

Association of single nucleotide polymorphisms in the diamine oxidase gene with diamine oxidase serum activities

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To cite this article: Maintz L, Yu C-F, Rodríguez E, Baurecht H, Bieber T, Illig T, Weidinger S, Novak N. Association of single nucleotide polymorphisms in the diamine oxidase gene with diamine oxidase serum activities. *Allergy* 2011; DOI: 10.1111/j.1398-9995.2011.02548.x.

Keywords

diamine oxidase; histamine; histamine intolerance; luciferase assay; single nucleotide polymorphism.

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Accepted for publication 3 January 2011

DOI:10.1111/j.1398-9995.2011.02548.x

Edited by: Marek Sanak

Abstract

Background: Histamine intolerance (HIT) is associated with an excess of histamine because of an impaired function of the histamine-degrading enzyme diamine oxidase (DAO). The genetic background of HIT is unknown yet.

Methods: Case-control association study of all haplotype tagging and four previously reported *DAO* SNPs and one *HNMT* Single nucleotide polymorphism with symptoms of HIT and DAO serum activity in 484 German individuals including 285 patients with clinical symptoms of HIT and 199 controls.

Results: Diamine oxidase serum activity was significantly associated with seven SNPs within the *DAO* gene. The minor allele at rs2052129, rs2268999, rs10156191 and rs1049742 increased the risk for a reduced DAO activity whereas showing a moderate protective effect at rs2071514, rs1049748 and rs2071517 in the genotypic ($P = 2.1 \times 10^{-8}$, 7.6×10^{-10} , 8.3×10^{-10} , 0.009, 0.005, 0.00001, 0.006, respectively) and allelic genetic model ($P = 2.5 \times 10^{-11}$, 5.4×10^{-13} , 8.9×10^{-13} , 0.00002, 0.006, 0.0003, 0.005, respectively). Reporter gene assays at rs2052129 revealed a lower promoter activity ($P = 0.016$) of the minor allele. DAO mRNA expression in peripheral blood mononuclear cells of homozygous carriers of the minor allele at rs2052129, rs2268999, rs10156191 was lower ($P = 0.002$) than homozygous carriers of the major allele. Diamine oxidase variants were not associated with the HIT phenotype *per se*, only with DAO activity alone and the subgroup of HIT patients displaying a reduced DAO activity.

Conclusions: DAO gene variants strongly influence DAO expression and activity but alone are not sufficient to fully effectuate the potentially associated disease state of HIT, suggesting an interplay of genetic and environmental factors.

Histamine intolerance (HIT) is characterized by an imbalance between histamine and the capacity for histamine degradation (1, 2). Diamine oxidase (DAO/ABP1, histaminase) as a secretory protein metabolizes extracellular, histamine-

N-methyltransferase (HNMT) as a cytosolic protein intracellular histamine (2–4). A malfunction of histamine catabolism results in the accumulation of high levels of histamine, leading to gastrointestinal symptoms, headache, flushing, rhinitis, asthma-like wheezing, and less often to cardiovascular symptoms, urticaria, pruritus and others (1, 2, 5). Symptoms are usually triggered by ingestion of histamine-rich food, alcohol, drugs releasing histamine or blocking DAO, while they improve under a histamine-free diet (1, 2, 5, 6). Approximately 1% of the population suffer from HIT, most of them being middle-aged women (5).

Abbreviations

CI, confidence interval; DAO, diamine oxidase; LD, linkage disequilibrium; Het, heterozygous; HIT, histamine intolerance; HNMT, histamine-*N*-methyltransferase; Hom, homozygous; OR, odds ratio; PBMCs, peripheral blood mononuclear cells; SNP, single nucleotide polymorphism.

An impaired enzymatic histamine degradation caused by gastrointestinal diseases with a decreased production of DAO by the altered enterocytes or competitive inhibition of DAO by other biogenic amines, alcohol or drugs have been proposed to contribute to the pathogenesis of HIT (2, 5–7).

However, little is known about primary factors that possibly influence interindividual variation in DAO and HNMT levels and activity, and a potential genetic susceptibility to symptoms of HIT. The expression and activity of other metabolizing enzymes, e.g. lactase have been shown to be strongly influenced by common genetic polymorphisms. The expression of DAO shows high interindividual variations in the gut (3), and single nucleotide polymorphisms (SNPs) in the *DAO* gene have been associated with inflammatory and neoplastic gastrointestinal diseases (8–12). Recently, two *DAO* SNPs have been associated with reduced DAO activities in healthy subjects (13).

The human *DAO* gene spans approximately 10 kbp, is located on chromosome 7q35 and contains five exons and four introns (3).

Histamine-*N*-methyltransferase is widely expressed in human tissues (4) and appears to have a role for histamine degradation in the airways. Rs11558538:C>T (Thr105Ile) is a functional relevant polymorphism of the *HNMT* gene, and the 314T allele has been significantly associated with asthma in Caucasian patients. Conversely, this association could not be replicated in a Japanese, German pediatric (14) and Indian population (12). The *HNMT* gene maps to chromosome 2q22, spans approximately 53 kbp (15) and consists of six exons and five introns (12).

To evaluate the candidacy of *DAO* and *HNMT* gene variants as risk factors for impaired DAO function or HIT, we investigated *DAO* haplotype-tagging SNPs as well as SNPs previously associated with gastrointestinal diseases and reduced DAO serum activities (13) and the *HNMT* SNP in patients with HIT and controls. Furthermore, we examined the functional relevance of selected SNPs by analysing DAO mRNA expression in Peripheral blood mononuclear cells (PBMCs), measuring DAO serum activity, and studying *DAO* promoter activity by Luciferase reporter gene assays.

Methods

Study population

A total of 285 patients with HIT (244 women, 41 men, mean age 47.9 ± 13.8 years; age range 17–78) and 199 healthy controls with no history of HIT symptoms (123 women, 76 men, mean age 42.0 ± 19.0 years; age range 18–91) were investigated. The subjects were recruited at the Department of Dermatology and Allergy in Bonn and at the Department of Allergy and Internal Medicine in Schmallenberg between 2004 and 2009. HIT was defined as (i) report of 2 or more positive symptoms of HIT such as gastrointestinal symptoms, chronic and premenstrual headache, flush and intolerance of histamine-rich food, alcohol and drugs releasing histamine or blocking DAO; (ii) improvement of symptoms under histamine-free diet, antihistamines, mast cell stabilizers and/or supplementa-

tion of DAO (5). In ten patients with HIT, the diagnosis was further supported by a positive reaction to a double-blind placebo-controlled provocation with 1 mg histamine dihydrochloride/kg body weight in gelatine capsules (placebo: corn starch capsules) (16). The phenotype of about half of the study population has already been described previously (17) (Table S1).

Diamine oxidase serum activity was determined by a radioactive extraction assay using radiolabelled putrescine-dihydrochloride as a substrate according to the manufacturer's instructions (Immunodiagnostik AG, Bensheim, Germany), and DAO activity lower than 10 U/ml was considered as decreased (5). All study methods followed the Declaration of Helsinki protocols and have been approved by the local ethics committee. Written informed consent was obtained from all participants prior to the beginning of the study.

Genotyping

Genomic DNA was isolated from peripheral blood as described previously (18). In order to adequately capture the genetic variance across the *DAO* gene region, we selected 8 tagging SNPs calculated with Haploview 4.0 (pairwise tagging with cutoffs $r^2 > 0.8$; minimum minor allele frequency (MAF) ≥ 0.05 and HW P -value ≥ 0.05) according to the hap-map project (HapMap Data Rel 22/phase II Apr07, on NCBI B36 assembly, dbSNP b126) and four SNPs in the *DAO* gene previously reported to be associated with gastrointestinal diseases (8, 9, 19).

Assays for SNP genotyping were developed for rs11558538 (C314T) in the *HNMT* gene (14) and 12 SNPs in the *DAO* gene: rs2052129 (G4586T), rs2268999 (A594T), rs10156191 (C47T), rs1049742 (C995T), rs2071514 (G1329A), rs1049748 (T2444C), rs1049793 (C4106G), rs12539 (C4808T), rs2071517 (G4810A), rs4725970 (C5053T), rs4725376 (G5729A) and rs11978239 (A6764G) (position based on translation initiation site in exon 2) (Fig. S1, Table S2). Polymerase chain reaction (PCR) and extension primers were designed using the MassARRAY Assay Design 3.0 software (Sequenom, San Diego, CA, USA). PCR and extension reactions were performed according to the manufacturer's instructions, and extension product sizes were determined by mass spectrometry using the Sequenom iPLEX system (Sequenom, San Diego, CA, USA) (20).

Reporter gene assays

Briefly, HEK293 cells were transiently co-transfected with a reporter construct containing either G or T at the location of rs2052129 (21) fused to firefly luciferase gene and a control renilla luciferase plasmid. After 24 h, firefly luciferase activity in cell lysates was determined, related to renilla luciferase activity, and relative luciferase activity was normalized to the activity of a pGL2-Basic vector (Data S1).

Isolation of PBMCs and quantitative real-time PCR

Briefly, mRNA was isolated from PBMCs of patients with HIT homozygous for the minor allele at rs2052129,

rs2268999 and rs10156191, patients with HIT and controls homozygous for the major allele at these loci and relative DAO mRNA expression on PBMCs was evaluated using quantitative real-time PCR (Data S2) (21, 22).

Statistical analysis

Differences in the phenotype characteristics were calculated with the Chi-squared test for qualitative values, Mann-Whitney *U*-test for DAO serum activity and DAO mRNA expression on PBMCs (SPSS 17.0) and Wilcoxon signed rank test for normalized luciferase activity (SPSS Inc., Chicago, IL, USA). *P*-values are two sided and subject to a significance level of 5%.

Deviation from Hardy-Weinberg equilibrium (HWE), linkage disequilibrium (LD), genotyping success and minor allele frequencies were assessed with HAPLOVIEW 3.32 (<http://www.broad.mit.edu/node/443>) (23). First unadjusted analyses of the genotype distribution of SNPs in patients with HIT compared to CTRs and subjects with reduced DAO compared to normal DAO serum activities were conducted with the Chi-squared test (SPSS 17.0). In a second step, variants were tested for association using logistic regression, assuming a genotypic and allelic (additive) model adjusted for age and gender as covariates. For the genotypic model, the three-categorical genotype was modelled by two dummy variables, thus analysing effects of the heterozygote and homozygote genotypes separately. The effect of every polymorphism on square-root-transformed DAO activity was analysed using linear regression. As residuals from linear regression models on DAO activity violated the normality assumption and DAO activity can also be zero, the square root is an appropriate transformation of DAO activity. Furthermore, logistic regressions on low compared to normal DAO activity using the clinical cut-off value of 10 U/ml were performed. All statistical analyses were carried out with R 2.8.1 (R Foundation for Statistical Computing, Vienna, Austria) (24) unless otherwise stated.

Results

Higher frequency of gastrointestinal symptoms, chronic and premenstrual headache, dysmenorrhoea, drug, food and alcohol intolerance in subjects with reduced DAO activity and histamine intolerance

Diamine oxidase serum activity was significantly lower in patients with HIT ($P < 1 \times 10^{-6}$) and reduced in 49.5% of patients with HIT and 17.1% of controls ($P = 2.9 \times 10^{-13}$). This was mirrored by a significantly higher occurrence of clinical symptoms of HIT such as gastrointestinal symptoms ($P < 1.0 \times 10^{-16}$), chronic headache ($P < 1.0 \times 10^{-16}$), premenstrual headache and dysmenorrhoea ($P = 1.1 \times 10^{-16}$), flushing ($P < 1.0 \times 10^{-16}$), urticaria/angioedema ($P = 3.3 \times 10^{-16}$), and intolerance of the triggering factors drugs, histamine-rich food and alcohol ($P < 1.0 \times 10^{-16}$ each) compared to controls. Interestingly, patients with HIT also displayed significantly more frequently a positive family history for HIT ($P = 3.1 \times 10^{-12}$). Further analysis of the effects of DAO

activity on the manifestation of symptoms in the whole population revealed also a significant lower DAO activity of subjects suffering from gastrointestinal symptoms ($P < 0.0001$), chronic headache ($P = 2.0 \times 10^{-4}$), premenstrual headache and dysmenorrhoea ($P = 0.04$), flushing ($P = 1.0 \times 10^{-4}$), and intolerance of drugs ($P = 8.0 \times 10^{-4}$), food and alcohol ($P < 0.0001$ each) and a significantly higher frequency of the aforementioned symptoms in subjects with a reduced compared to normal DAO activity (Table S1).

Genotyping results

Except of rs1049742, observed genotype frequencies of all SNPs did not deviate significantly from expected frequencies under the HWE assumption nor from other populations with European ancestry (http://hapmap.org/cgi-perl/gbrowse/hapmap_B36/ and http://www.ncbi.nlm.nih.gov/SNP/snp_ref) (Tables S2 and S3 and Table 1).

Polymorphisms in the DAO gene are associated with the activity of the histamine-degrading enzyme DAO

Associations of DAO gene variants with DAO serum activity were analysed in the whole population (cases and controls) and in cases and controls separately. All significant effects were DAO specific and of approximately equal size in both cases and controls and the whole population. If not stated otherwise, results obtained in the whole population are reported [results for the subgroups: Table 2 (additive model) and Table S4 (genotype model)].

In the unadjusted analysis, DAO serum activity was significantly associated with five SNPs within the DAO gene. Reduced activity was associated with rs2052129 ($P = 4.1 \times 10^{-11}$; $\chi^2 = 47.82$), rs2268999 ($P = 5.9 \times 10^{-12}$; $\chi^2 = 51.72$), rs10156191 ($P = 1.6 \times 10^{-12}$; $\chi^2 = 54.27$), and rs1049742 ($P = 2.0 \times 10^{-4}$; $\chi^2 = 17.25$) while rs1049748 ($P = 2.1 \times 10^{-5}$; $\chi^2 = 21.53$) was associated with an increased activity (Table 1). Refining the analysis and adjusting for sex and age, these polymorphisms remained significantly associated. The promoter SNP rs2052129 and the two polymorphisms rs2268999 (Intron 1), rs10156191 (Exon 1, Thr16Meth), which were strongly correlated with each other ($r^2 = 0.70$ – 0.98), as well as rs1049742 (Exon 2, Ser332Phe) (Fig. S2), displayed strong associations with an effect estimate ranging of -0.72 to -0.74 and a *P*-value of 2.5×10^{-11} for rs2052129 (Table 2). All four SNPs were in high LD ($D' = 0.88$ – 1.0) (Fig. 1). The genotypic model revealed that for these SNPs the risk in carriers of two risk alleles (minor alleles) was approximately double the risk in heterozygotes (e.g. effect estimate -0.68 for GT and -1.50 for TT at rs2052129) and thus indicated that the data fitted very well to an additive model (Table 2).

In contrast, the more distal variants rs2071514 (Exon 2, Ala414Ala), rs1049748 (Exon 3, Pro574Pro) and rs2071517 (5'UTR) were associated with increased DAO activities (effects of 0.34 – 0.36 , $P = 0.0003$ – 0.006). For these variants, which were in strong LD with each other ($D' = 0.98$ – 1.0), the genotype model revealed comparable effects for heterozygous and homozygous carriers of the risk alleles implicating

Table 1 Genotype distribution of *DAO* and *HNMT* SNPs in patients with HIT and healthy controls, and of patients with a normal (≥ 10 U/ml) activity of *DAO* when compared to those with a reduced (< 10 U/ml) *DAO* activity

SNP	Geno- type	CTR (<i>n</i> = 199)	HIT (<i>n</i> = 285)	<i>P</i> -value	χ^2	DAO ≥10 U/ml (<i>n</i> = 309)	DAO < 10 U/ml (<i>n</i> = 175)	<i>P</i> -value	χ^2
		<i>n</i> (%)	<i>n</i> (%)			<i>n</i> (%)	<i>n</i> (%)		
DAO gene									
rs2052129	GG	116 (58.3)	166 (58.2)	0.12	5.87	211 (68.3)	71 (40.6)	4.1×10^{-11}	47.82
	GT	73 (36.7)	91 (31.9)			89 (28.8)	75 (42.9)		
	TT	10 (5.0)	23 (8.1)			8 (2.6)	25 (14.3)		
rs2268999	AA	115 (57.8)	161 (56.6)	0.47	2.97	209 (67.6)	67 (38.3)	5.9×10^{-12}	51.72
	AT	69 (34.7)	93 (32.6)			88 (28.5)	74 (42.3)		
	TT	15 (7.5)	28 (9.8)			11 (3.6)	32 (18.3)		
rs10156191	CC	114 (57.3)	160 (56.1)	0.63	2.23	208 (67.3)	66 (37.7)	1.6×10^{-12}	54.27
	CT	70 (35.2)	95 (33.3)			90 (29.1)	75 (42.9)		
	TT	15 (7.5)	28 (9.8)			11 (3.6)	32 (18.3)		
rs1049742	CC	173 (86.9)	239 (83.9)	0.35	3.67	278 (90.0)	134 (76.6)	2.0×10^{-4}	17.25
	CT	23 (11.6)	41 (14.4)			29 (9.4)	35 (20.0)		
	TT	3 (1.5)	2 (0.7)			1 (0.3)	4 (2.3)		
rs2071514	GG	130 (65.3)	190 (66.7)	0.34	3.75	193 (62.5)	127 (72.6)	0.056	6.96
	GA	64 (32.2)	80 (28.10)			103 (33.3)	41 (23.4)		
	AA	5 (2.5)	12 (4.2)			12 (3.9)	5 (2.9)		
rs1049748	TT	72 (36.2)	100 (35.1)	0.67	2.14	88 (28.5)	84 (48.0)	2.1×10^{-5}	21.53
	TC	95 (47.7)	137 (48.1)			169 (54.7)	63 (36.0)		
	CC	32 (16.1)	45 (15.8)			51 (16.5)	26 (14.9)		
rs1049793	CC	94 (47.2)	139 (48.8)	0.54	2.59	153 (49.5)	80 (45.7)	0.55	2.09
	CG	90 (45.2)	119 (41.8)			129 (41.7)	80 (45.7)		
	GG	15 (7.5)	24 (8.4)			26 (8.4)	13 (7.4)		
rs12539	CC	123 (61.8)	173 (60.7)	0.43	2.55	177 (57.3)	119 (68.0)	0.12	5.98
	CT	60 (30.2)	98 (34.4)			111 (35.9)	47 (26.9)		
	TT	13 (6.5)	11 (3.9)			16 (5.2)	8 (4.6)		
rs2071517	GG	131 (65.8)	188 (66.0)	0.48	2.99	193 (62.5)	126 (72.0)	0.07	6.46
	GA	63 (31.7)	83 (29.1)			103 (33.3)	43 (24.6)		
	AA	5 (2.5)	11 (3.9)			12 (3.9)	4 (2.3)		
rs4725970	CC	123 (61.8)	173 (60.7)	0.43	2.55	177 (57.3)	119 (68.0)	0.12	5.98
	TC	60 (30.2)	98 (34.4)			111 (35.9)	47 (26.9)		
	TT	13 (6.5)	11 (3.9)			16 (5.2)	8 (4.6)		
rs4725376	AA	52 (26.1)	72 (25.3)	0.67	2.13	83 (26.9)	41 (23.4)	0.44	2.56
	AG	101 (50.8)	144 (50.5)			158 (51.1)	87 (49.7)		
	GG	46 (23.1)	66 (23.2)			67 (21.7)	45 (25.7)		
rs11978239	AA	94 (47.2)	138 (48.4)	0.58	2.51	153 (49.5)	79 (45.1)	0.50	2.32
	AG	90 (45.2)	120 (42.1)			129 (41.7)	81 (46.3)		
	GG	15 (7.5)	24 (8.4)			26 (8.4)	13 (7.4)		
HNMT gene									
rs11558538	CC	161 (80.9)	223 (78.2)	0.40	3.30	246 (79.6)	138 (78.9)	0.65	1.81
	CT	35 (17.6)	57 (20.0)			58 (18.8)	34 (19.4)		
	TT	3 (1.5)	2 (0.7)			4 (1.3)	1 (0.6)		

CTR, controls; HIT, histamine intolerance; DAO, diamine oxidase.

a dominant mode of action. No associations were seen for the other SNPs investigated.

Polymorphisms in the *DAO* gene are not associated with HIT *per se*, but with HIT and reduced *DAO* serum activities

No significant associations of the investigated SNPs in the *DAO* gene with the HIT phenotype *per se* were seen. If the phenotype was narrowed down to HIT combined with reduced

DAO serum activity, significant associations with the three SNPs, which also showed the strongest associations with *DAO* serum activity, were observed. Results from the additive model showed an odds ratio (OR) of 1.82 (95% CI, 1.27; 2.60; $P = 0.001$) for rs2052129, 1.86 (95% CI, 1.33; 2.62; $P = 3.5 \times 10^{-4}$) for rs2268999, and 1.88 (95% CI, 1.33; 2.64; $P = 3.1 \times 10^{-4}$) at rs10156191. There was no association of rs11558538 in the *HNMT* gene with HIT or *DAO* serum activity (Table S5).

Table 2 Association analysis of *DAO* and *HMMT* SNPs with *DAO* serum activity in patients with histamine intolerance (cases), healthy controls (controls) and the whole study population using an additive genetic model

Minor allele	Function*	All subjects		Cases		Controls	
		Est (95% CI)	P-value	Est (95% CI)	P-value	Est (95% CI)	P-value
DAO							
rs2052129	Near gene-5	-0.72 (-0.93; -0.51)	2.5×10^{-11}	-0.73 (-0.96; -0.49)	3.9×10^{-9}	-0.68 (-0.99; -0.36)	4.0×10^{-5}
rs2268999	Intron	-0.73 (-0.93; -0.54)	5.4×10^{-13}	-0.77 (-0.99; -0.55)	6.4×10^{-11}	-0.62 (-0.92; -0.33)	6.0×10^{-5}
rs10156191	Missense	-0.73 (-0.92; -0.53)	8.9×10^{-13}	-0.77 (-0.99; -0.55)	5.9×10^{-11}	-0.61 (-0.90; 0.31)	9.0×10^{-5}
rs1049742	Missense	-0.74 (-1.08; -0.40)	2.0×10^{-5}	-0.78 (-1.19; -0.38)	1.9×10^{-4}	-0.66 (-1.15; -0.17)	8.4×10^{-3}
rs2071514	Coding synonymous	0.34 (0.10; 0.58)	5.8×10^{-3}	0.31 (0.02; 0.59)	0.03	0.42 (0.06; 0.78)	0.03
rs1049748	Coding synonymous	0.36 (0.16; 0.55)	3.0×10^{-4}	0.36 (0.13; 0.59)	2.1×10^{-3}	0.34 (0.06; 0.61)	0.02
rs1049793	Missense	-0.02 (-0.23; 0.19)	0.86	-0.04 (-0.29; 0.21)	0.76	0.002 (-0.31; 0.32)	0.99
rs12539	Untranslated-3	0.20 (-0.03; 0.43)	0.09	0.21 (-0.07; 0.49)	0.14	0.11 (-0.21; 0.43)	0.50
rs2071517	Near gene-3	0.35 (0.10; 0.59)	5.4×10^{-3}	0.32 (0.04; 0.61)	0.03	0.42 (0.06; 0.79)	0.02
rs4725970	Near gene-3	0.20 (-0.03; 0.43)	0.09	0.21 (-0.07; 0.49)	0.14	0.11 (-0.21; 0.43)	0.50
rs4725376	Unknown	-0.12 (-0.31; 0.07)	0.21	-0.11 (-0.34; 0.11)	0.33	-0.10 (-0.38; 0.18)	0.50
rs11978239	Unknown	-0.02 (-0.23; 0.19)	0.86	-0.05 (-0.23; 0.21)	0.73	0.02 (-0.30; 0.33)	0.92
HMMT							
rs11558538	Missense	-0.01 (-0.32; 0.30)	0.96	-0.05 (-0.42; 0.32)	0.79	0.12 (-0.32; 0.57)	0.59

For each marker, the regression model was adjusted for age and sex. Estimated linear effects (Est) on squareroot-transformed diamine oxidase (DAO) activity were calculated for the whole study population and for cases and controls separately. CI, Confidence interval.

*Function retrieved from UCSC genome browser assembly March 2006, dbSNP build 130 (<http://genome.ucsc.edu>): untranslated-3 = variation in transcript but not in coding regional interval, near gene-5/ near gene-3 = variation within 2000 bases of gene, but not in transcript, coding synonymous = no change in peptide for allele with respect to reference assembly, missense (coding non synonymous) = change in peptide for allele with respect to reference assembly, unknown = no known functional classification.

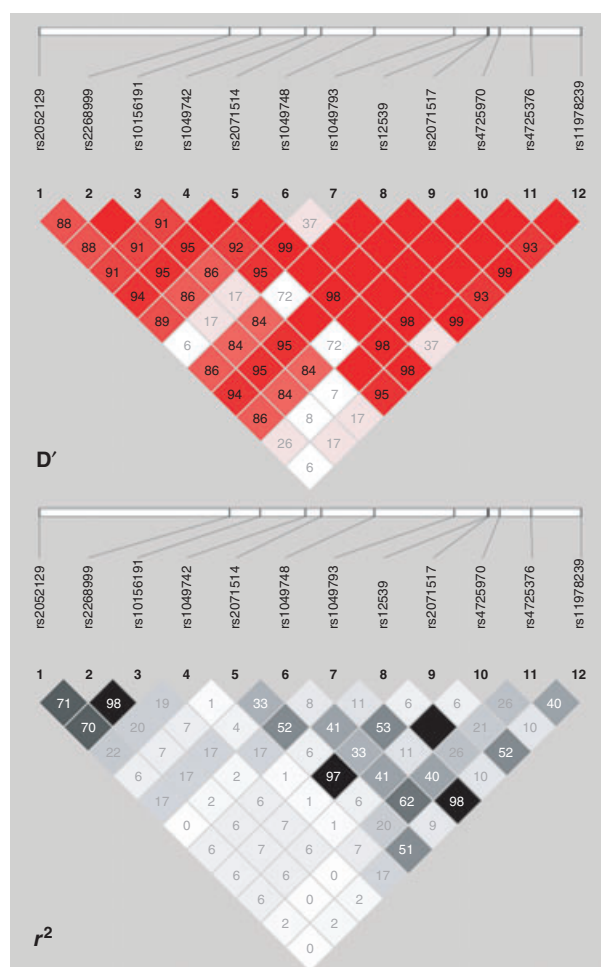


Figure 1 Linkage disequilibrium plot of the SNPs in the *DAO* gene based on the measure of normalized linkage disequilibrium deviation (D') (top – red colour scheme) and r^2 (below – grey colour scheme).

Moderate association of polymorphisms in the *DAO* gene with drug intolerance, but not with other single traits of histamine intolerance

There was an association of moderate significance of drug intolerance with rs2071514 (OR = 1.58; 95% CI 1.11; 2.25; P = 0.001); rs1049793 (OR = 1.43; 95% CI 1.05; 1.95; P = 0.02); rs2071517 (OR = 1.58; 95% CI 1.11; 2.26; P = 0.01) and rs11978239 (OR = 1.41; 95% CI 1.03; 1.92; P = 0.03). No associations were seen for the other SNPs investigated in the *DAO* or the *HNMT* gene with drug intolerance or other single traits (Table S6).

Reporter gene assays revealed a lower promoter activity of the T allele at rs2052129

To test the functional significance of the *DAO* promoter SNP rs2052129 associated with DAO serum activities, we cloned 981-bp fragments of the promoter sequence from individuals

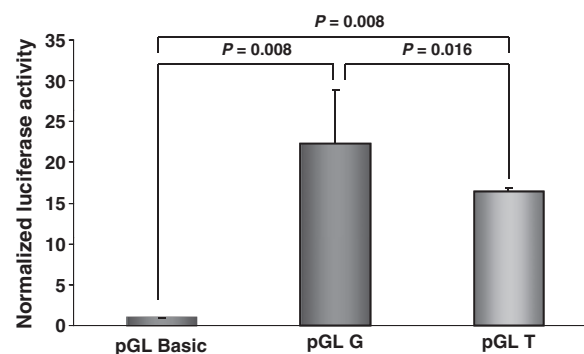


Figure 2 The promoter variant rs2052129 alters basal diamine oxidase (*DAO*) promoter activity. HEK293 cells were transiently co-transfected with a reporter construct containing either G or T at the location of rs2052129 fused to firefly luciferase gene and a control renilla luciferase plasmid. After 24 h, firefly luciferase activity in cell lysates was determined, related to renilla luciferase activity, and relative luciferase activity was normalized to the activity of a pGL2-Basic vector. Mean and SEM of eight independent co-transfection experiments are shown.

homozygous for either T or G at this SNP, and used these to drive luciferase expression in HEK293 cells. Transcriptional activities of the *DAO* promoter fragments with the minor allele (T) at rs2052129 are lower than those of fragments with the major allele (G) (P = 0.016) (Fig. 2).

Significantly lower relative DAO mRNA expression on PBMCs of patients with the risk genotype compared to wild-type controls

We observed a significantly lower DAO mRNA expression (P = 0.002) in PBMCs of homozygous carriers of the minor allele at rs2052129, rs2268999 and rs10156191 and a reduced DAO serum activity, when compared to patients with HIT and controls homozygous for the major allele at these loci and normal DAO serum activity (Fig. 3).

Discussion

Although a clear-cut definition and reliable epidemiologic data are still missing, HIT is increasingly being recognized as a distinct clinical entity and common cause of food intolerance. Despite evidence for a genetic predisposition to the development of other intolerances, the high interindividual variations in the expression of DAO in the gut (3) and the association of SNPs in the *DAO* gene with gastrointestinal or psychiatric diseases (8–12, 25), a potential genetic component in HIT has not been investigated yet. Recently, two *DAO* SNPs were shown to be associated with changes in DAO enzyme kinetics (13).

We could show a significant association of DAO serum activity with seven *DAO* SNPs. The promoter variant rs2052129 and two correlated more distal variants, rs2268999 and rs10156191, showed strong and highly significant effects on DAO serum activity, and were associated with

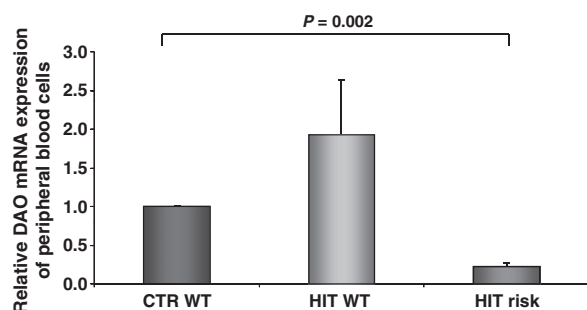


Figure 3 Association of diamine oxidase (*DAO*) polymorphisms with decreased *DAO* mRNA expression in PBMCs. Significantly lower relative *DAO* mRNA expression was observed in PBMCs of individuals homozygous for the risk-associated minor alleles at rs2052129, rs2268999 and rs10156191 (HIT RISK) when compared to individuals homozygous for the major allele ($n = 6$ for each group; mean value of relative mRNA expression and SEM).

HIT symptoms in patients with decreased *DAO* levels. Moreover, the minor allele of rs2052129 had reduced *DAO* promoter activity compared to the major allele in a reporter gene assay. The major allele G at rs2052129 in the quasi-palindromic sequence (imperfect inverted repeats) permits the formation of a hairpin structure (26). Nuclear acid structure studies have demonstrated the importance of the G–C base pair in stabilizing the RNA hairpin structure (27). According

to the thermal stability of hairpins, G allele at rs2052129 contributes to the formation of five trans G–C base pairs in the stem helix and leads to a stable hairpin structure, while the T allele confers a partially unpaired stem helix and diminishes the stability of the hairpin. Furthermore, the hairpin structure has been reported to play important biological roles in offering binding sites for proteins in the regulation of transcription and translation (28, 29). Thus, the stable secondary hairpin structure offered by G allele at rs2052129 might be involved in chromatin remodelling to facilitate the recruitment of transcription factors such as CREB, NFAT, AP1, SP1 to their binding sites to promote *DAO* gene transcription (Fig. 4). Mutagenesis mediated by imperfect inverted sequence configuring hairpin structures has been associated with human hereditary diseases (30, 31).

Furthermore, *DAO* mRNA expression was significantly decreased in PBMCs from homozygous carriers of the minor allele at rs2052129, rs2268999 and rs10156191, compared to homozygous carriers of the major allele. However, there was a higher reduction of mRNA transcripts than of the transcriptional activity of the promoter T variant which might result from a further post-transcriptional attenuation by the intron 1 SNP. Furthermore, some HIT subjects displayed mRNA abundance much exceeding the one found in controls, which eventually points to the heterogeneity of the syndrome.

Moreover, we also observed a significant association of a second group of highly correlated variants, rs2071514 (Exon 2,

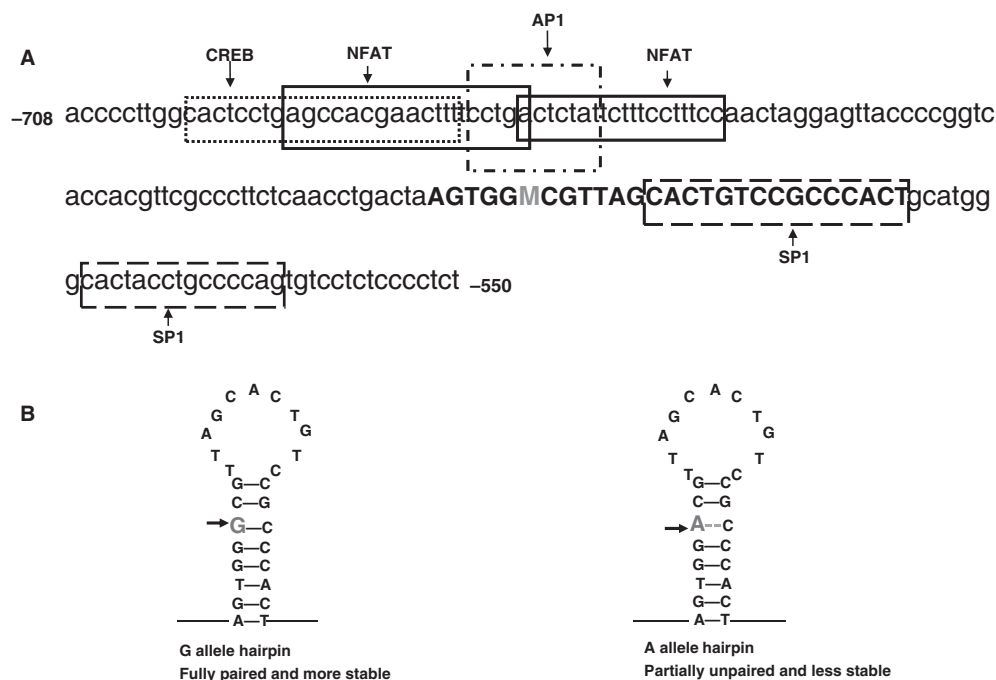


Figure 4 Impact of rs2052129 in quasi-palindromic sequence adopting a hairpin conformation. (A) The diagram illustrates a quasi-palindromic sequence (indicated in bold letters) in the promoter including rs2052129 (showed as M) and *in silico* (Gene2promoter

and GEMSLauncher) predicted transcription factor binding sites nearby. (B) G allele confers five trans G–C pairs in the stem helix to form a fully paired and stable hairpin structure, whereas the A allele causes a partially unpaired stem to destabilize the hairpin structure.

Ala414Ala), rs1049748 (Exon 3) and rs2071517 (3'UTR), with increased *DAO* levels. On the basis of LD measures, we believe those two regions represent distinct association signals, and thus, *DAO* levels seem to be influenced at least by two regulatory segments.

Our results confirm the previously reported association of rs10156191, but not the association of rs1049793 with reduced *DAO* serum activities (13). This might be partly explained by differences in both study design and populations. Although the previous study genotyped 134 subjects, they measured *DAO* serum activity only in 37 subjects (13) using a colorimetric method, whereas we compared genotype and *DAO* activity in a considerably larger population ($n = 484$) with a REA (32). However, more studies are needed to replicate the observed associations and clarify these differences.

In line with a reported lack of association of rs11558538 in the *HNMT* gene with migraine as a key symptom of HIT (33), we did not find any associations of this SNP with HIT in our study.

Our study has potential limitations that may influence the interpretation of our results. We recognize that HIT is a complex and largely unexplored disease, which is difficult to diagnose precisely. A reliable biomarker for the diagnosis is missing, as plasma histamine is very unstable and *DAO* serum activity is decreased only in half of the patients with HIT, but also in about 17% of healthy controls. Currently, standardized histamine provocations are being established for the diagnosis of HIT (16). We did only perform double-blind, placebo-controlled provocations as additional diagnostic criteria in ten patients, as because of the great effort, time and costs they are not practicable in larger epidemiologic studies. However, we tried to narrow down the phenotype as far as possible but at reasonable expense. By demanding presentation of ≥ 2 typical symptoms, improvement by histamine-free diet and antihistamines, measurement of *DAO* serum activities and exclusion of IgE-mediated food allergies and mastocytosis, we think that our cases were well defined.

We did not observe any significant association of *DAO* SNPs with the HIT phenotype alone. Only further specification of the phenotype including only patients with a reduced *DAO* serum activity compared to controls revealed an association with rs2052129, rs2268999, rs10156191. According to the hypothesis of Mendelian randomization, a causal variation in the genome should influence both the levels of a biomarker (*DAO*) and the risk of disease (HIT) (34). In our present study, among the SNPs associated with *DAO* levels, only three showed weak evidence for an association with HIT symptoms. Thus, different variants in the *DAO* gene are related to *DAO* levels, but not sufficiently to bring about the potentially associated disease state HIT. This finding does not contradict the concept of Mendelian randomization, because it has to be considered that intermediate-trait-associated genes are not necessarily directly associated with higher-order more remote disease traits in question, especially because their effects are observed also in healthy and in genetically at risk, but clinically unaffected individuals, and both effect sizes (that of the genotype on the intermediate

trait and that of the intermediate trait on the clinical endpoint) might be modest only (34, 35).

Further strengths of our study include the LD-based, haplotype-tagging approach, which offered good coverage of the common genetic variation across *DAO*, and our experiments indicating a functional role of the associated *DAO* promoter polymorphism rs2052129.

In summary, our results provide evidence for a genetic predisposition towards reduced *DAO* activities, which might contribute to clinical symptoms of HIT, but might also affect its role as a regulating enzyme in rapidly proliferating tissues (12). However, *DAO* gene variants alone are not sufficient to fully effectuate the potentially associated disease state of HIT, suggesting the requirement of environmental cofactors such as potential modifications in the alternate *N*-methyltransferase pathway, vesicular trafficking of both enzymes and the amine or the uptake of histamine by enterocytes.

Replication of our data in independent cohorts, which is ultimately the true test of any gene association study, are needed, as well as prospective studies in which genotyping is used as diagnostic tool to estimate the risk as well as the degree of impairment of *DAO* activity to evaluate a putative predictive value and usefulness of genetic testing.

Acknowledgments

We thank Dr. Friedrich Riffelmann (Department of Allergology and Internal Medicine, Fachkrankenhaus Kloster Grafschaft, Schmallingenberg) for his help with the recruitment of the patients.

Funding

This work was supported by grants from the Deutsche Forschungsgemeinschaft (DFG NO454/2-4, NO454/1-4; SFB704 TPA4) and a BONFOR grant of the University of Bonn. N.N. is supported by a Heisenberg Professorship of the DFG NO454/5-2. L.M. is supported by a BONFOR research fellowship of the University of Bonn. S. W. is supported by a Heisenberg fellowship of the DFG (WE 2678/4-1). Costs of the genotyping have been partly covered by Immundiagnostik AG.

All authors had full access to all data and take responsibility for the integrity of the data and the accuracy of the analysis.

Conflict of interest

We declare that we have no conflict of interest.

Author's contributions

Study concept and design: Novak, Maintz, Baurecht, Yu, Weidinger. Acquisition of data: Yu, Maintz. Recruitment of patients: Maintz. Genotyping: Rodríguez, Illig. Analysis and interpretation of data: Novak, Maintz, Yu, Baurecht, Weidinger. Drafting of the manuscript: Novak, Maintz, Yu, Weidinger. Critical revision of the manuscript for important intellectual content: Bieber. Obtained funding: Novak.

Supporting Information

Additional Supporting Information may be found in the online version of this article found at: www.wileyonlinelibrary.com

Data S1. Methods 1: Reporter gene assays.

Data S2. Methods 2: Isolation of peripheral blood mononuclear cells and quantitative real-time PCR analysis of DAO mRNA expression.

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Table S6. Association analysis of DAO and HNMT SNPs with single traits of histamine intolerance.

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**ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE DIAMINE
OXIDASE GENE WITH DIAMINE OXIDASE SERUM ACTIVITIES**

Revised online Repository

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eMethods 2: Isolation of peripheral blood mononuclear cells and quantitative real-time PCR analysis of DAO mRNA expression..

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Figure E1.

Overview of the gene structure and location of the 12 *DAO* SNPs investigated.

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Table E1. Phenotype characteristics of the study population.

Table E2. SNPs in the *DAO* and *HNMT* gene with dbSNP accession numbers (rs#), localization, and genotyping primers.

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Table E4. Association analysis of *DAO* and *HNMT* SNPs with DAO serum activity in the whole study population using a genotype model.

Table E5. Association analysis of *DAO* SNPs with HIT and decreased DAO serum activity versus controls using a genotype model and an additive genetic model.

Table E6: Association analysis of *DAO* and *HNMT* SNPs with single traits of histamine intolerance

emethods 1: Reporter gene assays

Primers for amplifying 981bp fragments were designed to clone the promoter region of the human DAO gene using genomic DNA from homozygous G or homozygous T carriers of SNP rs2052129 (5'-ACCGACGCGTCAATCCCTGAGCAACCAAAC-3' and 5'-TTCGCTCGAGGCACCTTCTGTGATGTGCTG-3') (21), (Genomatix Gene2promoter data base (Accession No. GXP_60303)). This fragment contained about 750bp 5' flanking region (including SNP rs2052129), a very short first exon and part of the first intron. Amplified fragments were cloned into luciferase reporter vector pGL2-Basic to generate two firefly luciferase reporter vectors, pGL2-DAO/G and pGL2-DAO/T, which differed only in one base. The sequencing and gene bank blast results showed that the cloned fragment was identical to the region (11124841 – 11125821) of NT_007914.14. The day before transfection, 1.5×10^5 Human Embryonic Kidney 293 (HEK293) cells (CRL-1537, ATCC, LGC Promochem, Teddington, United Kingdom) were seeded into 24-well culture plates in fresh Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal calf serum (FCS). 90% confluent HEK293 cells were transiently transfected with 0.8 μ g of one of the reporter constructs or pGL-Basic and 0.1 μ g of thymidine kinase-driven Renilla plasmid (pTK-RL, Promega, Madison, USA) as internal control of transfection efficiency by using lipofectamineTM 2000 (Invitrogen, Carlsbad, California). After 24 hours, cells were lysed and dual-luciferase measurement was performed utilizing the Dual-Luciferase Reporter Assay System (Promega). Relative luciferase activity was normalized to the level of the pGL2-Basic transfected sample (defined as 1.0). Eight independent experiments were performed in duplicates. Putative transcription factor binding sites were identified in silico using the 'Gene2promotor' and 'GEMS Launcher' software (Genomatix Software GmBH, Munich, Germany).

eMethods 2: Isolation of PBMCs and quantitative real-time PCR analysis of DAO mRNA expression.

We investigated the DAO mRNA expression of peripheral blood mononuclear cells (PBMCs) of HIT patients homozygous for the minor allele rs2052129, rs2268999 and rs10156191, and a reduced DAO serum activity compared to HIT patients and healthy controls homozygous for the major allele at these loci and a normal DAO serum activity (n=6 for each group). These SNPs were chosen because these were the SNPs showing the strongest association to DAO activity and the only SNPs, which were associated with the phenotype of HIT with reduced DAO serum activities. PBMCs have been isolated from heparin blood with the Lymphoprep gradient technique as described in the manufacturer's protocol (Axis Shield, Oslo, Norway). Total RNA was isolated from PBMCs using the RNeasy Mini kit (Quiagen, Hilden, Germany) including digestion of genomic DNA. 300ng of total isolated RNA was subjected to cDNA synthesis in a total reaction volume of 20µl of TaqMan reverse transcription reagents with random hexamers according to the manufacturer's instructions (Applied Biosystems, Darmstadt, Germany). The prepared cDNA was diluted with 20µl DNase-free water and 9µl of diluted cDNA was amplified for 40 quantification cycles using DAO taqman probe (Hs00175631_m1) or 18s taqman probe (4310893) in a total volume of 20µl of TaqMan assay Master Mix buffer system (Applied Biosystems) according to the recommendations of the manufacturer in an ABI Prism 7300 Sequence Detection System (Applied Biosystems). All analyses were conducted in duplicates and the experimental performance followed the MIQE guidelines (22). Expression of DAO was normalized to endogenous control 18s and the expression of CTR group was arbitrarily set to 1. Relative quantification and calculation of the range of confidence was performed using the comparative $\Delta\Delta CT$ method (23).

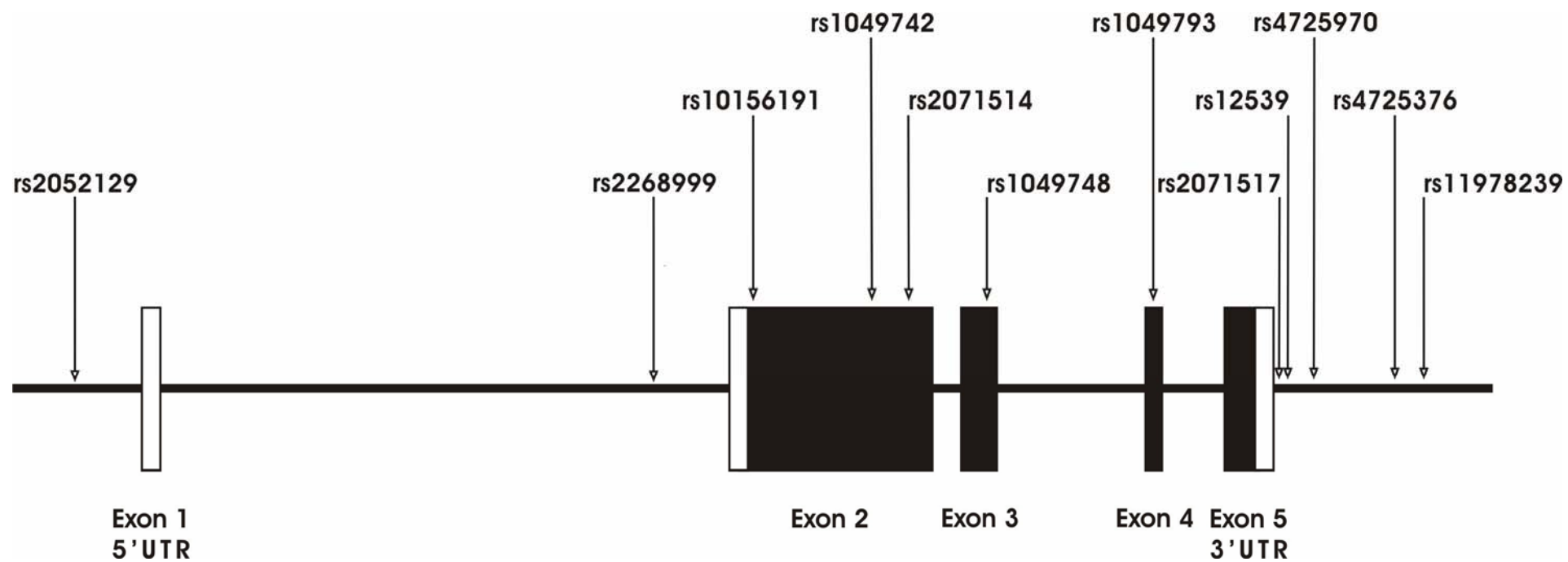


Figure E1. Overview of the gene structure and location of the 12 *DAO* SNPs investigated (NCBI RefSeq ID NM_001091)

Table E1. Phenotype of patients with histamine intolerance (HIT, n=285) and healthy controls (CTR, n=199), and of subjects with a normal (≥ 10 U/ml) diamine oxidase (DAO) serum activity (n=309) compared to those with a reduced (<10 U/ml) DAO activity (n=175).

Phenotype characteristics	CTR	HIT			DAO ≥ 10 U/ml	DAO <10 U/ml		
	n (%)	n (%)	χ^2	P-value	n (%)	n (%)	χ^2	P-value
gastrointestinal symptoms	20 (10.1)	219 (84.6)	250.36	$<1.0 \times 10^{-16}$	130 (43.9)	109 (66.1)	20.80	$<.0001$
chronic headache/migraine	26 (13.1)	176 (64.2)	123.35	$<1.0 \times 10^{-16}$	114 (37.7)	88 (51.2)	8.06	.005
premenstrual headache/dysmenorrhea	21 (10.6)	116 (43.4)	68.36	1.1×10^{-16}	77 (25.6)	60 (35.5)	14.65	.001
flushing	8 (4.1)	159 (58.5)	146.06	$<1.0 \times 10^{-16}$	92 (30.9)	76 (44.2)	8.41	.004
urticaria/angioedema	6 (3.1)	85 (35.0)	66.90	3.3×10^{-16}	54 (18.8)	37 (23.9)	1.81	.39
drug intolerance	19 (9.5)	167 (60.7)	126.84	$<1.0 \times 10^{-16}$	107 (35.2)	79 (45.4)	4.85	.03
intolerance of histamine-rich food	11 (5.5)	236 (86.4)	302.12	$<1.0 \times 10^{-16}$	131 (43.2)	116 (67.4)	25.76	$<.0001$
alcohol intolerance	18 (9.0)	219 (81.1)	273.78	$<1.0 \times 10^{-16}$	130 (43.3)	107 (62.6)	16.13	$<.0001$
positive family history for histamine intolerance	13 (6.5)	90 (33.6)	48.61	3.1×10^{-12}	53 (17.8)	50 (29.4)	8.41	.01
mean DAO serum activity [U/ml]	20.65 $\pm$ 13.28	11.32 $\pm$ 8.28		$<1 \times 10^{-6}$				
reduced DAO serum activity <10 U/ml	34 (17.1)	141 (49.5)	53.25	2.9×10^{-13}				

Table E2. SNPs in the *Diamine oxidase (DAO)* and *histamine N-methyltransferase (HNMT)* gene with dbSNP accession numbers (rs#), localization, and genotyping primers.

dbSNP rs#	Alleles	Amino acid change	Gene region	Polymerase chain reaction primer 5'→3'		Extension primer 5'→3'	Call-rate	MAF	HWE
DAO									
rs2052129	G>T	-	5'	2 nd 1 st	ACGTTGGATGAGGTAGTGCCCATGCAGTG ACGTTGGATGTTCCAAC TAGGAGTTACCCC	GCAGTGGGCGGACAGTGCTAACG	0.9938	0.2337	0.7319
rs2268999	A>T	-	Intron1	2 nd 1 st	ACGTTGGATGCTAGGAGCTAAGCACTGTTG ACGTTGGATGCACGCTGCTTAAACTCTCTG	CACTGTTGTCATTATTTTCATTTTA	0.9938	0.2487	0.3080
rs10156191	C>T	Thr16 Met	Exon 2	2 nd 1 st	ACGTTGGATGGTGGCTGCCATCCTGATGC ACGTTGGATGTAGGTCTGAAAACACCCCTG	CCATCCTGATGCTGCAGA	0.9959	0.2513	0.3584
rs1049742	C>T	Ser332 Phe	Exon 2	2 nd 1 st	ACGTTGGATGAGGGCAACGCTGTGCTCTAC ACGTTGGATGCCAAGTGCACGTT CAGGAC	CCGGCTGCGCTCCTCCT	0.9938	0.0729	0.0414
rs2071514	G>A	Ala443 Ala	Exon 2	2 nd 1 st	ACGTTGGATGGGCGGCACTTTAATTCCAAC ACGTTGGATGACAGTTGAAGTTGTCCGCAG	GGTGGCTTCAACTTCTATGC	0.9938	0.1859	0.3788
rs1049748	T>C	Pro574 Pro	Exon 3	2 nd 1 st	ACGTTGGATGAGTGGATCTGCAGGCGGTA ACGTTGGATGCCTTCCGCTTCAAAGGA	TGGTAAAGAGCAGGTA CTT	0.9938	0.3995	0.9435
rs1049793	C>G	His645 Asp	Exon 4	2 nd 1 st	ACGTTGGATGGAAACTGCTCAAAGACCACG ACGTTGGATGTGTGCAGCAGCAGCATCTAC	AAAGACCACGGGCGGGT	0.9938	0.3015	0.2982
rs12539	C>T	-	Exon 5	2 nd 1 st	ACGTTGGATGGCAAGAAGCAGCACACAGAG ACGTTGGATGAGTT CCTCCCAGGAAGCCCA	GCAGAGCGAGGCTCTGAG	0.9876	0.2194	0.1369
rs2071517	G>A	-	3'	2 nd 1 st	ACGTTGGATGGTGTTCCTACACACATGGC ACGTTGGATGCACTGGGGCAGACAATAAAC	CACATGGCCCTGTGCCTCCC	0.9938	0.1834	0.4225
rs4725970	C>T	-	3'	2 nd 1 st	ACGTTGGATGAACAATCTGATAGGTGCAGG ACGTTGGATGCTACTGCAGTCCAGAAAAGC	AGGTGCAGGATTT CAGC	0.9876	0.2194	0.1369
rs4725376	G/A	-	3'	2 nd 1 st	ACGTTGGATGTCAGCACACAGAGTATCCTC ACGTTGGATGATGAACTGTGGCACAACCTC	GTATCCTCATTCGTGTGTTTCT	0.9938	0.4849	0.8214
rs11978239	A>G	-	3'	2 nd 1 st	ACGTTGGATGGAGCCTCCATTAGGAACAAG ACGTTGGATGACCACAACTTAGTGGCCTC	GACCTCAGATGGTAAGAGAAT	0.9938	0.3015	0.2982
HNMT									
rs11558538	C>T	Thr105 Ile	Exon 4	2 nd 1 st	ACGTTGGATGATGGTGTGT CACCTCTTCTC ACGTTGGATGGATGTCTCCTTATGCCAAGC	TGTATTTTAGAGCTTGTAGCCAAGA	0.9938	0.1030	0.4956

Minor allele frequency (MAF) and Hardy-Weinberg-Equilibrium (HWE) were calculated for control subjects, callrate for all subjects.

Table E3. Genotype distribution of *DAO* and *HNMT* SNPs in patients with HIT and healthy controls considering their activity of the histamine degrading enzyme DAO (normal: ≥ 10 U/ml; reduced: < 10 U/ml).

SNP	CTR (n=199);n (%)						HIT (n=285);n (%)					
	DAO ≥ 10 U/ml (n=165)			DAO < 10 U/ml (n=34)			DAO ≥ 10 U/ml (n=144)			DAO < 10 U/ml (n=141)		
<i>DAO</i>	wt	het	hom	wt	het	hom	wt	het	hom	wt	het	hom
rs2052129	103 (62.4)	56 (33.9)	6 (3.6)	13 (38.2)	17 (50.0)	4 (11.8)	108 (75.0)	33 (22.9)	2 (1.4)	58 (41.1)	58 (41.1)	21 (14.9)
rs2268999	104 (63.0)	52 (31.5)	9 (5.5)	11 (32.4)	17 (50.0)	6 (17.6)	105 (72.9)	36 (25.0)	2 (1.4)	56 (39.7)	57 (40.4)	26 (18.4)
rs10156191	103 (62.4)	53 (32.1)	9 (5.5)	11 (32.4)	17 (50.0)	6 (17.6)	105 (72.9)	37 (25.7)	2 (1.4)	55 (39.0)	58 (41.1)	26 (18.4)
rs1049742	148 (89.7)	16 (9.7)	1 (0.6)	25 (73.5)	7 (20.6)	2 (5.9)	130 (90.3)	13 (9.0)	0 (0.0)	109 (77.3)	28 (19.9)	2 (1.4)
rs2071514	105 (63.6)	56 (33.9)	4 (2.4)	25 (73.5)	8 (23.5)	1 (2.9)	88 (61.1)	47 (32.6)	8 (5.6)	4 (2.8)	33 (23.4)	102 (72.3)
rs1049748	53 (32.1)	85 (51.5)	27 (16.4)	19 (55.9)	10 (29.4)	5 (14.7)	35 (24.3)	84 (58.3)	24 (16.7)	65 (46.1)	53 (37.6)	21 (14.9)
rs1049793	81 (49.1)	73 (44.2)	11 (6.7)	13 (38.2)	17 (50.0)	4 (11.8)	72 (50.0)	56 (38.9)	15 (10.4)	67 (47.5)	63 (44.7)	9 (6.4)
rs12539	98 (59.4)	52 (31.5)	12 (7.3)	25 (73.5)	8 (23.5)	1 (2.9)	79 (54.9)	59 (41.0)	4 (2.8)	94 (66.7)	39 (27.7)	7 (5.0)
rs2071517	106 (64.2)	55 (33.3)	4 (2.4)	25 (73.5)	8 (23.5)	1 (2.9)	87 (60.4)	48 (33.3)	8 (5.6)	101 (71.6)	35 (24.8)	3 (2.1)
rs4725970	98 (59.4)	52 (31.5)	12 (7.3)	25 (73.5)	8 (23.5)	1 (2.9)	79 (54.9)	59 (41.0)	4 (2.8)	94 (66.7)	39 (27.7)	7 (5.0)
rs4725376	44 (26.7)	82 (49.7)	39 (23.6)	8 (23.5)	19 (55.9)	7 (20.6)	39 (27.1)	76 (52.8)	28 (19.4)	33 (23.4)	68 (48.2)	38 (27.0)
rs11978239	81 (49.1)	73 (44.2)	11 (6.7)	13 (38.2)	17 (50.0)	4 (11.8)	72 (50.0)	56 (38.9)	15 (10.4)	66 (46.8)	64 (45.4)	9 (6.4)
<i>HNMT</i>												
rs11558538	132 (80.0)	31 (18.8)	2 (1.2)	29 (85.3)	4 (11.8)	1 (2.9)	114 (79.2)	27 (18.8)	2 (1.4)	109 (77.3)	30 (21.3)	0 (0.0)

Table E4. Association analysis of *DAO* and *HNMT* SNPs with DAO serum activity in the whole study population using a genotype model

SNP DAO	Location	Effects	DAO serum activity	P-value
			Est (95% CI)	
rs2052129	chr7:150179905	het. vs. wt.	-0.68 (-0.96; -0.40)	1.8 x10 ⁻⁶
		hom. vs. wt.	-1.50 (-2.02; -0.99)	2.1 x10 ⁻⁸
rs2268999	chr7:150183898	het. vs. wt.	-0.74 (-1.01; -0.46)	2.4 x10 ⁻⁷
		hom. vs. wt.	-1.46 (-1.92; -1.01)	7.6 x10 ⁻¹⁰
rs10156191	chr7:150184538	het. vs. wt.	-0.72 (-1.00; -0.45)	4.0 x10 ⁻⁷
		hom. vs. wt.	-1.46 (-1.92; -1.01)	8.3 x10 ⁻¹⁰
rs1049742	chr7:150185486	het. vs. wt.	-0.70 (-1.08 ; -0.31)	4.9 x10 ⁻⁴
		hom. vs. wt.	-1.73 (-3.03; -0.43)	9.2 x10 ⁻³
rs2071514	chr7:150185820	het. vs. wt.	0.42 (0.13; 0.71)	5.1 x10 ⁻³
		hom. vs. wt.	0.42 (-0.31; 1.15)	.26
rs1049748	chr7:150186935	het. vs. wt.	0.65 (0.36; 0.94)	1.0 x10 ⁻⁵
		hom. vs. wt.	0.56 (0.17; 0.96)	5.5 x10 ⁻³
rs1049793	chr7:150188598	het. vs. wt.	0.01 (-0.27; 0.29)	.93
		hom. vs. wt.	-0.09 (-0.60; 0.42)	.74
rs12539	chr7:150189299	het. vs. wt.	0.18 (-0.11; 0.48)	.21
		hom. vs. wt.	0.42 (-0.21; 1.04)	.19
rs2071517	chr7:150189332	het. vs. wt.	0.41 (0.12; 0.70)	5.9 x10 ⁻³
		hom. vs. wt.	0.46 (-0.29; 1.21)	.23
rs4725970	chr7:150189544	het. vs. wt.	0.18 (-0.11; 0.48)	.21
		hom. vs. wt.	0.42 (-0.21; 1.04)	.19
rs4725376	chr7:150190220	het. vs. wt.	0.04 (-0.28; 0.37)	.79
		hom. vs. wt.	-0.25 (-0.64; 0.13)	.20
rs11978239	chr7:150191255	het. vs. wt.	0.01 (-0.27; 0.29)	.93
		hom. vs. wt.	-0.09 (-0.60; 0.42)	.74
HNMT				
rs1155853	chr2:138476119	het. vs. wt.	-0.05 (-0.39; 0.29)	0.77
		hom. vs. wt.	0.34 (-0.99; 1.67)	0.61

Est=estimated linear effect on squareroot-transformed DAO activity, CI=Confidence interval.

Linear regression analysis on squareroot-transformed DAO serum activity for every marker adjusted for age and sex.

Table E5. Association analysis of *DAO* SNPs with HIT and decreased *DAO* serum activity versus controls using a genotype model and an additive genetic model.

SNP <i>DAO</i>	Location	HIT+DAO<10 U/ml vs CTR				
		Genotype model			Additive model	
		Effects	OR (95% CI)	P-value	OR (95% CI)	P-value
rs2052129	chr7:150179905	het. vs. wt.	1.55 (0.95; 2.54)	.08	1.82 (1.27; 2.60)	1.1 x10 ⁻³
		hom. vs. wt.	4.11 (1.72; 9.83)	1.5 x10 ⁻³		
rs2268999	chr7:150183898	het. vs. wt.	1.67 (1.01; 2.77)	.04	1.86 (1.33; 2.62)	3.5 x10 ⁻⁴
		hom. vs. wt.	3.82 (1.78; 8.18)	5.8 x10 ⁻⁴		
rs10156191	chr7:150184538	het. vs. wt.	1.70 (1.03; 2.80)	.04	1.88 (1.33; 2.64)	3.1 x10 ⁻⁴
		hom. vs. wt.	3.86 (1.80; 8.28)	5.4 x10 ⁻⁴		
rs1049742	chr7:150185486	het. vs. wt.	1.75 (0.93; 3.33)	.09	1.44 (0.83; 2.48)	.19
		hom. vs. wt.	0.80 (0.12; 5.43)	.82		
rs2071514	chr7:150185820	het. vs. wt.	0.78 (0.47; 1.30)	.34	0.80 (0.52; 1.25)	.33
		hom. vs. wt.	0.80 (0.17; 3.70)	.78		
rs1049748	chr7:150186935	het. vs. wt.	0.62 (0.38; 1.03)	.06	0.79 (0.57; 1.09)	.15
		hom. vs. wt.	0.71 (0.36; 1.42)	.33		
rs1049793	chr7:150188598	het. vs. wt.	1.01 (0.63; 1.62)	.97	0.96 (0.66; 1.39)	.81
		hom. vs. wt.	0.82 (0.32; 2.11)	.68		
rs12539	chr7:150189299	het. vs. wt.	0.82 (0.49; 1.36)	.44	0.80 (0.54; 1.19)	.27
		hom. vs. wt.	0.62 (0.22; 1.75)	.37		
rs2071517	chr7:150189332	het. vs. wt.	0.72 (0.43; 1.21)	.21	0.81 (0.52; 1.27)	.36
		hom. vs. wt.	1.01 (0.25; 4.11)	.99		
rs4725970	chr7:150189544	het. vs. wt.	0.82 (0.49; 1.36)	.44	0.80 (0.54; 1.19)	.27
		hom. vs. wt.	0.62 (0.22; 1.75)	.37		
rs4725376	chr7:150190220	het. vs. wt.	1.13 (0.64; 1.99)	.67	1.18 (0.85; 1.64)	.32
		hom. vs. wt.	1.39 (0.73; 2.67)	.32		
rs11978239	chr7:150191255	het. vs. wt.	1.03 (0.64; 1.65)	.92	0.97 (0.67; 1.40)	.85
		hom. vs. wt.	0.83 (0.32; 2.12)	.70		

OR=odds ratio, CI=Confidence interval. Logistic regression analysis for every marker adjusted for age and sex.

Table E6: Association analysis of *DAO* and *HNMT* SNPs with single traits of histamine intolerance

Trait	Gene SNP	<i>DAO</i>												<i>HNMT</i>
		rs 2052129	rs 2268999	rs 10156191	rs 1049742	rs 2071514	rs 1049748	rs 1049793	rs 12539	rs 2071517	rs 4725970	rs 4725376	rs 11978239	rs 11558538
Gastro-intestinal symptoms	OR	0.97	0.97	0.97	0.95	1.13	1.09	0.96	0.96	1.14	0.96	1.03	0.97	1.04
	95% CI	0.72; 1.32	0.72; 1.30	0.73; 1.30	0.59; 1.55	0.80; 1.59	0.83; 1.43	0.72; 1.30	0.69; 1.34	0.81; 1.62	0.69; 1.34	0.78; 1.35	0.72; 1.31	0.67; 1.61
	P	0.87	0.82	0.86	0.85	0.50	0.54	0.81	0.82	0.45	0.82	0.84	0.85	0.86
Chronic headache	OR	0.92	0.97	0.97	1.21	0.97	1.02	0.99	1.02	0.97	1.02	0.97	0.98	1.16
	95% CI	0.68; 1.24	0.73; 1.29	0.73; 1.29	0.75; 1.93	0.69; 1.35	0.78; 1.33	0.74; 1.32	0.74; 1.39	0.69; 1.36	0.74; 1.39	0.75; 1.27	0.73; 1.31	0.76; 1.77
	P	0.57	0.82	0.82	0.43	0.85	0.89	0.94	0.93	0.85	0.93	0.84	0.88	0.49
Flushing	OR	0.95	1.02	1.01	0.78	1.16	0.94	0.99	0.75	1.20	0.75	1.21	1.00	0.88
	95% CI	0.69; 1.30	0.75; 1.37	0.75; 1.36	0.47; 1.31	0.82; 1.64	0.71; 1.25	0.73; 1.34	0.54; 1.06	0.85; 1.70	0.54; 1.06	0.92; 1.60	0.74; 1.35	0.55; 1.39
	P	0.73	0.92	0.96	0.35	0.40	0.67	0.92	0.10	0.30	0.10	0.17	0.98	0.58
Urticaria/ Angio-edema	OR	0.82	0.85	0.84	0.95	1.03	1.26	0.94	1.28	1.00	1.28	0.86	0.93	0.79
	95% CI	0.55; 1.22	0.59; 1.24	0.58; 1.22	0.52; 1.73	0.68; 1.56	0.90; 1.76	0.65; 1.35	0.87; 1.87	0.65; 1.51	0.87; 1.87	0.61; 1.19	0.64; 1.33	0.45; 1.39
	P	0.32	0.40	0.35	0.86	0.88	0.18	0.73	0.21	0.98	0.21	0.36	0.68	0.41
Drug intolerance	OR	0.88	0.94	0.95	1.10	1.58	1.22	1.43	0.85	1.58	0.85	0.82	1.41	1.17
	95% CI	0.64; 1.21	0.69; 1.27	0.71; 1.29	0.66; 1.84	1.11; 2.25	0.92; 1.62	1.05; 1.95	0.60; 1.19	1.11; 2.26	0.60; 1.19	0.62; 1.09	1.03; 1.92	0.75; 1.84
	P	0.44	0.69	0.76	0.70	0.01	0.17	0.02	0.34	0.01	0.34	0.17	0.03	0.48
Intolerance of histamine-rich food	OR	1.04	1.05	1.05	0.92	0.95	1.06	0.88	1.08	0.97	1.08	1.03	0.89	1.03
	95% CI	0.76; 1.41	0.78; 1.41	0.79; 1.42	0.57; 1.51	0.67; 1.33	0.81; 1.40	0.65; 1.19	0.77; 1.49	0.69; 1.37	0.77; 1.49	0.78; 1.35	0.66; 1.20	0.66; 1.60
	P	0.82	0.75	0.72	0.75	0.75	0.67	0.41	0.66	0.87	0.66	0.85	0.43	0.91
Alcohol intolerance	OR	0.89	0.98	0.99	1.03	1.15	1.00	1.06	0.88	1.12	0.88	1.01	1.07	1.02
	95% CI	0.65; 1.21	0.73; 1.33	0.73; 1.33	0.63; 1.70	0.81; 1.64	0.76; 1.33	0.78; 1.45	0.63; 1.23	0.78; 1.59	0.63; 1.23	0.77; 1.34	0.79; 1.46	0.65; 1.59
	P	0.44	0.91	0.94	0.91	0.43	0.98	0.69	0.45	0.54	0.45	0.92	0.66	0.93
Positive family history for HIT	OR	1.14	1.13	1.14	1.56	0.97	0.82	1.14	0.79	0.93	0.79	1.06	1.12	1.12
	95% CI	0.80; 1.62	0.81; 1.59	0.81; 1.60	0.93; 2.61	0.65; 1.45	0.59; 1.13	0.81; 1.60	0.53; 1.18	0.62; 1.40	0.53; 1.18	0.77; 1.45	0.79; 1.58	0.68; 1.86
	P	0.46	0.48	0.44	0.09	0.88	0.23	0.47	0.25	0.72	0.25	0.73	0.52	0.65

HIT= histamine intolerance; OR=odds ratio, CI=Confidence interval, $P=P$ -value.

Logistic regression analysis for every marker adjusted for age and sex.