

Expression of histidine decarboxylase messenger RNA and histamine N-methyltransferase messenger RNA in nasal allergy

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N. HIRATA, K. TAKEUCHI, K. UKAI and Y. SAKAKURA

Department of Otorhinolaryngology Mie University School of Medicine, Edobashi, Tsu, Mie, Japan

Summary

Background Histamine is an important chemical mediator in allergic rhinitis. Histamine is synthesized by L-histidine, catalysed by L-histidine decarboxylase (HDC) and metabolized mainly by histamine N-methyltransferase (HMT). The patients with allergic rhinitis may have altered histamine metabolism in the nasal tissue.

Objective The objective was to compare the expression of HDC mRNA and HMT mRNA in nasal mucosa with normal individuals and patients with allergic rhinitis.

Methods We extracted RNA from scrapings of inferior turbinate mucosa of 11 patients with allergic rhinitis and from 11 normal subjects and from surgically dissected inferior turbinate mucosa of 11 patients. HDC, HMT and β -actin mRNA were amplified by reverse transcription-polymerase chain reaction (RT-PCR).

Results The mean $\pm$ SD of HDC/ β -actin ratios were 0.58 ± 0.28 for the scrapings of normal subjects, 0.93 ± 0.38 for the scrapings of the patients with allergic rhinitis and 1.41 ± 0.26 for the inferior turbinates of the patients with allergic rhinitis. The mean $\pm$ SD of HMT/ β -actin ratios were 1.66 ± 0.27 for the scrapings of normal subjects, 0.93 ± 0.20 for the scrapings of the patients with allergic rhinitis and 0.65 ± 0.28 for the inferior turbinates of the patients with allergic rhinitis. The differences among the three groups were statistically significant.

Conclusions Increased HDC mRNA expression and decreased HMT mRNA expression may be playing some role in the hyperresponsiveness of patients with allergic rhinitis.

Keywords: L-histidine decarboxylase, histamine N-methyltransferase, mRNA, allergic rhinitis, reverse transcription-polymerase chain reaction, nasal scrapings, inferior turbinates

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Introduction

Histamine is a chemical mediator released from basophils or mast cells [1,2] and plays an important role in eliciting nasal symptoms of allergic rhinitis: pruritus, sneezing, rhinorrhoea and congestion [3]. The formation of histamine from L-histidine is catalysed by L-histidine decarboxylase (HDC; L-histidine carboxy-lyase, EC 4.1.1.22) [4]. In rodents, the enzyme is primarily localized in the brain (hypothalamic

neurons and projections to other brain regions) [5], the glandular regions of the stomach [6], and fetal liver [7]. The released histamine is thought to be catabolized rapidly *in vivo*, mainly through two metabolic pathways. One is transmethylation to telemethylhistamine, catalysed by histamine N-methyltransferase (HMT; S-adenosyl-L-methionine: histamine N-methyltransferase, EC 2.1.1.8), and the other is oxidative deamination to imidazole acetaldehyde, catalysed by diamine oxidase (DAO; diamine, oxygen oxidoreductase, EC 1.4.3.6) which is also called histaminase [8–10]. Human HDC and HMT genes have been isolated and cloned by screening human genomic libraries with human HDC and HMT cDNA

Correspondence: N. Hirata, Department of Otorhinolaryngology, Mie University School of Medicine, 2–174 Edobashi, Tsu, Mie 514–8507, Japan.

probes, respectively [11,12]. The cDNA clone of human HDC and HMT have been isolated from a cDNA library of human basophilic leukaemia cells and kidney, respectively [13–15]. The DAO gene has not been cloned yet.

Hisamatsu [16] reported that the histaminase activity in nasal mucous membrane was increased in allergic patients. Abe *et al.* [17] reported that increases in histamine content and HDC and HMT activities were observed in the nasal mucosa and lung of TDI-sensitized guinea-pigs. Yamauchi *et al.* [18] reported that the HDC mRNA level was increased in the patients with bronchial asthma. These previous reports suggest that histamine metabolism may be altered in allergic rhinitis. We hypothesized that the expression of HDC mRNA is increased and that of HMT mRNA and DAO mRNA are decreased in nasal mucosa of patients with allergic rhinitis compared with normal subjects.

According to previous reports [17,19], the activity of the HMT is more than that of the DAO in the nasal mucosa, and they suggest that HMT plays a more important role in histamine degradation in the human nasal mucosa than DAO.

The present study was designed to demonstrate and compare the expression of HDC mRNA and HMT mRNA in nasal mucosa from normal subjects and patients with allergic rhinitis to clarify the features of histamine metabolism in allergic rhinitis. As it is impossible to obtain large specimens from human subjects, we amplified mRNA by a reverse transcription-polymerase chain reaction (RT-PCR).

Materials and methods

Subjects

Scrapings were obtained using sterile surgical curettes (cupsize, 2.5 × 3.5 mm, Nakatsuji, Japan) from nasal mucosa in the inferior turbinates of 11 healthy volunteers (18–45 years of age; mean age ± SD, 22.1 ± 7.8 years, five men and six women) and 11 patients with allergic rhinitis (16–67 years of age; mean age ± SD, 28.1 ± 14.2 years, seven men and four women) and human inferior turbinates were obtained at the time of surgery from 11 patients with allergic rhinitis (17–67 years of age; mean age ± SD, 32.5 ± 18.1 years, seven men and four women) under topical anaesthesia by 4% xylocaine. Both scrapings and inferior turbinate mucosae were obtained from seven patients.

The diagnosis of allergic rhinitis to house dust mite (HDM) was based on a compatible history, anterior rhinoscopic examination and the following three criteria: (1) eosinophilia in nasal smears, (2) a radioallergosorbent (RAST) class 2 or more for HDM, and (3) a positive nasal provocation test for HDM. These tests were performed several weeks before obtaining samples. The patients with

allergic rhinitis were moderately symptomatic and received neither antihistaminic therapy nor topical steroid in the 6 months before the study. The normal individuals had none of nasal symptoms, physical examination findings peculiar to allergic rhinitis or significant eosinophils in nasal scrapings. The specimens of nasal mucosa were collected in the summer, because some of the allergic patients suffer from pollinosis in the spring.

Informed consent was obtained from each patient and healthy volunteer, and the protocol was approved by the Ethics Committee, Mie University School of Medicine.

Isolation of total cellular RNA

After samples were scraped or resected, they were immediately put into ice-cold denaturing solution (4 mol/L guanidinium thiocyanate, 25 mmol/L sodium citrate pH 7.0, 0.5% sarcosyl, 0.1 mol/L 2-mercaptoethanol). After vortexing or homogenizing the mixtures, we followed a single-step method of RNA isolation by Chomczynski [20]. The 1 µg of RNA pellets were resuspended in 20 µL of 40 mmol/L TRIS-HCl (pH 7.5), 6 mmol/L MgCl₂, added 1 U of DNase I and incubated at 37 °C for 10 min to distinguish RNA from contaminating genomic DNA. The RNA was extracted with phenol/chloroform/isoamyl alcohol (25:24:1 v/v/v), ethanol-precipitated, centrifuged at 10 000 g for 10 min, rinsed with 70% ethanol and dried.

Preparation of oligonucleotide primers

Oligonucleotide primers were constructed from the published cDNA sequences of HDC [11,13], HMT [12,14,15] and β -actin cDNAs [21]. The HDC, HMT and β -actin primers were selected to span intron-exon boundaries to distinguish between amplification of mRNA and any contaminating genomic DNA, and these two pairs of primers were selected to be as close as possible to the poly A tail to increase the efficiency of the RT-PCR using oligo d(T) as a primer for reverse transcription. The sequence of the HDC primers were 5' – CTGGCAGCAAGGAAGAACA – 3' (sense) corresponding to bases 577–596 of the cloned full-length sequence and 5' – AGGAGTCGGCATACTCAATC – 3' (antisense) which anneals to bases 978–997. The HMT primers were 5' – GAGGAGCTTGTTTTCTGACC – 3' (sense) corresponding to bases 15–34 of the cloned full-length sequence and 5' – GTCCCACTTTTGAAGCTCCT – 3' (antisense) which anneals to bases 392–411. The β -actin primers were 5' – ATGGATGATGATATCGCCGCG – 3' (sense) and 5' – ATGTCGTCCCAAGTTGGTGACGAT – 3' (antisense). The predicted sizes of the amplified HDC, HMT and β -actin DNA products were 421, 397 and 254 bp, respectively.

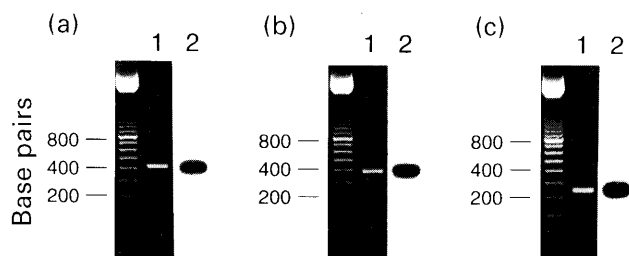


Fig. 1. Amplification products obtained with HDC (a) HMT (b) and β -actin (c) specific primers and RNA derived from nasal scrapings from a patient with allergic rhinitis. Lane 1 shows the ethidium bromide staining patterns and lane 2 shows the autoradiographic signals obtained following Southern transfer of the amplified products onto nylon membranes, and hybridization with end-labelled sense oligonucleotide probes whose sequences were internal to the primers. Amplification was for 32, 32 and 27 cycles, respectively.

Reverse transcription of RNA

The RNA pellets prepared as above were reverse transcribed into cDNAs using GeneAmp RNA PCR Kit (Takara Bio-medicals, Tokyo, Japan). Oligo d(T)₁₆ primer was used for generation of cDNAs. After incubation at 42°C for 60 min, the samples were heated for 5 min at 99°C to terminate the reactions, and were stored at -20°C until used.

Amplification of HDC, HMT and β -actin cDNAs

Fresh tubes containing the upstream and downstream primers (25 μ mol/L of each primer) spanning the given sequence for amplification, 200 μ mol/L of each dNTP (dATP, dCTP, dGTP, dTTP), 50 mmol/L KCl, 10 mmol/L TRIS-HCl, pH 8.3, 1.5 mmol/L MgCl₂, 1 U of *Thermus aquaticus* (Taq) DNA polymerase were heated to 94°C, and each 0.1 μ g of cDNA was added to a final volume of 50 μ L. The reaction mixture was then added 1 drop of mineral oil and amplified in Perkin-Elmer/Cetus (Norwalk, CT, USA) thermal cycler. The amplification profile consisted of denaturation at 94°C for 45 s, primer annealing at

55°C for 45 s, and extension at 72°C for 90 s. The reaction was terminated by chilling at 4°C. The negative controls contained all the necessary components for PCR except template DNA.

Previous studies have shown that the amount of amplified fragments is proportional to the quantity of input mRNA during the exponential phase of the amplification process [22,23]. We have also examined every five cycles of amplification to determine the point at which amplification is within the exponential phase. cDNAs from two normal subjects and two patients with allergic rhinitis were amplified for this purpose. We confirmed that the amplification of HDC, HMT and β -actin mRNA remained in the exponential phase at 32, 32 and 27 cycles, respectively. The reaction mixtures were amplified for each cycles.

The PCR products were electrophoresed through a 1.5% (w/v) agarose gel containing 0.5 μ g/mL of ethidium bromide in 40 mmol/L TRIS-acetate buffer, pH 8.0, containing 1 mmol/L EDTA, and blotted onto nylon membranes.

Hybridization with end-labelled oligonucleotide probes

In the experiments to validate the PCR products, a sense 28-mer oligonucleotide probe internal to the amplified fragment of HDC sequence 5' - TCTGCCTGTGGATGA-CAACTTCTCACTC - 3' corresponding to nucleotide 732~759 of the cDNA sequence and a sense 30-mer probe of HMT sequence 5' - CTTGTAGCCAAGACATC-GAACCTCGAGAAC - 3' corresponding to nucleotide 301~330 were synthesized. The oligonucleotide probe used to validate the β -actin PCR fragment was 5' - GTCCACTCTCAATCACTCTCAGTTCTTTGA - 3'. One hundred ng of each probe were end-labelled with 25 μ Ci of [γ -³²P]ATP (sp. act 3000 Ci/mmol) using 10 U of T4 polynucleotide kinase in the presence of 70 mmol/L TRIS-HCl, pH 7.6, 10 mmol/L MgCl₂, and 5 mmol/L dithiothreitol in a final volume of 20 μ L. The nylon membranes were prehybridized in 30 mL of buffer composed of 0.25% (w/v) sodium dodecyl sulphate (SDS), 1.2X standard saline citrate (SSC), 2X Denhardt's solution and heat-denatured salmon sperm DNA (10 μ g/mL) at 59°C for HDC, 65°C for HMT

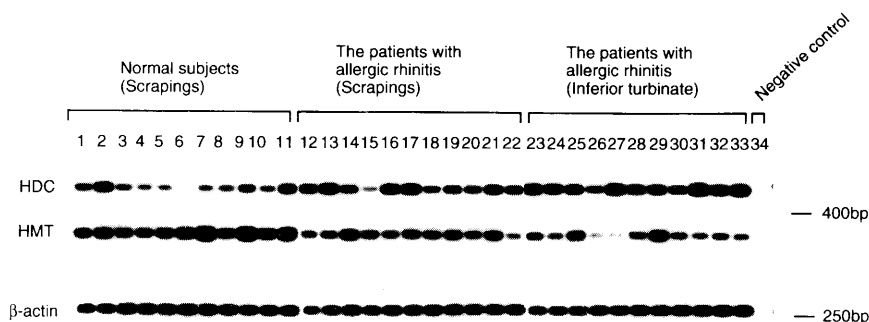


Fig. 2. Southern blotting bands of amplification products of HDC mRNA (the upper row), HMT mRNA (the middle row) and β -actin mRNA (the lower row) are shown in the vertical lane 1-34. Lane 1-11: scrapings of the normal subjects, lane 12-22: scrapings of the patients with allergic rhinitis, lane 23-33: inferior turbinates of the patients with allergic rhinitis, lane 34: negative control containing no DNA.

or at 61 °C for β -actin for 1 h. Hybridization was conducted in the presence of the each labelled oligonucleotide probe in the same solution as in prehybridization by incubation at the same temperature as in prehybridization for 2 h. Before autoradiography the blots were washed three times in 2X SSC and 0.05% SDS for 10 min at 60 °C. Intensity of the bands of HDC, HMT and β -actin in autoradiographs was quantified using a densitometer.

Statistical analysis of data

The Mann–Whitney *U*-test was used to test for differences HDC/ β -actin and HMT/ β -actin ratio between the two groups. Correlation coefficients and associated tests of significance were computed using standard linear regression methods. Significance was accepted at the 5% level of confidence.

Results

Verification of PCR products as predicted segments of spliced HDC, HMT and β -actin mRNA

As can be seen in the Fig. 1(a,b,c) (lane 1), the amplification products of HDC, HMT and β -actin were identical to the predicted sizes for the mRNA template of 421, 397 and 254 bp, respectively. In Fig. 1(a,b,c) (lane 2) show that hybridization of Southern blots of the amplification products with end-labelled HDC, HMT and β -actin oligonucleotide probes produced hybridization signals that corresponded in size to the ethidium bromide-stained gel, thus confirming that the amplified sequences consisted of authentic HDC, HMT and β -actin sequences. The bands of HDC, HMT and β -actin were observed in all the samples (Fig. 2). Although some lanes appear to have no obvious bands for HDC and HMT mRNA, faint bands could be observed on original films. The HDC, HMT and β -actin probes did not hybridize to unrelated DNA molecules under the condition used (data not shown).

Kinetics and yields of HDC, HMT and β -actin mRNA

As illustrated in Fig. 3, the amplification of HDC, HMT and β -actin mRNA remained in an exponential phase at 32, 32 and 27 cycles, respectively.

Comparison of the expression of HDC mRNA and HMT mRNA in normal subjects and patients with allergic rhinitis

The HDC, HMT and β -actin mRNA were amplified separately for 32, 32 and 27 cycles, respectively. Samples from the normal subjects and the patients were always run in parallel. The HDC/ β -actin and HMT/ β -actin ratios were quantified in the 11 healthy volunteers and the 11 patients

with allergic rhinitis. As shown in the Fig. 4, the mean $\pm$ SD of HDC/ β -actin ratios were 0.58 ± 0.28 for the scrapings of normal subjects, 0.93 ± 0.38 for the scrapings of patients with allergic rhinitis and 1.41 ± 0.26 for the inferior turbinates of patients with allergic rhinitis. The mean $\pm$ SD of HMT/ β -actin ratios were 1.66 ± 0.27 for the scrapings of normal subjects, 0.93 ± 0.20 for the scrapings of patients with allergic rhinitis and 0.65 ± 0.28 for the turbinates of patients with allergic rhinitis. The group of patients with

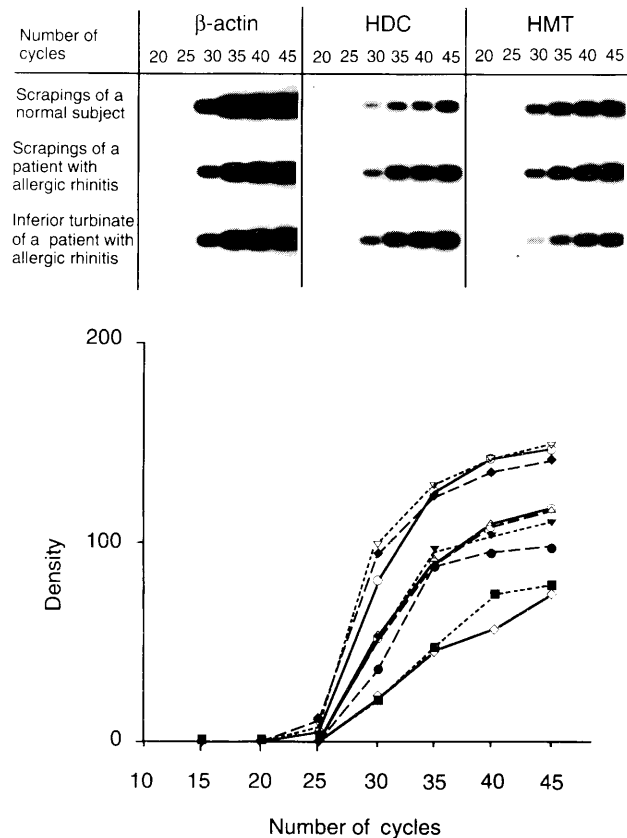


Fig. 3. Amplification of HDC, HMT and β -actin mRNA. Top, cDNA derived from scrapings of a normal subject or from a patient with allergic rhinitis were amplified with HDC, HMT and β -actin specific primers for the indicated number of cycles. The nylon membrane containing the HDC, HMT and β -actin cDNA fragments were exposed to X-ray film for 6.5 h at -80°C with an intensifying screen. The figure shows the corresponding autoradiograms. Bottom, representation of HDC, HMT and β -actin amplification, as measured by a densitometer. —○—, β -actin of a normal subject (scrapings); ---◆---, β -actin of a patient with allergic rhinitis (scrapings); ---▽---, β -actin of a patient with allergic rhinitis (inferior turbinate); ---◇---, HDC of a normal subject (scrapings); ---●---, HDC of a patient with allergic rhinitis (scrapings); ---▼---, HDC of a patient with allergic rhinitis (inferior turbinate); ---△---, HMT of a normal subject (scrapings); ---○---, HMT of a patient with allergic rhinitis (scrapings); ---■---, HMT of a patient with allergic rhinitis (inferior turbinate).

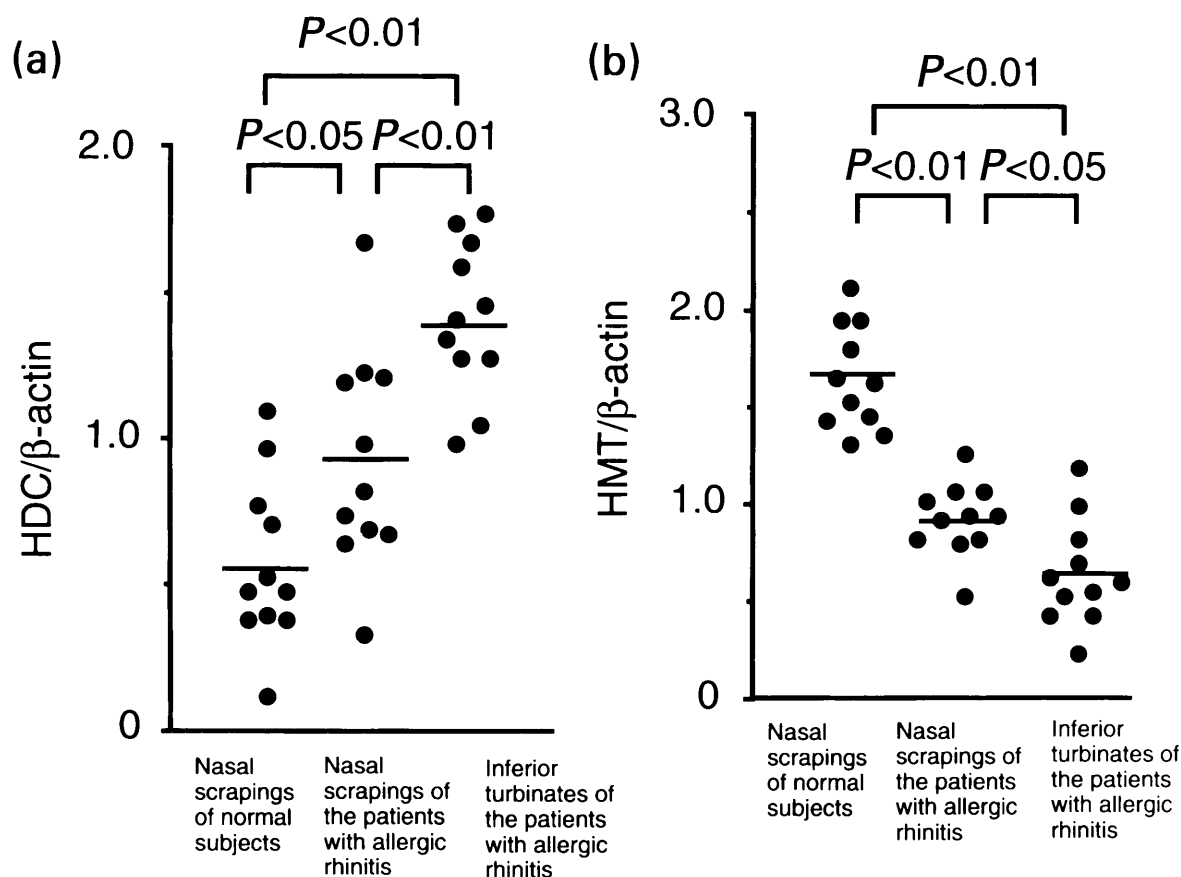


Fig. 4. Differences in the HDC (a) and HMT (b) mRNA level in the scrapings from normal subjects and the scrapings and the inferior turbinates from the patients with allergic rhinitis. The quantitative results are expressed as ratios of the intensity of the HDC and HMT bands at 32 cycles to the intensity of the β -actin bands at 27 cycles.

allergic rhinitis had higher HDC/ β -actin ratios and significantly lower HMT/ β -actin ratios compared with the normal subjects ($P < 0.05$, $P < 0.01$). In patients with allergic rhinitis, the scrapings had lower HDC/ β -actin ratios and higher HMT/ β -actin ratios compared with the inferior turbinates ($P < 0.01$, $P < 0.05$).

Correlation between HDC/ β -actin ratio and HMT/ β -actin ratio

We have examined relationships between the HDC/ β -actin ratio and HMT/ β -actin ratio in the scrapings. As can be seen in Fig. 5, the HMT/ β -actin ratio was positively correlated to the HDC/ β -actin ratio ($r = 0.708$, $P < 0.05$) in normal subjects. While in the patients with allergic rhinitis, the HMT/ β -actin ratio was not correlated to the HDC/ β -actin ratio ($r = -0.008$, $P = 0.98$).

Discussion

It has been speculated that hypersensitivity of the nasal

mucosa in allergic rhinitis and the trachea in asthma is due to the activity of the HDC and the HMT [17,19,24–27]. To our knowledge, this study is the first report which specifically demonstrated the expression of HDC mRNA and HMT mRNA in nasal scrapings and mucosae of inferior turbinates of both patients with allergic rhinitis and normal individuals.

In this study, we developed a semiquantitative assay for HDC mRNA and HMT mRNA to examine the expression of the HDC and HMT in nasal mucosa immediately after sampling from the inferior turbinates. The expression of HDC and HMT mRNA was quantified by RT-PCR. Such an approach has been shown to be a useful method for detecting small amounts of transcripts when applied to the quantification of RNAs from small number of cells or to characterize low abundant transcripts [28–30]. We treated the RNA pellets with DNase I in order to remove contaminating genomic DNA and confirmed that this treatment can abolish the amplification of β -actin genomic DNA (386 bp) (data not shown). And we confirmed that the amplification of HDC, HMT, and β -actin mRNA remained in an

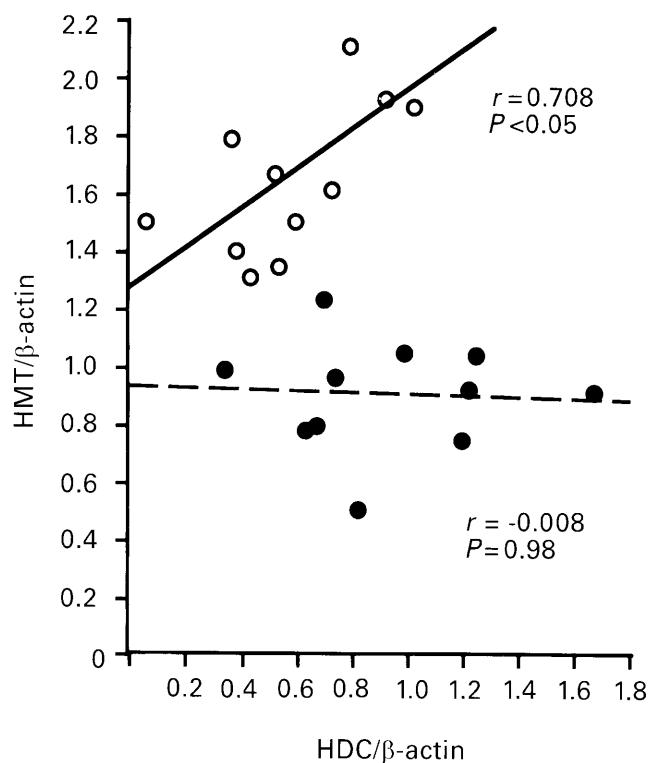


Fig. 5. Correlation between the HMT/ β -actin ratio and HDC/ β -actin ratio. Open circles and solid line: scrapings of normal subjects, closed circles and dashed line: scrapings of the patients with allergic rhinitis.

exponential phase at 32, 32, and 27 cycles, respectively. Therefore we believe that Figs 1 and 2 show only the bands of HDC, HMT, and β -actin mRNA and could accomplish a semiquantitative assay.

There are some reports in HDC and HMT [16–18]. We hypothesized that the expression of HDC mRNA is increased and HMT mRNA is decreased in nasal mucosa of patients with allergic rhinitis compared with normal subjects, and the results were that in nasal scrapings and inferior turbinates, the patients with allergic rhinitis had higher HDC/ β -actin ratios and significantly lower HMT/ β -actin ratios compared with normal subjects.

The content of histamine in normal human skin tissue was reported to be parallel to the number of infiltrating mast cells [31]. Pipkorn *et al.* [32] reported strong correlations among mast cell density, symptoms, and histamine content of the nasal mucosa in hay fever patients. Naclerio *et al.* [33] also reported that histamine concentrations in nasal lavage were increased by nasal provocation with antigen, and that this increase in histamine levels appeared to be associated with the activation of mast cells. As HDC is also contained in mast cells [2], increased HDC mRNA in the nasal mucosa of the patients with allergic rhinitis may depend on the mast cell density as well as individual mast cell activity.

The eosinophil is considered to be an important cell in allergic rhinitis. Secretory products from eosinophils such as major basic protein and eosinophil cationic protein are both powerful toxins that can cause destruction and desquamation of the epithelium [34,35]. Lim *et al.* [36] reported that although acute challenges did not change the epithelium, epithelial attrition was evident in the mucosa of the perennially allergic group of subjects. Therefore, the decreased HMT mRNA in the patients with perennial allergic rhinitis may be a consequence of epithelial cell change. Likewise, Nakazawa *et al.* [25] reported that the HMT activity was decreased in the infected animals and that may be a consequence of epithelial cell injury. The sequences of the two published human kidney HMT cDNAs differ at two nucleotides [14,15]. Thus, the allelic variations in HMT genes may be responsible for the difference in HMT mRNA levels.

It is reported that HDC localizes in mast cells and basophils [1,2]. Cytology of nasal scrapings shows several mast cells [37], and there are more of these cells in the submucosa than in the epithelium [38]. Okayama *et al.* [39] have reported that HMT mRNA was localized in cells in the epithelium and submucosa, and densely in endothelial cells of vessels and Ohnishi *et al.* [40] have reported that HMT mRNA was more abundant in the epithelium than in the endothelium. We performed haematoxylin and eosin stain of surgically dissected inferior turbinate mucosa and confirmed that there was scarce epithelia in those specimens (data not shown). So we believe that scrapings mainly contain the epithelium, and resected specimens from inferior turbinates mainly contain the submucosal tissue. The result that scrapings had lower HDC/ β -actin ratios and higher HMT/ β -actin ratios compared with the inferior turbinate is compatible with the previous reports [38–40]. But we cannot draw a conclusion on which cells expressed HDC and HMT mRNA.

The responsiveness to intranasally challenged histamine is increased in perennial rhinitis [41]. This nasal hyper-responsiveness has been explained by an increase in the number of surface basophilic cells [42], of sensory nerve receptor sensitivity [43], and possibly of H_1R mRNA [44]. We suggest that decreased HMT mRNA in patients with allergic rhinitis may also be responsible for the hyper-responsiveness. This is supported by the evidence that HMT does limit the contractile response of guinea-pig trachea to histamine and hyperresponsiveness to histamine is caused by removal of HMT [40].

Kawabori *et al.* [38] reported that allergic patients had higher histamine content in their nasal mucosa than normal subjects. It is reported that histamine is synthesized by HDC [4] and is mainly degraded by HMT in human nasal mucosa [19]. We suggest that increased HDC mRNA expression and decreased HMT mRNA expression are responsible for

increased histamine contents in the nasal mucosa of the patients with allergic rhinitis.

In conclusion, we demonstrated in this study that patients with allergic rhinitis had increased expression of HDC mRNA and decreased expression of HMT mRNA in comparison with normal subjects and that the expression of HMT mRNA was positively correlated to the expression of HDC mRNA in the normal subjects, but the correlation was not significant in the patients with allergic rhinitis. We suggest that increased HDC mRNA expression and decreased HMT mRNA expression may play a role in the development of nasal symptoms in the patients with allergic rhinitis.

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