



Association of *CRTH2* gene polymorphisms with the required dose of antihistamines in patients with chronic urticaria

Introduction: Chronic urticaria (CU), defined as the recurring incidence of wheals with or without angioedema for more than 6 weeks, is a common disorder associated with mast cell activation, degranulation, and histamine release. Considering the association between the *CRTH2* gene and mast cells, we investigated the association of this gene polymorphism with the CU phenotype and antihistamine drug requirement in patients with CU. **Materials & methods:** Two groups consisting of 384 patients with CU and 231 patients as normal controls (NCs) were enrolled from the Department of Allergy and Rheumatology, Ajou University Hospital, Suwon, Korea. Two polymorphisms of the *CRTH2* gene, -466T>C and -129C>A were genotyped using primer extension methods. **Results:** No significant differences were detected in the genotype and allele frequencies of the two *CRTH2* polymorphisms between the CU and NC groups, and no significant associations were observed with clinical parameters, such as atopy status, serum total IgE, prevalence of autoantibodies and duration of CU. However, CU patients with homozygous TT genotypes had significantly higher dose requirements of antihistamines to control the CU symptoms (164.56 ± 115.62 vs 137.38 ± 90.15 loratadine equivalents, mg/week) than those with the CT and CC genotypes ($p = 0.025$). The luciferase activity was significantly enhanced in the construct containing *CRTH2* 466C compared with the -466T-containing construct ($p < 0.001$). Co-transfection experiments with GATA-3 (300 ng) and the -466T and -466C *CRTH2* alleles revealed that the *CRTH2* -466T allele produced a greater increase in induction of luciferase activity than the -466C allele ($p < 0.001$). **Conclusion:** The *CRTH2* -466T>C gene polymorphism may not affect on the phenotype of CU, but contributes to the required dose of antihistamines in patients with CU.

KEYWORDS: antihistamine drugs • chronic urticaria • *CRTH2* • polymorphism

Nami Shrestha Palikhe,
Seung-Hyun Kim,
Young-Min Ye,
Gyu-Young Hur,
Bo-Young Cho &
Hae-Sim Park*

*Author for correspondence:
Department of Allergy and
Rheumatology, Ajou University
School of Medicine,
Youngtonggu Wonchon Dong
San 5, Suwon, Korea
Tel.: +82 031 219 5196;
Fax: +82 031 219 5154;
hspark@ajou.ac.kr

Chronic urticaria (CU), defined as dermal edema resulting from vascular dilatation and leakage of fluid into the skin in response to molecules released from mast cells, lasts for 6 weeks or more [1]. The pathogenic mechanism remains obscure even though skin mast cells and basophils are the key cells involved in CU. Clinical studies have shown that H1 antihistamines are highly effective for the inhibition of the hives/wheals and pruritus associated with CU [2]. Based on the severity of the disease, antihistamines are usually the drug of choice.

Prostaglandin D2 (PGD2), a product of the arachidonic acid/cyclooxygenase pathway, is generated at sites of inflammation and is believed to play an important regulatory role in inflammatory and immune responses. Predominantly released by activated mast cells [3], PGD2 has a prominent role in allergic responses [4–6] due to its ability to induce leukocyte extravasations and by acting as a chemoattractant for subsets of helper-inducer T lymphocytes (Th2) cells, eosinophils and basophils [7–9]. At present two different PGD2 receptors, the D prostanoid

receptor (DP) and the more recently described chemoattractant receptor-homologous molecule expressed on Th2 lymphocytes (CRTH2) are known. CRTH2 is a member of the G protein-coupled, seven transmembrane receptor family. The biological functions of CRTH2 are much less defined. In certain human cells, activation of CRTH2 leads to a decrease in cAMP levels [10,11] and triggers a pertussis-sensitive Ca signal [7,8], suggesting that CRTH2 couples to a G protein of the $G_{ai/o}$ class. CRTH2 levels effect on circulating T cells have been shown to be correlated with the severity of atopic dermatitis [12,13].

The importance of PGD2 in inflammatory cell activation and recruitment *in vitro* and *in vivo* has been reported in several studies [9,14–17]. Recent reports emphasize the importance of CRTH2 in allergic inflammation, suggesting that CRTH2 signaling may occur in the absence of PGD2 production and independently of PGD synthase [14,15]. However, the exact correlation between CRTH2 and Th2 cells in various disease settings remains to be elucidated [3]. Moreover,

evidence indicates that genetic alteration of *CRTH2* is related to allergic asthma. Sequence analysis of SNPs in the gene encoding *CRTH2* has revealed variants that are associated with a higher degree of bronchial hyper-responsiveness and the occurrence of near-fatal asthma [18]. *CRTH2* polymorphisms influence the susceptibility and severity of asthma and may serve as genetic markers for identifying high-risk individuals. Although this gene was suggested as having a potential role in the pathogenesis of asthma, no study has examined the association between *CRTH2* polymorphisms and CU.

Previous studies also revealed an effect of PGD2 on histamine release from basophil and mast cells [19,20]. Therefore, this study was designed to evaluate whether genetic variation in the *CRTH2* gene confers a risk for developing CU. We investigated the association of *CRTH2* polymorphisms with the phenotype of CU and the required dose of antihistamines in patients with CU.

Materials & methods

■ Study subjects

Two groups, consisting of 384 patients with CU and 231 patients from a normal control (NC) group were enrolled at the Department of Allergy and Rheumatology, Ajou University Hospital, Suwon, Korea. The CU phenotype was defined as those having CU for more than 6 weeks in duration and the subjects were excluded if they were associated with food allergy, urticarial vasculitis, physical urticaria, infectious agents, parasites or autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis and Graves' diseases. The NC group consisted of individuals having no personal or family history of allergic diseases, or aspirin or other drug hypersensitivity. They were chosen as the control group because this has been the most adopted population for comparative case-association studies [21–24]. Informed consent was obtained from all subjects and the Institutional Review Board of Ajou University Hospital approved the study. Skin prick tests were performed with 55 common aeroallergens (Bencard Co., West Sussex, UK), and atopy was defined as one or more positive reactions to common inhalant allergens. Two thyroid autoantibodies, antithyroglobulin and antimicrosomal antibodies, which are known to be associated with CU phenotype in our previous report [25], were detected using anti-thyroglobulin and anti-thyroid peroxidase radioimmunoassay (BRAHMS AG, Hennigsdorf, Germany), respectively.

■ SNP identification & genotyping

For SNP identification, genomic DNA donated from 40 healthy Korean volunteers were used. Genomic DNA was prepared from peripheral blood samples using the Puregene DNA purification kit (Gentra, MN, USA) according to the manufacturer's protocol. A region of *CRTH2*, upstream of exon 2 and ATG, was sequenced to examine SNPs using the ABI Prism® 3100 DNA analyzer (Applied Biosystems, CA, USA). Two sets of primers: forward, 5'-CTGTTTATGGTCGGCACA-3' and reverse, 5'-ATGACTCCATTCTCCACCA-3' were designed for the amplification and sequencing analyses and were based on GenBank sequences (GenBank ID: 11251). Sequence variants were verified using chromatograms. SNP information of *CRTH2* was obtained from the SNP database in NCBI. Numbering of two SNPs was relative to ATG in this paper.

We genotyped two polymorphisms of *CRTH2* using a primer extension method and the SNAPshot ddNTP primer extension kit. (Applied Biosystems, USA). The region of genomic DNA spanning the polymorphic site was amplified using PCR with one phosphothiolated primer and one regular PCR primer. The forward and reverse primer sequenced for the intron region of *CRTH2* -466T>C gene was forward 5'-GATGCTGTGGGAAGGGGC-3', reverse 5'-AGAGCACTGGCGGGTGAA-3', extension primer 5'-CTCCATCGCC-CAGGCCAGCAGAAT-3' and 129C>A were forward 5'-TAAATGACTTGCCCAAGCA-3', reverse 5'-GACCCTGCCCAGGAGATG-3' extension primer CCAATGTGACCCACGAA-TATATTGG.

■ Antihistamine drug requirement

In all of the patients whose follow-up duration was longer than 1 year, drug requirements for antihistamines (H1 and H2) and steroids were calculated and compared between the genotypes of the *CRTH2* -466T>C polymorphism.

Since loratadine is the most commonly used H1-antihistamine drug for treatment of CU in Korea, the daily doses for other antihistamine drugs were calculated based on the equivalent dose of loratadine 10 mg/day. We used a dose conversion table prescribed by the hospital for analysis of the total doses of antihistamine drugs. For each tablet, prescribed dose mentioned was obtained from Korean FDA guidelines [101]. Loratadine (Clarityne) one tablet (10 mg) was equal to one loratadine dose; bepotastine (Talion) one tablet (10 mg) was equal to half

loratadine; chlorpheniramine (Peniramin) one tablet (2 mg) was equal to one third loratadine; ebastine (Ebastel®) one tablet (10 mg) was equal to one loratadine; epinastine (Alesion®) one tablet (10 mg) was equal to half loratadine; fexofenadine (Allegra) one tablet (180 mg) was equal to one loratadine; and hydroxyzine (Ucerax®) one tablet (25 mg) was equal to one third loratadine.

The required antihistamine doses were calculated from the total antihistamine doses used for control of CU during the period of treatment, presented as the equivalent dose (mg/week) of loratadine for the H1 antagonist and cimetidine for the H2 antagonist. Similarly, the dose requirement of total (oral) steroids was calculated as the prednisolone equivalent (average dose, mg/week) dose (prednisolone 1 tablet equal to one tablet deflazacort, and equal to 1 tablet methylprednisolone).

■ Plasmid construction

A 762-bp fragment of the human *CRTH2* gene was prepared by PCR amplification from either -466C homozygous or -466T homozygous human genomic DNA as a template (forward primer, 5'-CTGTTTATGGTTCGGCACA-3'; reverse primer, 5'-ATGACTCCATTCTCCACCA-3'). Each of the PCR products was gel-purified with an agarose-gel purification kit (iNtRON, Daejeon, Korea) and then ligated into the TA vector (Invitrogen, CA, USA). Next, the plasmid was digested with *KpnI* and *XhoI* restriction enzymes (Takara, Shuzo, Japan) and ligated into similarly digested pGL3-basic luciferase reporter vector (Promega, WI, USA) using T4 DNA ligase (iNtRON). All constructs were verified by direct sequencing. Plasmid DNAs were prepared from these constructs using the Endo Free Plasmid Maxi kit (Qiagen, Hilden, Germany). Plasmid DNA concentration and purity were assessed using UV spectrophotometry and agarose gel electrophoresis.

■ Transfection & luciferase assay

Human mast cell line (HMC)-1 cells were transfected by using microporator MP-100 (Digital Bio Technology, Seoul, Korea) and PMK-1096 (Digital Bio Technology) according to the manufacturer's instructions. Briefly, 24-well plates were filled with 1000 µl of culture medium containing 10% fetal bovine serum (FBS) without antibiotics. Plates were preincubated in a humidified 37°C and 5% CO₂ incubator. HMC-1 cells (2 × 10⁵), reporter plasmid DNA of *CRTH2* (1 µg), and Renilla plasmid DNA (5 ng) were resuspended in 10 µl of Solution R.

Co-transfection with GATA-3 binding protein (GATA-3) was carried out with reporter plasmid DNA (1 µg; *CRTH2* promoter 0.7 µg, GATA-3 0.3 µg) and Renilla plasmid DNA (5 ng). The incorporation tube was filled with 3 ml of Solution E. The cell and DNA mixtures were inserted into the microporator pipette station. The cells were shocked using 1750 V/20 mA/plus number 1. The samples were transferred to the preincubated 24-well plate. Plates were incubated for 24 h. Cells were then lysed with 100 µl of passive lysis buffer (Promega) per well. From the 100-µl lysate, a 20-µl aliquot was assayed for luciferase activity using a Dual-Luciferase® reporter assay kit (Promega) and Clarity™ (Bio-Tek, VT, USA). The pGL3-Control (Promega) and the promoter-less pGL3-basic vectors were used as positive and negative controls, respectively. Transfection efficiency was determined by measuring Renilla activity using a Dual-Luciferase reporter assay kit (Promega) after co-transfection of both the reporter construct and Renilla control vector into the cell line.

■ Statistical analysis

Significant departures of the genotype frequency from Hardy–Weinberg equilibrium at the polymorphic site were tested using χ^2 analyses. Differences in the mean values of phenotypic characteristics between CU and NC were compared using analysis of variance (ANOVA) and *t*-tests. Differences in genotype frequency between the two groups were examined with the χ^2 test using SPSS (SPSS Inc., IL, USA), and three logistic regression models (co-dominant, dominant, and recessive) were used after accounting for age and sex as covariates. Relative luciferase activity was determined by an independent *t*-test. The level of statistical significance was set at $p < 0.05$.

Results

■ Allele & genotype frequencies of the *CRTH2* gene

Two SNPs, -466T>C and -129C>A, were identified in the intron region (upstream of exon 2 and ATG) of the *CRTH2* gene. No significant differences were observed in the genotype and allele frequencies of *CRTH2* polymorphisms between the CU and NC groups ($p > 0.05$) (TABLE 1). The genotype distribution of the two polymorphisms did not depart significantly from Hardy–Weinberg equilibrium ($p > 0.05$). No significant associations were noted with clinical parameters such as atopy status, serum total IgE or prevalence of autoantibodies (TABLE 2).

Table 1. Allele and genotype frequency of chronic urticaria.

	Genotype	CU (frequency, %)	NC (frequency, %)	p-value
<i>CRTH2</i> -466T>C	TT	192 (50.00%)	103 (44.60%)	0.174
	CT	166 (43.20%)	109 (47.20%)	0.358
	CC	26 (6.80%)	19 (8.20%)	0.224
	q	0.284	0.318	0.192
<i>CRTH2</i> -129C>A	CC	333 (94.10%)	129 (97.00%)	0.269
	AC	21 (5.9%)	4 (3.00%)	NA
	AA	–	–	0.269
	q	0.3	0.15	0.275

Each p-value was calculated with codominant, dominant, and recessive models. Logistic regression analysis was applied to control for age and sex as covariables.
CU: Chronic urticaria; NA: Not available; NC: Normal control; q: Minor allele frequency.

■ Association of the *CRTH2* gene with antihistamine requirement

The *CRTH2* polymorphism -466T>C showed a significant difference in the required dose of antihistamines. Patients with CU having homozygous TT genotypes needed significantly higher doses of antihistamines (164.56 ± 115.62 versus 137.38 ± 90.15 loratadine equivalents, mg/week) to control CU compared with those with CT and CC genotypes ($p = 0.025$; see TABLE 3). Such association was not observed in the case of -129C>A (data not shown). No significant differences were detected in requirements of total (oral plus systemic) steroids (9.66 ± 21.37 vs 8.32 ± 12.7 prednisolone equivalents, mg/week) and H2 receptor antagonists (1019.62 ± 1079.66 vs 924.77 ± 1396.11 cimetidine equivalents, mg/week) between the two groups.

■ Functional analysis by Dual-Luciferase reporter assay

We checked *CRTH2* endogenous gene expression in different cell lines; epithelial cell line (Bease-2b), human bronchial epithelial cell line (A549), human acute monocytic leukemic cell line (THP-1), human leukemic monocyte lymphoma cell line (U937), human epithelial carcinoma cell line (Hela), HMC-1 and human promyelocytic leukemic cells (HL-60) (FIGURE 1). The HMC-1 cell line was chosen to measure the promoter activity as this cell line represents an appropriate mast cell model.

To examine whether the *CRTH2* -466T>C polymorphism is associated with altered promoter activity, a construct composed of the *CRTH2* sequence and a luciferase reporter gene was transfected into the HMC-1 cell line. Reporter activities were compared between constructs containing either *CRTH2* -466T or *CRTH2* -466C in the gene. The luciferase activity was enhanced significantly in the construct containing *CRTH2* -466C when compared with the -466T-containing construct ($p < 0.001$; see FIGURE 2). Furthermore, a survey of a transcription factor database conducted using the consite program suggested that the -466T>C polymorphism generates a GATA-3 transcription factor-binding motif (FIGURE 3A) [102]. The co-transfection experiment with GATA-3 containing the *CRTH2* -466T and the -466C alleles demonstrated that the *CRTH2* -466T allele produced a greater increase in induction of luciferase activity compared with the *CRTH2* -466C allele ($p < 0.001$; see FIGURE 3B & 4).

Discussion

We investigated the genetic polymorphisms of *CRTH2* for an association with CU compared with the NC group in a Korean population. This is the first study to screen and investigate the SNPs of *CRTH2* in patients with CU. However, no significant differences were found in allele and genotype frequencies of the SNPs

Table 2. Clinical characteristics of study subjects according to genotype in *CRTH2* -466T>C polymorphism.

Clinical characteristics	<i>CRTH2</i> -466T>C		p-value
	TT	CT or CC	
Sex (male/total)	89/191 (46.60%)	84/192 (43.80%)	0.608
Atopy (presence/total)	94/160 (58.8%)	86/153 (56.2%)	0.732
Antithyroglobulin antibody (positive/total)	18/130 (13.8%)	24/124 (19.4%)	0.243
Antimicrosomal antibody (positive/total)	17/130 (13.1%)	17/124 (13.7%)	1.000
Anti- <i>Helicobacter pylori</i> antibody (positive/total)	24/36 (66.7%)	25/51 (49%)	0.127

between the study groups, suggesting that these gene polymorphisms may not be related to CU phenotypes in this Korean population.

We also analyzed the antihistamine drug requirement between two groups of study subjects and found that the *CRTH2*-466T genotype was associated with a higher dose requirement of antihistamines to control CU. A previous study reported that the PGD2-*CRTH2* system plays a significant role in chronic allergic skin inflammation, and therefore, *CRTH2* may represent a novel therapeutic target for the treatment of human atopic dermatitis [26]. In this study, the higher dose requirement of antihistamine drug, calculated as equivalent doses of loratadine in the TT genotype, can be correlated with the differential role of the PGD2-*CRTH2* receptor for the histamine release observed in the *CRTH2*-466T>C polymorphism.

In the present *in vitro* functional study, the *CRTH2* polymorphism enhanced the transcriptional activity in the human mast cell line HMC-1. We selected the HMC-1 cells for measuring promoter activity since this type is the key effector cell for CU. However, we also used other cells such as jurkat-T cells, human monocyte cells, U937 cells and replicated similar results (data not shown). Mast cell activation is important in the promotion of Th2 accumulation, independent of antigen presentation by dendritic cells. Studies in IgE transgenic mice have shown that immunological activation of mast cells can lead to recruitment of Th2 cells into the airways in the absence of antigen-specific T-cell activation [27,28]. In this study, the -466C allele of *CRTH2* showed an increased promoter activity, which may induce increased expression of *CRTH2* on inflammatory cells and suppress the histamine release from skin mast cells, and require less antihistaminic drug. PGD2 has been shown *in vitro* to suppress the antigen-induced histamine release from RBL-2H3 cells, suggesting that PGD2 inhibits IgE-mediated scratching by suppressing histamine release from mast cells [29]. The study showed that PGD2 significantly shortened the basophil life-span, suggesting differential modulation of human basophil functions through PGD2 receptors including *CRTH2* [20]. The study also showed that the *CRTH2* agonist, 13,14-dihydro-15-keto-PGD2 (DK-PGD2), as well as the nonspecific agonist, PGD2, enhanced basophilic degranulation and histamine release that was antagonized by ramatroban (3R-[[4-fluorophenyl]sulfonyl]

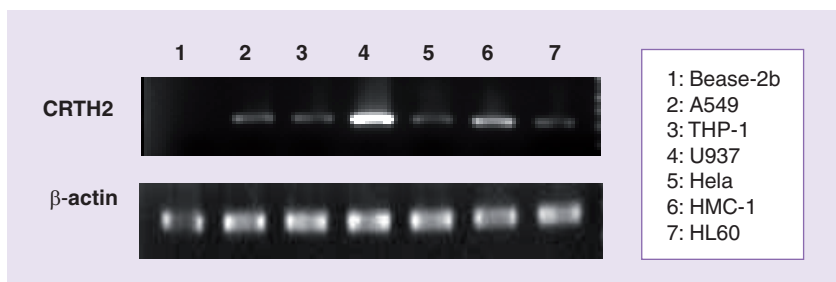


Figure 1. *CRTH2* expression in different cell lines. Normalization was performed with β -actin.

amino]-1,2,3,4-tetrahydro-9H-carbazole-9-propanoic acid), a *CRTH2* antagonist. By contrast, the lower promoter activity of the *CRTH2*-466T allele may exhibit decreased expression of *CRTH2* on inflammatory cells, and may be responsible for the increased histamine release and higher required dose of

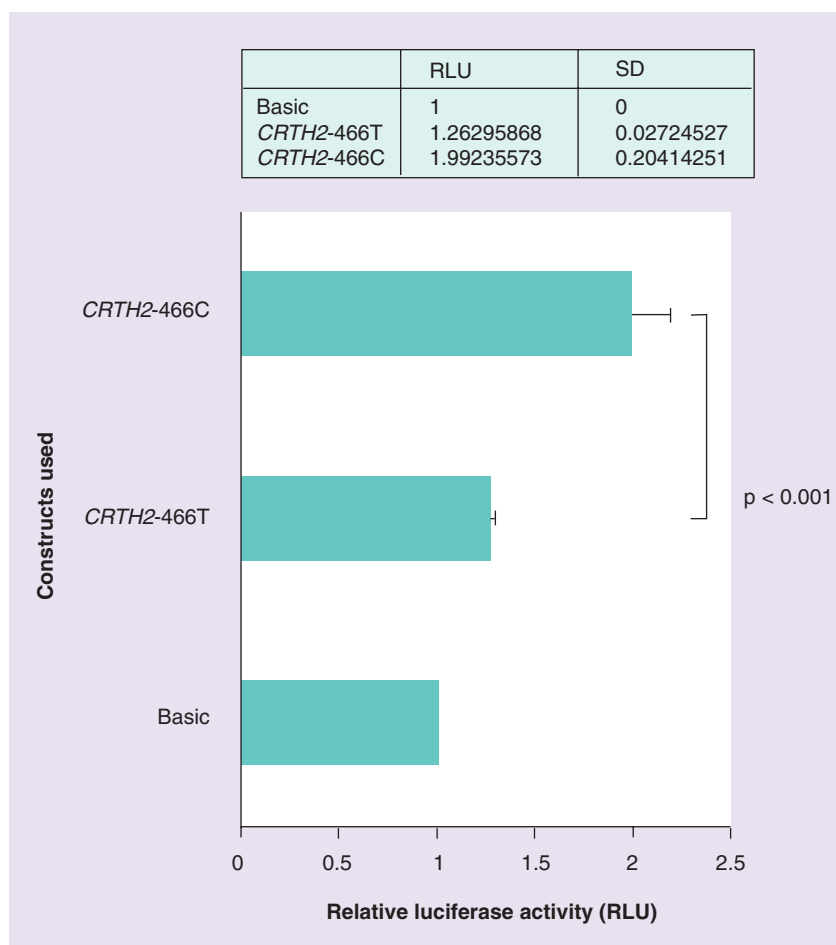


Figure 2. Promoter activity assay of the human *CRTH2* gene polymorphism in HMC-1 cells. The RLU is represented as the ratio of the activity to the luciferase activity in the cells transfected with the promoterless basic vector, pGL3B. Data are the mean $\pm$ standard deviation values of three independent experiments in total, triplicated for each sample, $n = 9$. Statistical analysis was performed using an independent *t*-test.

HMC: Human mast cell; RLU: Relative luciferase activity; SD: Standard deviation.

Table 3. Dose requirement of steroids and antihistamines according to genotype of *CRTH2* -466T>C.

Parameters	<i>CRTH2</i> -466T>C		p-value
	TT	CT or CC	
Cimetidine (average dose, mg/week)	1019.62 ± 1079.66 /66	924.77 ± 1396.11/86	0.6
Loratadine (average dose, mg/week)	164.56 ± 115.62/156	137.38 ± 90.15/143	0.025
Duration of urticaria (years)	2.62 ± 4.47	2.51 ± 4.01	0.85
Total doses of steroid administered (average dose, mg/week)	9.66 ± 21.36/117	8.32 ± 12.7/106	0.574

The values are presented as mean ± standard deviation. Values in bold indicate significant p value.

antihistamines. From this observation, we concluded that the major T allele in the *CRTH2* -466T>C polymorphism may affect histamine release from skin mast cells. In fact the

data from the innate immunity program for genomic application (IIPGA) [103] has shown that in cell lines from European Americans (n = 23) and African-Americans (n = 23) the major allele was *CRTH2* -466C. Therefore, our present data may be specific for the Korean population only.

GATA-3 is a transcription factor critical to the differentiation of the Th2 phenotype [30]. Since the *CRTH2* -466T contains the GATA-3 sites; we investigated whether GATA-3 could induce *CRTH2* wild-type expression at the transcription level. We performed cotransfection of *CRTH2* with GATA-3 and compared the promoter activity of *CRTH2* -466T and -466C allele by calculating the induction fold ratio of with and without cotransfection with GATA-3 for each allele (Figure 4). Our co-transfection experiments with GATA-3 displayed significantly higher luciferase induction in the wild-type of *CRTH2* than in the mutant. Thus, GATA-3 protein binding may be responsible for the enhanced effect of transcriptional activity in wild-type *CRTH2*. Previously, a causative link was reported between *CRTH2* expression and GATA-3 upregulation [31]. The main functions established for GATA-3 are exclusively in the T-cell lineage, including T-lineage specification and peripheral T-helper type 2 (Th2) differentiations [32]. Even though a previous study has shown that mature mast cells differentiated from mouse embryonic stem cells do not express GATA-3 [33], new data suggest that GATA-3 may also have a determining function in mast cell lineage development [34]. Therefore, the GATA-3 responsive site created by the *CRTH2* -466T allele may play a role in Th2 cells. This could be the SNP effect on Th2 cell expression of *CRTH2* and the increased dose requirement for antihistamine indicates there was more PGD2 released from mast cells and propagation of the Th2 immune response. Further study, such as electrophoretic mobility shift assays (EMSA), is required to investigate the role of GATA-3 for each

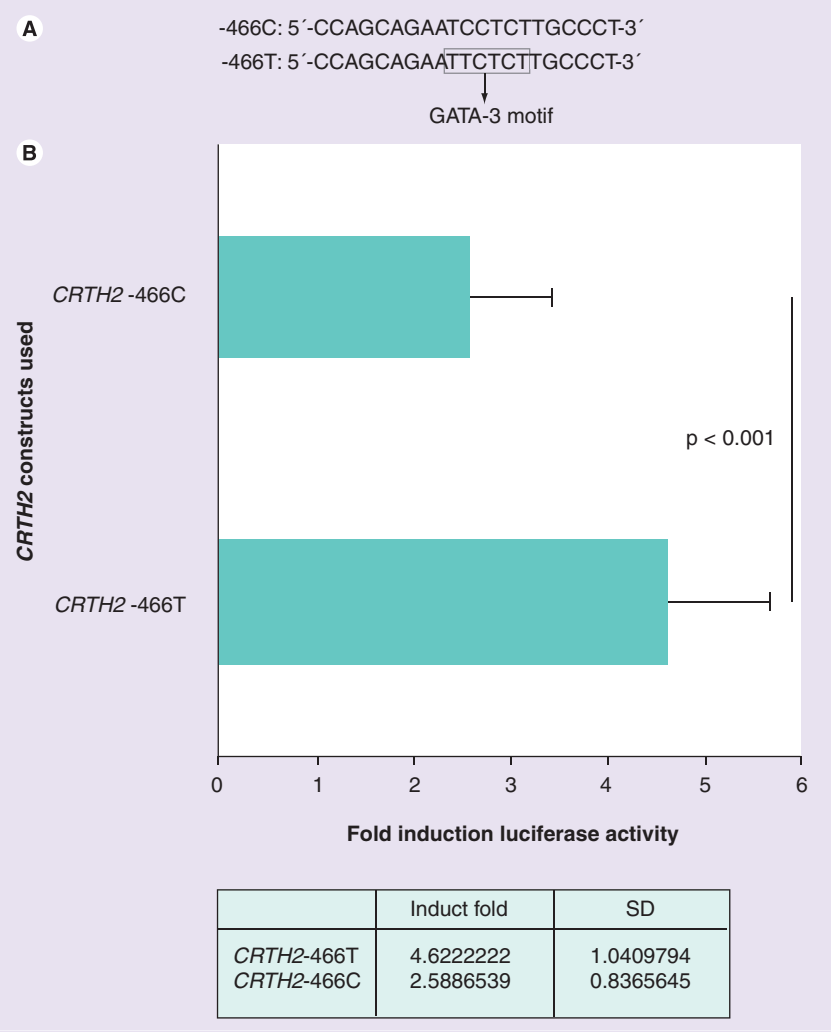


Figure 3. Cotransfection of the human *CRTH2* gene polymorphism with GATA-3 in HMC-1 cells. (A) The *CRTH2* -466T>C polymorphism generates a GATA-3 transcription factor binding motif. (B) Promoter activity of *CRTH2* gene cotransfected with GATA-3 in HMC-1 cells. The RLU is represented as the ratio of the RLU (the RLU is represented as the ratio of the activity to the luciferase activity in the cells transfected with the promoterless basic vector, pGL3B) of *CRTH2* T and C allele cotransfected with and without GATA-3. Data are the mean ± SD values of three independent experiments in total, triplicated for each sample, n = 9. Statistical analysis was performed using an independent t-test. HMC: Human mast cell; RLU: Relative luciferase activity; SD: Standard deviation.

allele, as the previous study has demonstrated that the GATA-3 binds to a probe from the *CRTH2* promoter by EMSA [35]. Furthermore, it is essential to investigate the regulatory role of other transcriptional factors, variability of cytokine release and *CRTH2* expression *in vivo* in terms of genetic polymorphisms.

Our data at present poses a few limitations. The exact prevalence of CU has not been reported in our population because CU is often a remitted disease. Moreover, the sample sizes enrolled in this study are thought to be a large cohort based on a homogenous population to compare genotype frequency. Despite the fact that a few genotype data were missed due to assay failure, our data cannot be overlooked for the association of CU with antihistamine drug requirement which was further confirmed by *in vitro* functional studies.

In conclusion, these findings suggest that the *CRTH2* polymorphism is important in determining the antihistamine dose requirement in patients with CU via regulation of *CRTH2* expression, and may be used as a pharmacogenetic marker for predicting antihistamine requirements in those patients.

Conclusion

We conclude that the *CRTH2* -466T>C gene polymorphism may not affect the phenotype of CU, but contributes to the requirement of antihistamines in patients with CU via *CRTH2* expression.

Financial & competing interests disclosure

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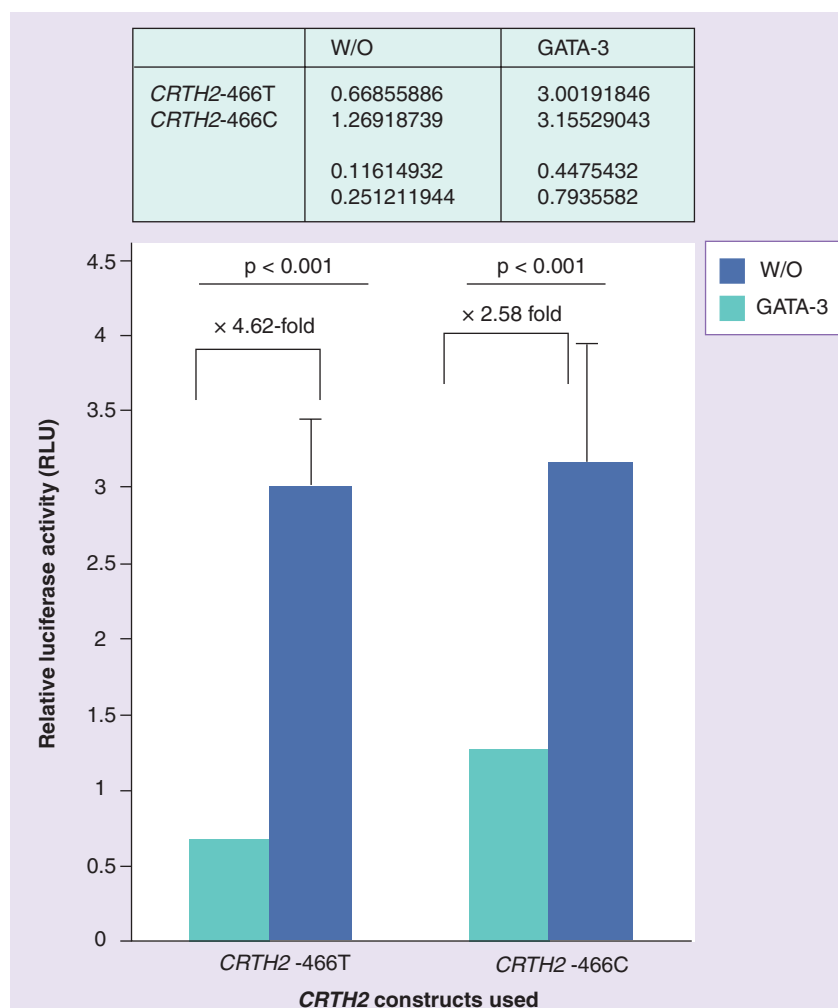


Figure 4. Promoter activity of *CRTH2* gene with and without cotransfected GATA-3 in HMC-1 cells. Data are the mean $\pm$ standard deviation values of three independent experiments in total, triplicated for each sample, $n = 9$. Statistical analysis was performed using an independent *t*-test. HMC: Human mast cell.

financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- The pathogenic mechanism of chronic urticaria (CU) is not well understood even though skin mast cells and basophils are the key cells involved. In this study genetic polymorphism of the *CRTH2* gene and the antihistaminic requirement for treatment of patients with CU were studied.

The key findings of this study are:

- Patients with CU with homozygous TT genotypes required significantly higher doses of antihistamines to control CU symptoms (164.56 ± 115.62 vs 137.38 ± 90.15 loratadine equivalents, mg/week) than those with the CT and CC genotypes.
- Luciferase activity was significantly enhanced in the construct containing *CRTH2* -466C when compared with the -466T-containing construct.
- Co-transfection experiments with GATA-3 (300 ng) containing the *CRTH2* -466T and -466C alleles showed that the *CRTH2* -466T allele produced a greater increase in luciferase activity than the *CRTH2* -466C allele.
- These findings suggest that the *CRTH2* gene polymorphism is important in determining the antihistamine dose requirement in patients with CU via regulation of *CRTH2* expression, and may be used as a pharmacogenetic marker for predicting antihistamine requirements in those patients.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all

human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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