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BRIEF COMMUNICATION

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Lack of association between atopic asthma and polymorphisms of the histamine H1 receptor, histamine H2 receptor, and histamine N-methyltransferase genes

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Histamine functions as one of the major mediators in allergic diseases such as rhinitis, asthma, urticaria, and anaphylaxis when released from mast cells and basophils after cross-linking surface-bound IgE by allergen. The effects of histamine are mediated through three types of receptors, the H1 receptor (HRH1), H2 receptor (HRH2) and H3 receptor (Hill 1990). HRH1 mediates the proinflammatory actions of histamine in the cytokine release and adhesion process (Bachert 1998; Banu and Watanabe 1999), while HRH2 suppresses the production of the Th1-inducing cytokine interleukin-12, resulting in a shift of the Th1/Th2 balance toward Th2 dominance (van der Pouw Kraan et al. 1998). Furthermore, a linkage study identified Chromosome 3p25, where *HRH1* is localized, as a candidate gene region for atopy in the Japanese population (Yokouchi et al. 1999).

The polymorphism data reported in this paper have been submitted to the HGBASE database and have been assigned the accession numbers SNP000005191, SNP000005192, and SNP000005193

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Released histamine is catabolized principally by two enzymes, histamine N-methyltransferase (HNMT) and diamine oxidase. HNMT plays an important role in degrading histamine and in regulating the airway response to histamine. The *HNMT* 314C/T polymorphism, which results in a 105Thr/Ile amino acid change, has been shown to induce a functional difference; HNMT with Ile105 had a significantly lower activity than with Thr105 (Preuss et al. 1998; Yamauchi et al. 1994). Thus, since HRH1/HRH2 and HNMT are considered to contribute to the pathogenesis of allergies, certain variations in their genes might be responsible for an atopic predisposition. In this study, we first screened for polymorphisms in *HRH1* and *HRH2* genes in the Japanese population and performed association studies between the polymorphisms of *HRH1*, *HRH2* and *HNMT* genes and atopic asthma.

The subjects in this study comprised 100 patients with atopic asthma and 100 normal schoolchildren in the northern Kyushu area of Japan. Atopic asthma was defined as bronchial asthma with one or more of the following findings: high IgE concentrations (>400 IU/ml) and a positive radioallergosorbent test (score >2+) in response to one or more inhalant allergens. The age at onset (mean ± SD) of atopic asthma was 2.8 ± 2.5 years. The IgE level was 1123 ± 1146 IU/ml. Asthma severity was evaluated using the Classification of International Guidelines (International Consensus report on the Diagnosis and Treatment of Asthma 1992).

Screening for variation in *HRH1* and *HRH2* genes was carried out first. To screen the entire protein-coding region (exon 2) of the *HRH1* gene, we constructed three sets of polymerase chain reaction (PCR) primers, 5′-TCATCACCCAAGTCTCTGACC-3′ and 5′-GCCTGCATGTGCACAATATC-3′ for the first segment, 5′-GTCTTGAAGTCACCATCCCA-3′ and 5′-GCGGTTCATGTGCAACCCAG-3′ for the second segment, and 5′-TCGAACGGACTCAGATACCA-3′ and 5′-CACAGGCCTTCGTCCTCTAT-3′ for the third segment. The PCR profiles used were as follows:

first fragment, initial denaturation at 94 °C for 5 min, followed by 30 cycles at 94 °C for 60 s, 60 °C for 60 s and 72 °C for 120 s; second and third fragments, initial denaturation at 94 °C for 5 min, followed by 30 cycles at 94 °C for 60 s, 60 °C for 60 s and 72 °C for 60 s with the final extension at 72 °C for 5 min. To cover 1211 base pairs (bp) of the protein-coding region of the *HRH2* gene, PCR was carried out with the primer pair 5′-TTGTCACATTGGGAGCAGA-3′ and 5′-TCATTCCCATCAGTAGCATCC-3′ under the condition of initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 60 s, 60 °C for 60 s, and 72 °C for 60 s. PCR products were purified and analyzed by direct sequencing using a fluorescent dideoxy-terminator method and an ABI 310 DNA sequencer (Perkin-Elmer, Foster City, Calif.).

Sequencing analysis of exon 2 of the *HRH1* gene in 20 Japanese children revealed two polymorphisms in the gene. One was a novel polymorphism, a cytosine (C) to thymine (T) substitution at nucleotide position –17 bp from the first ATG within the noncoding region of exon 2. The other was a guanine (G) to adenine (A) substitution at nucleotide position 1045 (codon 349) with an amino acid change from Asp to Asn, as previously reported (Shimizu et al. 1995). We were unable to detect any previously reported polymorphisms of the *HRH2* gene (Orange et al. 1996) at nucleotide positions 398, 525, 620, 649, 692, and 802 by sequencing analysis in 25 Japanese children. We detected two novel polymorphisms, a G to A substitution at nucleotide position 543 and a C to T substitution at 826, neither of which introduced an amino acid change.

To examine a possible contribution of these polymorphisms to atopic predisposition, we performed an association study with –17C/T, 1045G/A of the *HRH1* gene, 543G/A of the *HRH2* gene, and a known 314C/T polymorphism of the *HNMT* gene using PCR-single-strand conformation polymorphism and PCR-restriction fragment length polymorphism (Fig. 1). The allelic frequencies of these polymorphisms are shown in Table 1. There were no differences in the genotype or allelic frequencies of histamine *HRH1* –17C/T, *HRH2* 543G/A, and *HNMT* 314C/T polymorphisms between the 100 atopic patients and 100 control subjects (Table 1). These polymorphisms were not associated with the age

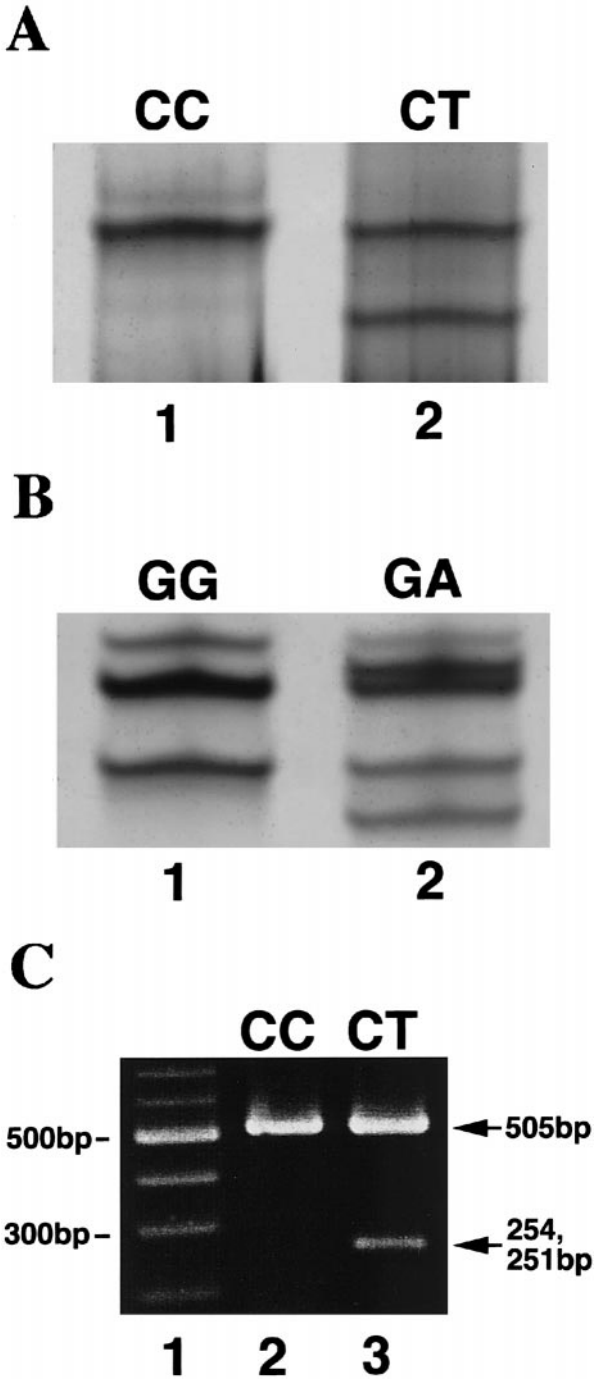

Fig. 1 Single-strand conformation polymorphism (SSCP) analysis of the –17C/T polymorphism of the *HRH1* gene (**A**) and the 543G/A polymorphism of the *HRH2* gene (**B**), and PCR-restriction fragment length polymorphism analysis of the *HNMT* gene (**C**). **A** PCR was performed with the primer pair 5′-TCATCACCCAAGTCTCTGACC-3′ and 5′-GCTCGGGTCTTGGTACGAT-3′. PCR products 512 base pairs (bp) long were digested with the restriction enzyme, *Pvu*II (Boehringer Mannheim, Mannheim, Germany) to shorten the fragments optimal for SSCP analysis, using GenePhor (Amersham Pharmacia Biotech, Uppsala, Sweden) (*lane 1* CC genotype, *lane 2* CT genotype). **B** DNA was amplified by PCR with the primer pair of 5′-ACCAGCAAGGGCAATCATAC-3′ and 5′-TGCCTTCCAGGAGCTAATGT-3′. SSCP analysis was performed using the same conditions as for *HRH1* (*lane 1* GG genotype, *lane 2* GA genotype). **C** DNA was amplified by PCR with the primer pair 5′-CTGCCCAGGGTTCTTGTAAT-3′ and 5′- GGCAGATCATGGTCACTTGT-3′ for 35 cycles at 94 °C for 60 s, 60 °C for 60 s and 72 °C for 60 s. The PCR product was digested with *EcoR*V (Takara Shuzo, Otsu, Japan) and analyzed on 2% agarose gels. The CC genotype without an *EcoR*V restriction site shows a 505-bp band after digestion (*lane 2*), while the CT genotype exhibits 505-bp, 254-bp, and 251-bp bands after digestion (*lane 3*) (*lane 1* shows the molecular size marker)

Table 1 *HRH1*, *HRH2*, and *HNMT* genotypes in atopic asthma patients

	Control	Patients	*P*-value
HRH1 gene: polymorphism –17			
CC	94	96	0.51[a]
CT	6	4	
TT	0	0	
C/T (%)	97/3	98/2	0.52
HRH2 gene: polymorphism 543			
GG	91	92	0.79[a]
GA	9	8	
AA	0	0	
G/A (%)	95/5	96/4	0.80
HNMT gene: polymorphism 314			
CC	90	92	0.62[a]
CT	10	8	
TT	0	0	
C/T (%)	95/5	96/4	0.62

[a] Analysis performed among the three genotypes

at onset or the severity of atopic asthma (data not shown). As the allelic frequency of 1045G/A of the *HRH1* gene in the patients and control subjects was estimated to be 0.995:0.005, this polymorphism was not evaluated as an association with asthma.

To date, several intensive studies have been conducted to elucidate genetic factors of bronchial asthma (Daniels et al. 1996; Doull et al. 1996). However, there have been no association studies between histamine receptors/*HNMT* and atopic asthma, despite their major contribution to allergies. Our study of three candidate atopy genes of *HRH1* (–17C/T), *HRH2* (543G/A), and *HNMT* (314C/T) failed to show any association of these genes with an increased risk for bronchial asthma or atopy or with the severity of atopic asthma. As the imbalances in allelic frequencies of *HRH1*, *HRH2*, and *HNMT* genes may influence the results of our association studies, a larger cohort might be needed to detect a small difference in disease frequencies or phenotypes between atopic asthma patients and control subjects. None of the six allelic variations of the *HRH2* gene found in Caucasians (Orange et al. 1996) were detected in Japanese subjects, indicating a difference in genetic background among races. Alternatively, if there are other ethnic groups with a higher frequency of these polymorphisms, an association study of these polymorphisms would be useful for clarifying the genetic background associated with the development of atopic asthma.

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