

No association of histamine-*N*-methyltransferase polymorphism with asthma or bronchial hyperresponsiveness in two German pediatric populations

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Histamine plays an important role in the allergic inflammation. Histamin *N*-Methyltransferase (HNMT) catalyses the major pathway of histamine metabolism in the human lung. A common functional single nucleotide polymorphism (SNP) within the *HNMT* gene (C314T) was recently related to asthma. We tested this SNP for associations with asthma and asthma associated traits in two German pediatric populations (1. MAS-cohort, $n = 888$, 85 children with asthma; 2. asthmatic children from Freiburg, $n = 176$). Non-asthmatic ($n = 515$) and non-atopic ($n = 211$) children from the MAS-cohort were used as controls. For genotyping melting curve analyses (Light Cycler System) were applied. In contrast to a previous study, no association of the *HNMT* 314T allele with asthma, bronchial hyperresponsiveness (BHR) or other asthma related phenotypes could be observed in either study population. We conclude that this SNP might not play a major role in the pathogenesis of asthma or BHR in German children.

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Histamine is an important preformed mediator in the allergic reaction. Released by mast cells and basophils after cross-linking of surface bound IgE by allergen it functions as a strong bronchoconstrictor in human lungs (1). A major pathway of histamine biotransformation in bronchial epithelium is the *N*-methylation of histamine catalysed by histamine-*N*-methyltransferase (HNMT). A single nucleotide polymorphism (SNP) within the *HNMT* gene has been identified (C314T) that results in an amino acid change (Thr105Ile) and has been shown to cause a significantly lower activity of the enzyme (2). Higher levels of histamine in the bronchial epithelium of individuals with this SNP might therefore influence their susceptibility for asthma or bronchial hyperresponsiveness (BHR). An association of asthma with this SNP was described by Yan et al. in Caucasian

subjects (3). The objective of our study was to evaluate the role of the C314T SNP in two German pediatric populations with regard to asthma and BHR.

The Multicenter Allergy Study (MAS) birth cohort has been described in detail previously (4, 5). Briefly, 1314 children born in 1990 were initially enrolled. The cohort infants were followed from birth to 7 yr of age. Yearly follow up visits included 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). Pulmonary function tests and histamine provocations were performed at the age of 7 yr. For histamine challenges a validated reservoir method (Pari Provocation Test 2, Pari, Starnberg,

Germany) was used as described before (5). 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 (PC₂₀ FEV₁). DNA has been collected from 888 children (85 asthmatics).

The phenotypes were defined as follows: Current asthma (age 7): one or more wheezing episode(s) during the previous 12 months (4). Allergic rhinitis (age 7): sneeze attacks, runny, blocked or itchy nose in the absence of a common cold during the previous 12 months plus sensitization against birch or grass pollen. Atopic dermatitis (AD): modified criteria of Haninfin and Rajka as described previously (5). Allergic sensitization: specific IgE ≥ 0.7 kU/l against at least one out of nine food and/or inhalant allergens. Control subjects: No symptoms of asthma or allergic rhinitis ever, no diagnosis of AD ever, IgE against any tested allergen < 0.35 kU/l at the age of 7 yr.

Additionally, a total of 176 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 (6)).

Genotyping of the *HNMT* C314T polymorphism was performed as follows: Polymerase chain reaction (PCR) was used to amplify Exon 4 of *HNMT*. The forward and reverse primers used for PCR were 5'-CAATTAAATGATGGTG TGTC A and 5'-TTTCTCCAACATTCTACTTT GGTA under the following conditions: 60 cycles at 94°C for 10 s, 52°C for 30 s, 72°C for 40 s followed by a 2 min extension at 72°C.

A pair of fluorescence resonance energy transfer (FRET) probes applied to the PCR product allowed to register allele specific melting curves with the LightCycler system (Roche Diagnostics, Mannheim, Germany). The donor probe was complementary to the wild-type sequence 5'-Ag-CTTgTAgCCAAgACATCgAACC-FL-3 (FL: 5,6-carboxyfluorescein attached to 3'-O-ribose) and the acceptor probe was 5'-LC-CgAgAACgT AAAGTTTgCTTggCAT-ph-3' (LC: LightCycler Red 705 attached to 5'-terminus; ph: 3'-phosphate. Probes were designed by Tib Molbiol, Berlin, Germany). The melting temperature of the Duplex formed by the mutant allele and the probes was 58.4°C. The more stable Duplex resulted in a melting temperature of 66.0°C.

Pearson's chi-squared test and non-parametric Mann-Whitney *U*-test were applied for the statistical analysis, *p*-values less than 0.05 were considered statistically significant.

We found the following allele frequencies: MAS-cohort: *HNMT* 314C: 0.88; *HNMT* 314T: 0.12; Freiburg asthmatics: *HNMT* 314C: 0.86; *HNMT* 314T: 0.14. Genotype frequencies and allele frequencies in asthmatics from both populations were not significantly different compared to the MAS non-atopic and non-asthmatic control groups (Table 1).

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 (PC₂₀ FEV₁/exercise induced fall in FEV₁) differed significantly between *HNMT* genotypes (data not shown).

We set out to analyse a possible association between *HNMT* alleles and asthma and BHR in two German pediatric populations. *HNMT* plays a major role in the metabolism of histamine in the human lung. A functional polymorphism within the *HNMT* gene had previously been shown to reduce the enzymatic activity (2) and to represent a risk factor for asthma (3). Yan et al. hypothesized that decreased enzyme activity might result in higher histamine levels in the lung, increased bronchoconstriction and, therefore, a higher susceptibility for asthma. This group found a significant increase in the frequency of the 314T allele in a group of Caucasian asthma patients. We could not confirm an effect of a certain *HNMT* genotype on asthma, allergic asthma, FEV₁ or BHR in two German pediatric populations. The *HNMT* 314T allele frequencies found in our two pediatric asthma populations were basically identical to those reported by Yan et al. (0.13 and 0.14, respectively), in contrast, the T-allele frequency in our 515 non-asthmatic pediatric control subjects (MAS-cohort) was higher (0.13) than in the control group (474

Table 1. *HNMT* C314T genotypes and allele frequencies in two German pediatric asthmatic populations and non-atopic and non-asthmatic control groups

	n	CC (%)	CT (%)	TT (%)	Allele frequency (T)
Asthmatics (MAS)	85	74	26	–	0.13
Asthmatics (Freiburg)	176	74	23	3	0.14
Non-asthmatic controls (MAS)	515	75	24	1	0.13
Non-atopic controls (MAS)	211	75	24	1	0.13

Caucasian blood donors) of the previous study (0.08). As children from the MAS-cohort were evaluated every year for asthma symptoms, we are confident about a negative asthma history although we cannot rule out that asthma may develop in some of the control children later in life. However, our results are in agreement with another negative report on the *HNMT* 314T allele and asthma (7). Sasaki et al. compared 100 Japanese atopic asthmatics with 100 normal Japanese schoolchildren. *HNMT*-314 T allele frequencies were 0.08 and 0.1, respectively.

Asthma and atopy are complex genetic traits with important gene–environmental interactions (8). Environmental factors may differ significantly among the study population tested by Yan et al. and our populations, such as exposure to allergens, viral and bacterial antigens. Also, different gene–gene interactions may underlie the discrepant results. Overall, our findings in two populations suggest that the *HNMT* C314T polymorphism is unlikely to be a major risk factor for asthma or BHR in German children. However, our study populations may not be large enough to detect minor effects of the *HNMT*-variant on pediatric asthma or asthma severity.

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References

1. CHUNG K, BARNES P. Other mediators of asthma. In: BARNES P, RODGER I, THOMPSON N, ed. *Asthma – Basic Mechanisms and Clinical Management*. 3rd edn. San Diego, CA, USA: Academic Press, 1998: 343–68.
2. PREUSS CV, WOOD TC, SZUMLANSKI CL, et al. Human histamine-*N*-methyltransferase pharmacogenetics: common genetic polymorphisms that alter activity. *Mol Pharmacol* 1998; 53: 708–17.
3. YAN L, GALINSKY RE, BERNSTEIN JA, LIGGETT SB, WEINSHILBOUM RM. Histamine *N*-methyltransferase pharmacogenetics: association of a common functional polymorphism with asthma. *Pharmacogenetics* 2000; 10: 261–6.
4. LAU S, NICKEL R, NIGGEMANN B, et al. The development of childhood asthma: lessons from the German Multi-centre Allergy Study (MAS). *Paediatr Respir Rev* 2002; 3: 265–72.
5. BERGMANN RL, EDENHARTER G, BERGMANN KE, et al. Atopic dermatitis in early infancy predicts allergic airway disease at 5 years. *Clin Exp Allergy* 1998; 28: 965–70.
6. SENGLER C, HEINZMANN A, JERKIC SP, et al. Clara cell protein 16 (CC16) gene polymorphism influences the degree of airway responsiveness in asthmatic children. *J Allergy Clin Immunol* 2003; 111: 515–9.
7. SASAKI Y, IHARA K, AHMED S, et al. Lack of association between atopic asthma and polymorphisms of the histamine H1 receptor, histamine H2 receptor, and histamine *N*-methyltransferase genes. *Immunogenetics* 2000; 51: 238–40.
8. SENGLER C, LAU S, WAHN U, NICKEL R. Interactions between genes and environmental factors in asthma and atopy: new developments. *Respir Res* 2002; 3: 7.