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Endogenous Histamine and Cortisol Levels in Subjects with Different Histamine N-Methyltransferase C314T Genotypes:

A Pilot Study

Yuen Yi Hon[1], **William J. Jusko**[2], **Hong-Hao Zhou**[3], **Guo-Lin Chen**[3], **Dong Guo**[3], **Gan Zhou**[3], **Vicky E. Spratlin**[1], and **Michael W. Jann**[1]

[1] Department of Clinical and Administrative Sciences, Mercer University Southern School of Pharmacy, Atlanta, Georgia, USA

[2] Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, State University of New York at Buffalo, Buffalo, New York, USA

[3] Pharmacogenetics Research Institute, Institute of Clinical Pharmacology, Central South University, Changsha, Hunan, People's Republic of China

Abstract

Background—Histamine N-methyltransferase (HNMT) catalyzes the methylation of histamine and plays an important role in histamine biotransformation in bronchial epithelium. Enzymatic activity of HNMT has been shown to be regulated by genetic factors, including polymorphisms in the *HNMT* gene. In this pilot study we determined endogenous levels of histamine and Cortisol in plasma and whole blood samples from subjects with different genotypes for the *HNMT* C314T polymorphism, and investigated whether these parameters differed between individuals with the *HNMT* CC genotype and those with the CT genotype.

Methods—Blood samples were collected from 48 unrelated volunteers (36 males, 12 females), aged 21-40 years, who participated in the study. PCR-restriction fragment length polymorphism analysis was used to determine *HNMT*C314T genotypes. Erythrocyte HNMT activity was determined as well as plasma and whole blood levels of histamine and Cortisol. Two-group comparisons of the various parameters were analyzed by Blocked Wilcoxon test and Wilcoxon Rank Sum test as appropriate.

Results—Thirty-seven subjects (24 Caucasians, three African Americans, one Middle Eastern, five Indians, three Chinese, and one Filipino) were found to have the homozygous CC genotype. Ten subjects (eight Caucasians, one Middle Eastern, and one Chinese) were heterozygous and one individual (Pakistani) was homozygous for the variant 314T allele. The frequency of *HNMT* CT heterozygotes in the small Caucasian cohort was 0.125. Median enzyme activity was significantly lower in subjects with the heterozygous CT genotype than in those with the homozygous CC genotype (485 vs 631 U/mL of red blood cells; p = 0.023). A broad range of histamine levels in

Correspondence and offprints: Dr *Yuen Yi Hon*, Clinical Pharmacokinetics Research Laboratory, Clinical Center Pharmacy Department, National Institutes of Health, Room 1N-257, Bldg 10, 10 Center Drive, Bethesda, MSC-1196, MD 20892, USA. chon@cc.nih.gov.

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plasma and whole blood was observed for all subjects. Whereas the median plasma histamine level was found to be higher in heterozygotes for the wild-type 314C allele than homozygotes (3.32 vs 2.30 nmol/L; p = 0.021), there was no difference between the two groups in histamine levels in whole blood. Cortisol levels were similar between individuals with the homozygous CC genotype and those with the heterozygous CT genotype.

Conclusion—Wide variability of plasma and whole-blood histamine levels was observed in subjects with different *HNMT*C3HT genotypes. Endogenous levels of histamine are likely to be affected by various genes and polymorphisms.

Histamine is an important mediator in the body; it is involved in the regulation of numerous physiological and pathophysiological processes, including gastric acid secretion, central nervous system functioning, bronchial asthma, and hypersensitivity reactions. Histamine is formed by decarboxylation of histidine, a pathway that is catalyzed by histidine decarboxylase,[1] and is metabolized by diamine oxidase and histamine N-methyltransferase (HNMT).[2] HNMT catalyzes the N-methylation of histamine and plays a dominant role in histamine biotransformation in bronchial epithelium.[3] It is expressed in human kidney, liver, spleen, prostate, ovary, colon, and spinal cord, with particularly high levels of expression in kidney and liver.[4] Enzymatic activity of HNMT has been shown to be regulated by inheritance,[5,6] and inter-individual variation of HNMT activity has been demonstrated in Caucasian and Han Chinese populations.[4,7]

The variability of the HNMT phenotype can be explained in part by polymorphisms in the *HNMT* gene. Thus far, a total of six single nucleotide polymorphisms (SNPs) have been reported in the *HNMT* gene, and most of these SNPs are in linkage disequilibrium. Five of these SNPs have been identified in the non-coding region, with three SNPs in the 5'-flanking region (T-1637C, T-463C, and C-41 IT) and two others in the 3'-untranslated region (A939G and A1097T).[4,7,8] In females, the T-1637C and T-463C tended to be associated with low HNMT activity, whereas the A939G and A1097T appeared to be associated with increased activity.[7] One SNP has been found in the coding region of the gene; a C to T transition at nucleotide 314 (in exon 4) causes an amino acid change of threonine (T) to isoleucine (I) at codon l 15. Transient expression of the 314T variant in COS-1 cells has been shown to result in low levels of immunoreactive HNMT protein and activity.[4] Moreover, lower levels of HNMT activity have been found in red blood cell (RBC) lysates from individuals who are heterozygous for the 314T allele than in those homozygous for the wild-type 314C allele.[7]

Whereas the genotype-phenotype relationship for the *HNMT* C314T polymorphism has been clearly demonstrated in previous studies,[4,9] the influence of *HNMT* C314T genotype on the enzyme substrate, histamine, has not been studied. To this end, we undertook a pilot investigation to determine the levels of histamine in plasma and whole blood samples in a small group of volunteers, and to examine the potential effects of *HNMT* genotype on endogenous histamine levels. Theoretically, individuals who carry the variant 314T allele have lower HNMT activity and, therefore, have higher histamine levels. Since the hypothalamic-pituitary-adrenal axis has been shown to be activated by intra-cerebroven-

tricular administration of histamine,'10121 we also measured plasma Cortisol levels in this study to determine if Cortisol is influenced by *HNMT* genotype.

Methods

Subject Enrollment

The pilot study was performed at the Center for Clinical Research, Mercer University Southern School of Pharmacy, Atlanta, Georgia, USA. Written informed consent was approved by the Mercer University Institutional Review Board for Research Involving Human Subjects and was obtained from all subjects prior to the study. This investigation was part of a main study to determine the pharmacokinetics and pharmacodynamics of a steroid in healthy subjects with different *HNMT* C314T genotypes. Healthy volunteers, aged 20-50 years, were eligible for this part of the study and were screened for *HNMT* genotype for the follow-up pharmacokinetic study. Women who participated in this study were not pregnant and had regular monthly menstruation.

Sample Collection and Preparation

A 15mL random blood sample was collected from each subject. Each sample was divided into four different tubes and prepared separately. From the original blood sample, 6mL was used for the determination of histamine and Cortisol levels. Of the 6mL, 4mL was collected into a pre-chilled plastic EDTA-containing collection tube. After centrifuging at 3000 rpm at 4°C for 15 minutes, plasma was carefully aspirated, frozen, and stored at −80°C for later determinations of plasma histamine and Cortisol levels. The remaining 2mL of blood was collected into a plastic heparin-containing collection tube at room temperature, transferred into a cryovial, frozen, and then stored at −80°C until measurement of the whole-blood histamine level.

The remaining 9mL of the original blood sample was divided into aliquots and used for *HNMT* genotype and enzyme activity assays. Of this 9mL, 4mL was collected into an EDTA-containing tube and placed on ice immediately. This sample was centrifuged at 3000 rpm at 4°C for 15 minutes. The buffy coat was aspirated, collected, and stored at −80°C until DNA extraction and genotype analysis were performed. The remaining 5mL of blood was placed into a heparin-containing tube and a RBC lysate was prepared as previously described elsewhere.[13] Briefly, RBCs were washed twice with normal saline. The packed RBCs (pRBCs) were resus-pended with 2mL of normal saline, and the hematocrit of the suspension was measured using a microcapillary tube after centrifuging in a micro-hematocrit centrifuge (Clay Adams, Parsippa-ny, NJ, USA). Ice-cold water (8mL) was then added into the suspension resulting in lysis of the RBCs. The lysate was then centrifuged at 13 OOOg for 10 min, following which 2 mL of the supernatant was collected into a cryovial, frozen, and stored at −80°C for HNMT phenotype analysis.

Polymerase Chain Reaction - Restriction Fragment Length Polymorphism Analysis of Histamine N-Methyltransferase (*HNMT*) C314T Genotype

DNA was extracted using the QIAmp® DNA blood mini kit (QIAGEN Inc., Valencia, CA, USA) and was stored at −20°C for later genotype analysis. The HNMT C314T

polymorphism was determined by PCR-RFLP analysis as previously described elsewhere.[14] In brief, exon 4 was amplified using M13-tagged intron-based primers, I3F(-119) forward and I4R191 reverse (generously provided by Dr Weinshilboum from the Mayo Clinic), using a PTC-100 Thermal Cycler (MJ Research, Waltham, MA, USA) and PCR Core System II (Promega, Madison, WI, USA). After amplification, 25μL of the reaction mixture was digested with EcoRV at 37°C for 4 hours. The final products were separated by agarose gel electrophoresis and visualized by staining with ethidium bromide. Whereas the PCR product from the wild-type 314C allele (476bp) remained intact, the variant 314T allele was digested to give fragments of 322 and 154bp.

Red Blood Cell HNMT Activity Assay

RBC HNMT activity was measured using a radiochemical assay, details of which have been described previously by Van Loon et al.[15] Our assay has been validated, optimized and used to determine HNMT activity in previous studies.[7,9] This assay is based on the conversion of histamine to radioactively labeled Nx-methylhistamine with (methyl-^{3}H) S-adenosylmethionine (AdoMet) as the methyl donor.[15] Radioactivity was measured using a Beckman LS3801 Liquid Scintillation Counter (Beckman Instruments, Fullerton, CA, USA). One unit of enzyme activity represents the formation of 1 pmol of NT-methylhistamine per hour of incubation at 37°C per mL of pRBCs. All samples were measured within a 2-week period in which the same batch of reagents, including the radiolabeled and non-radiolabeled AdoMet, was used. Almost all of the samples were measured on two or more occasions and inconsistent results were not accepted. Intra- and inter-day coefficients of variation (CV) were <10%.

Determination of Histamine and Cortisol Levels

Histamine levels were determined in plasma and whole blood using a commercial enzyme immunoassay kit (Histamine ELISA; Immunotech, Marseille, France) according to the manufacturer's instructions. The standard curve was fitted and the concentration in the samples was calculated by a four-parameter logistic function using SigmaPlot® 8.0 (SPSS Inc., Chicago, XL, USA). The lower limit of quantitation was 0.5 nmol/L. The intra- and inter-day CVs for the quality control sample were 9.24% and 10.6%, respectively. All samples were assayed in duplicate.

Plasma Cortisol levels were determined using a previously reported normal phase high-performance liquid chromatography procedure.[16] The lower limit of quantitation was 5 ng/mL. The intra- and inter-day CVs for low-, medium-, and high-quality control samples were all <7%.

Statistical Analyses

The differences in HNMT activity and whole-blood histamine levels between subjects with the CC genotype and those with the CT genotype were determined by Wilcoxon Rank Sum Test using STATISTICA 6.1 (StatSoft, Inc., Tulsa, OK, USA). Previously, it has been shown that hemolyzed plasma samples are associated with elevated plasma histamine levels.[17] Therefore, plasma samples were visually graded as 0, 1, and 2 according to the degree of hemolysis, and the effect of hemolysis on plasma histamine level was assessed by

Kruskal-Wallis ANOVA. The differences in plasma histamine levels and the ratio of whole-blood to plasma histamine levels (WB/P ratio) between the two genotype groups (CC and CT) were then analyzed with jespect to the degree of hemolysis by Blocked Wilcoxon Test. Since grade 2 hemolysis was observed in only a small number of samples, plasma samples were grouped as 'no hemolysis' or 'hemolysis,' and Blocked Wilcoxon was calculated for only two groups. Similarly, Cortisol levels exhibit circadian rhythm and are time dependent. The differences in Cortisol levels between males and females and between the two genotype groups were also analyzed by Blocked Wilcoxon. Samples collected from 6am to noon were classified as morning samples, and those collected from noon to 6pm were grouped as afternoon samples. Finally, the correlation between various parameters was assessed by Spearman rank correlation using STATISTICA 6.1. Statistical significance was set at $p < 0.05$ for all analyses.

Results

A total of 48 subjects were enrolled and participated in the study. Thirty-six were males and 12 were females. The median (range) age of the subjects was 25 (21–40) years, with no difference observed between males and females. The majority of the study participants were Caucasians (n = 32), three were African Americans, six were South West Asians (five Indians, one Pakistani), five were Southeast Asians (four Chinese, one Filipino), and two were of Middle Eastern origin. A total of 11 subjects were found to carry the variant 314T allele: ten were heterozygous CT (eight Caucasians, one Chinese, one Middle Eastern) and one was homozygous TT (Pakistani) [table I]. The frequency of the variant 314T allele in Caucasians was found to be 0.125, but this calculation was not done in the other ethnic groups because of their small sample sizes.

A wide range of HNMT activity was observed among all subjects; the median activity was found to be 556 U/mL pRBC (237-1879 U/mL pRBC; n = 46). Whereas there was no difference in HNMT activity between males and females, a difference in enzymatic activity was observed between subjects with the *HNMT* CC genotype and those with the CT genotype. The median [range] activity for *HNMT* CT heterozygotes (485 [237-1195] U/mL pRBC; n = 10) was significantly lower than that for individuals with the wild-type CC genotype (631 [295-1879] U/mL pRBC; n = 35) [p = 0.023] (figure 1). This difference between CC and CT genotypes was also observed within the subgroup of Caucasians included in the study (p = 0.017). For the one single subject who was homozygous for the 314T allele, HNMT activity was determined to be 323 U/mL pRBC.

Among the 48 subjects, seven (six Caucasians, one Asian) had a history of allergic diseases including asthma, allergic rhinitis, seasonal allergy, and atopic and contact dermatitis. One Caucasian was taking inhaled corticosteroids at the time of blood collection. Because the effects of allergic diseases and corticosteroid use on plasma histamine are unknown, data obtained from these eight subjects were excluded from subsequent analyses. Table II summarizes the results of plasma and whole-blood histamine levels, WB/P ratios, and Cortisol levels for the remaining 40 subjects who were not taking any medications at the time of the study. Wide variability was observed in all the parameters: there were 14-fold and 42-fold differences in the histamine levels in plasma and whole blood, respectively. No

differences were found in plasma and whole-blood histamine levels, or WB/P ratios, between males and females, whereas the median Cortisol level was found to be significantly lower in males than in females (118 vs 185 ng/mL; p = 0.006).

There was a difference in plasma histamine levels between subjects with the *HNMT* CC genotype and those with the CT genotype (table III). The median [range] plasma histamine level was 3.32 [1.77-1.49] nmol/L for heterozygotes and was significantly higher than that for homozygous wild-type individuals (2.30 [0.677-9.50] nmol/L) [p = 0.021]. The one subject with the homozygous TT genotype had a low plasma histamine level (1.21 nmol/L). When the results from this subject were included with those from the heterozygotes and compared with those of the wild-type subjects, a similar trend in the difference of plasma histamine was still observed but the p-value was slightly increased to 0.078. A subgroup analysis of plasma histamine levels in Caucasians revealed that the median level was not significantly different between the two genotype groups (3.33 vs 2.30 nmol/L; n = 25). Unlike plasma histamine, whole-blood histamine levels, WB/P ratios, and Cortisol levels were similar between individuals with the CC genotype and those with the CT genotype.

No significant correlation was observed between HNMT activity and plasma histamine levels, whole-blood histamine levels, or WB/P ratios. Likewise, Cortisol levels did not correlate with any of these parameters, including HNMT activity. Plasma histamine levels were found to correlate positively with whole-blood histamine levels (r_s = 0.464; p = 0.003) but negatively with WB/P ratio (r_s = -0.642; p < 0.001). A weak correlation between whole-blood histamine levels and WB/P ratio was also observed (r_s = 0.303; p = 0.058).

Discussion

This is the first pilot study to investigate endogenous levels of histamine and Cortisol in individuals with different *HNMT* C314T genotypes. Two-thirds of our study participants were Caucasian and the calculated frequency of the variant 314T allele in this subgroup was 0.125. Previously, genotyping of *HNMT* in a larger Caucasian population (237 blood donors) showed that the allelic frequency of the 314T variant was 0.08.[14] The frequency of this variant allele in our Caucasian cohort was higher, but it probably did not represent the true population value because of our limited sample size. One individual of Middle Eastern origin and one Pakistani individual were found to carry the C314T polymorphism. This indicates that the 314T variant allele exists in these populations, but its frequency in these two ethnic groups remains to be determined.

It is noted that HNMT activity was highly variable among subjects in this small study. Despite this, the median enzyme activity was found to be higher in individuals with the wild-type genotype than in heterozygotes. This result is comparable with previous findings showing the association of the C314T polymorphism with decreased HNMT activity.[4-9] However, it appears that this polymorphism could not account for all the variability in the HNMT phenotype, suggesting that additional molecular genetic mechanisms, such as SNPs in the 5'-flanking and 3'-untranslated regions, might also be involved in the regulation of HNMT activity.[7] A previous study demonstrated that HNMT activity was higher in male

than in female Chinese individuals.[9] This sex difference was not observed in our study in which most of the subjects were Caucasians.

In the current study, our aim was to investigate whether the C314T polymorphism was associated with differences in endogenous levels of histamine and Cortisol. We found a significant difference in plasma histamine levels between subjects with the CC genotype and those with the CT genotype. This result needs to be interpreted with caution for the following reasons. First, hemolysis occurred in about half of our samples. Non-parametric comparison showed differences in plasma histamine levels among samples with grade 0, 1, and 2 hemolysis (Kruskal-Wallis ANOVA; $p = 0.027$), with plasma histamine being highest in samples with hemolysis grade 2, followed by grade 1 and grade 0 samples. Although the use of Blocked Wilcoxon in our analysis accounted for some of the effects of hemolysis, the degree of hemolysis was arbitrarily assigned and the increases in plasma histamine levels were not adjusted for quantitatively. Second, the single homozygous TT individual had a surprisingly low plasma histamine level, which is inconsistent with the increased plasma histamine levels found in the CT heterozygotes. Lastly, but importantly, the difference was not observed in our small subgroup of Caucasians.

No significant correlation was observed between plasma histamine and HNMT activity, suggesting that the changes in plasma histamine levels might not be related to the alterations in HNMT phenotype. This lack of direct association may be related to the fact that endogenous histamine levels are affected by a number of factors, as evidenced by the wide range of histamine levels in our subjects, and HNMT activity is not the only determinant of these levels. It is known that the enzymes histidine decarboxylase and diamine oxidase are involved in the synthesis and degradation of histamine.[2] Any genotypic or phenotypic variations in these enzymes can also potentially affect the levels of plasma histamine. Recently, an *in silico* analysis of histamine-related gene sequences identified several SNPs in the histidine decarboxylase and diamine oxidase genes[18] Some of these SNPs were found to encode amino acid changes and may, therefore, have potential biological significance and affect plasma histamine levels.

Our reason to examine the association of the *HNMT* C314T polymorphism with Cortisol levels was based on the observation that intra-cerebroventricular administration of histamine activated the hypothalamic-pituitary-adrenal axis in animals.[10-12] Furthermore, intraperitoneal injection of histamine has been found to increase adrenocorticotrophic hormone release in rats.[19] We speculated that if histamine levels are higher due to the presence of the 314T variant allele, Cortisol levels will be increased subsequently. However, we failed to detect any differences in Cortisol levels between individuals who were heterozygous for the variant 314T allele and those who were homozygous for the wild-type 314C allele, suggesting that the C314T polymorphism may not be an important factor affecting basal levels of Cortisol in humans. Interestingly, results from our follow-up pharmacokinetic study showed that heterozygous CT individuals appeared less sensitive to the suppressive effects of methylprednisolone on Cortisol secretion than homozygous CC individuals (data not shown). The influence of the *HNMT*C314T polymorphism on Cortisol secretion after corticosteroid administration, however, requires further investigation. Finally,

a higher median level of Cortisol was found in female than in male subjects. The reason for this sex difference is unknown, as Cortisol levels are known to be similar between sexes.

Conclusion

There was a wide variability observed in the levels of histamine in plasma and whole blood from individuals with different *HNMT* C314T genotypes. Endogenous levels of histamine are likely to be affected by various genes and polymorphisms.

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References

1. Maslinski C. Histamine and its metabolism in mammals. Part I: chemistry and formation of histamine. Agents Actions. May; 1975 5(2):89–107. [PubMed: 1099882]
2. Maslinski C. Histamine and its metabolism in mammals. Part II: catabolism of histamine and histamine liberation. Agents Actions. Aug; 1975 5(3):183–225. [PubMed: 78663]
3. Okinaga S, Ohrui T, Nakazawa H, et al. The role of HMT (histamine N-methyl-transferase) in airways: a review. Methods Find Exp Clin Pharmacol. Nov; 1995 17(Suppl. C):16–20. [PubMed: 8750789]
4. Preuss CV, Wood TC, Szumlanski CL, et al. Human histamine N-methyltransferase pharmacogenetics: common genetic polymorphisms that alter activity. Mol Pharmacol. Apr; 1998 53(4):708–17. [PubMed: 9547362]
5. Scott MC, Van Loon JA, Weinshilboum RM. Pharmacogenetics of N-methylation: heritability of human erythrocyte histamine N-methyltransferase activity. Clin Pharmacol Ther. Mar; 1988 43(3): 256–62. [PubMed: 3345617]
6. Price RA, Scott MC, Weinshilboum RM. Genetic segregation analysis of red blood cell (RBC) histamine N-methyltransferase (HNMT) activity. Genet Epidemiol. 1993; 10(2):123–31. [PubMed: 8339926]
7. Chen GL, Wang H, Wang W, et al. Histamine N-methyltransferase gene polymorphisms in Chinese and their relationship with enzyme activity in erythrocytes. Pharmacogenetics. Jul; 2003 13(7):389–97. [PubMed: 12835614]
8. Wang L, Thomae B, Eckloff B, et al. Human histamine N-methyltransferase pharmacogenetics: gene resequencing, promoter characterization, and functional studies of a common 5′-flanking region single nucleotide polymorphism (SNP). Biochem Pharmacol. Aug; 2002 64(4):699–710. [PubMed: 12167489]
9. Chen GL, Wang W, Xu ZH, et al. Genotype-phenotype correlation for histamine N-methyltransferase in a Chinese Han population. Clin Chim Acta. Aug; 2003 334(1-2):179–83. [PubMed: 12867290]
10. Kjaer A, Larsen PJ, Knigge U, et al. Histaminergic activation of the hypothalamic-pituitary-adrenal axis. Endocrinology. Sep; 1994 135(3):1171–7. [PubMed: 8070360]
11. Tsujimoto S, Kamei C, Yoshida T, et al. Changes in plasma adrenocorticotropic hormone and Cortisol levels induced by intracerebroventricular injection of histamine and its related compounds in dogs. Pharmacology. Aug; 1993 47(2):73–83. [PubMed: 8102804]

12. Tsujimoto S, Okumura Y, Kamei C, et al. Effects of intracerebroventricular injection of histamine and related compounds on corticosterone release in rats. Br J Pharmacol. Jul; 1993 109(3):807–13. [PubMed: 8102936]

13. Weinshilboum RM, Raymond FA, Pazmino PA. Human erythrocyte thiopurine methyltransferase: radiochemical microassay and biochemical properties. Clin Chim Acta. May; 1978 85(3):323–33. [PubMed: 657528]

14. Yan L, Galinsky RE, Bernstein JA, et al. Histamine N-methyltransferase pharmacogenetics: association of a common functional polymorphism with asthma. Pharmacogenetics. Apr; 2000 10(3):261–6. [PubMed: 10803682]

15. Van Loon JA, Pazmino PA, Weinshilboum RM. Human erythrocyte histamine N-methyltransferase: radiochemical microassay and biochemical properties. Clin Chim Acta. Jul; 1985 149(2-3):237–51. [PubMed: 4028443]

16. Ebling WF, Szefler SJ, Jusko WJ. Analysis of cortisol, methylprednisolone, and methylprednisolone hemisuccinate: absence of effects of troleandomycin on ester hydrolysis. J Chromatogr. Feb; 1984 305(2):271–80. [PubMed: 6368578]

17. Laroche D, Dubois F, Gerard JL, et al. Radioimmunoassay for plasma histamine: a study of false positive and false negative values. Br J Anaesth. Apr; 1995 74(4):430–7. [PubMed: 7734264]

18. Igaz P, Fitzimons CP, Szalai C, et al. Histamine genomics in silico: polymorphisms of the human genes involved in the synthesis, action and degradation of histamine. Am J Pharmacogenomics. 2002; 2(1):67–72. [PubMed: 12083955]

19. Reilly MA, Sigg EB. Suppression of histamine-induced adrenocorticotropic hormone release by antihistamines and antidepressants. J Pharmacol Exp Ther. Sep; 1982 222(3):583–8. [PubMed: 6125582]

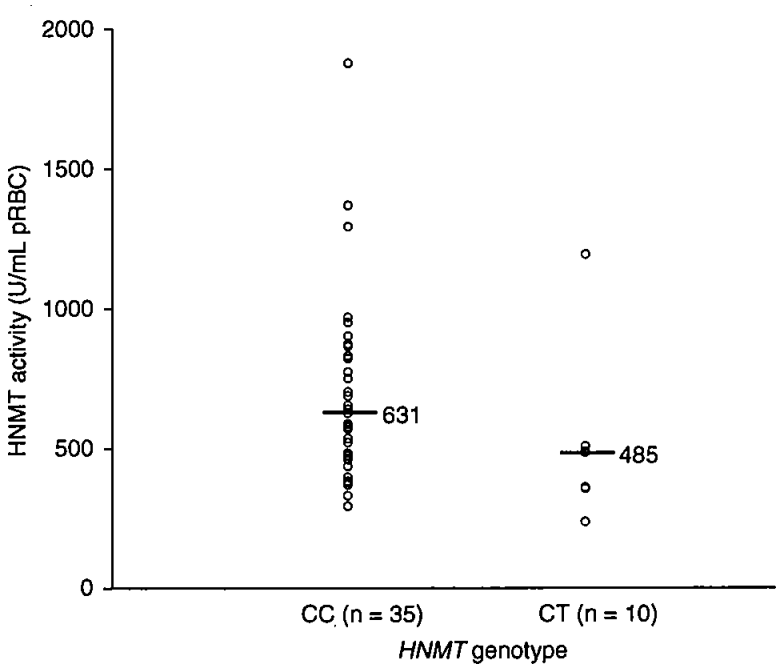

Fig. 1.

The differences in histamine N-methyltransferase (HNMT) activity between subjects with the *HNMT* CC genotype and those with the CT genotype (p = 0.023). pRBC = packed red blood cells.

Table I

Histamine N-methyltransferase (*HNMT*) C314T genotypes in all subjects

Ethnicity	Number			*HNMT* C314T genotype		
	total	male	female	CC	CT	TT
Caucasian	32	24	8	24	8	0
African American	3	1	2	3	0	0
Middle Eastern	2	2	0	1	1	0
Asian	11	9	2	9	1	1

Table II

Histamine and Cortisol levels in 40 subjects with no history of allergic diseases and not taking any medication at the time of the study

Subject group	Plasma histamine (nmol/L)[a]	Whole-blood histamine (nmol/L)[a]	Whole-blood/plasma histamine ratio	Cortisol (ng/mL)[a]
Males (n = 29)	2.31 (0.807–9.50)	305 (17.3–684)	121 (10.5–306)	118 (51.8–173)[b]
Females (n = 11)	2.43 (0.677–4.56)	347 (142–489)	165 (47.5–252)	185 (80.8–406)[b]
Total (n = 40)	2.32 (0.677–9.50)	315 (17.3–684)	141 (10.5–306)	126 (51.8–406)

[a] Levels of histamine and Cortisol are given as median (range).

[b] p = 0.006 between males and females by Blocked Wilcoxon as grouped by the time of the samples. Differences between the male and female groups for the other parameters were not statistically significant.

Table III

Histamine and Cortisol levels in 40 subjects with different histamine N-methyltransferase (*HNMT*) C314T genotypes

Genotype group	Plasma histamine (nmol/L)[a]	Whole-blood histamine (nmol/L)[a]	Whole-blood/plasma histamine ratio	Cortisol (ng/mL)[a]
CC (n = 31)	2.30 (0.677–9.50)[b]	334 (17.3–538)	145 (10.5–306)	134 (51.8–406)
CT (n = 8)	3.32 (1.77–4.49)[b]	283 (157–684)	102 (47.5–195)	120 (58.7–276)
TT(n= 1)	1.21	298	246	71.2

[a] Levels of histamine and Cortisol are given as median (range).

[b] p = 0.021 between *HNMT* CC and CT genotypes by Blocked Wilcoxon as grouped by the presence or absence of hemolysis. Differences between CC and CT groups for the other parameters were not statistically significant.