shown). Neither in the entire MAS-population nor in asthmatics of either study population the lung function parameter FEV₁ or degree of BHR (PC₂₀FEV₁/Exercise induced fall in FEV₁) differed significantly between *GSTP1* genotypes (data not shown).

Discussion

The aim of the study was to evaluate the role of both *GSTP1* polymorphisms in the development of asthma in children. Due to its role in the detoxification of ROS *GSTP1* represents a reasonable candidate gene. In addition several studies have demonstrated an impaired catalytic function of the variant enzyme (10).

We did not find an association of the Ile105Val polymorphism with bronchial asthma or airway hyper-responsiveness in two independent German populations of children. Also Ala114Val showed no association with the phenotypes under investigation.

The contrary findings could be due to different reasons. First, we used a pediatric population whereas most of the previous studies used populations of adult asthmatics. It is well known that pediatric and adult asthma differ in several clinical and genetic characteristics.

Second, asthma represents a very complex genetic disease with many candidate genes. These genes harbour only a small effect on the disease and thus can be easily missed.

Finally, as mentioned above, *GSTP1* might be especially important in the detoxification of inhalant pollutants. We do not have any data regarding the air pollution in the area of our populations and could not test for a gene-environment interaction. Maybe the most important environmental factor in this context is represented by exposure to passive smoking. Just very previously it has been shown that variants in other Glutathione-S-Transferases (GSTM1 and GSTT1) only confers risk to the development of asthma when the children were exposed to smoke (11). As we do not have data

about smoke exposure in our study populations we cannot exclude such an effect for *GSTP1*.

In conclusion polymorphisms within *GSTP1* do not represent a major genetic factor in the development of bronchial asthma in German children.

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