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C reactive protein (CRP) rs3093059C predicts poor mizolastine response in chronic spontaneous urticaria patients with elevated serum CRP level

CRP -757C/T affect mizolastine response in CSU

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Abstract

Chronic spontaneous urticaria (CSU) is a frequent disorder with recurrent itchy wheals and/or angioedema, and nearly 35% patients respond poorly to nonsedating H1 antihistamines treatment. *CRP* gene encodes the C reactive protein, which is involved in the pathogenesis of CSU. To investigate the impacts of *CRP* polymorphisms on the susceptibility and therapeutic efficacy in the South Han CSU patients, we enrolled 145 CSU patients in our study. After 4-week nonsedating H1 antihistamine monotherapy treatment, more than 50% reduction of the severity score is considered as effective, or else non-effective. The *CRP rs3093059T/C* and *rs2794521G/A* genotypes of patients were determined by Sequenom MassARRAY. Functional studies including relative luciferase assay and β -Hexosaminidase assay were conducted in HEK293T cells or RBL-2H3 cells to explore the function of variants. Forty (62.50%) CSU patients were effective when treated with mizolastine, and 55(72.4%) patients were effective in the desloratadine group. We found that the patients carried with *rs3093059TT* genotype was significantly associated with good response (OR=4.20, $p=0.015$), had lower serum CRP, IL-6 and TNF- α levels than the

CT/CC genotypes. *In vitro*, the rs3093059C allele exhibited significantly higher luciferase activity than wild allele ($p < 0.001$). From the β -Hexosaminidase assay, we observed the inhibiting degranulation effects by mizolastine and this effect is weakened when with a higher dose CRP in RBL-2H3 cells. Our findings suggested that CSU patients carrying the rs3093059C allele may respond poorly to mizolastine with elevated serum CRP level.

KEYWORDS

Chronic spontaneous urticaria (CSU); gene polymorphisms; mast cell; CRP; antihistamine

1 INTRODUCTION

Due to the influence of the environment, ethnic and other unknown factors, the prevalence of allergic diseases has increased by nearly seven-folds compared to ten years ago, including chronic urticaria^[1, 2]. It is reported that approximately 1-1.5% of the whole world population suffer from urticaria and 15-20% of them have chronic urticaria. Chronic urticaria is a common disorder with wheals, pruritus and occasional angioedema, lasting more than six weeks that occurs almost daily^[3-5]. Chronic spontaneous urticaria (CSU) is the most common type of chronic urticaria among the young people in China according to a multicenter hospital-based study^[6]. Due to the long-term recurrence, extensive lesions and pruritus, as well as unstable drug efficiency, the impact on the quality of CSU patients' daily life is equal to ischaemic

heart disease,^[7-10] and the annual economic costs reached almost 10,000 Chinese yuan for each CSU patients^[11]. Additionally, nearly 35% patients respond in vain on CSU therapy and efficacy varies between different patients^[12]. Therefore, we should take measures to optimize and achieve better clinical outcomes for CSU patients.

Previous studies pointed out that mast cells and basophils play significant roles in the pathogenesis of CSU^[13, 14]. For instance, it has been reported that the coagulation system and complement system participate in the mast cell activation in CSU patients^[15, 16]. C reactive protein (CRP), encoded by the *CRP* gene, is greatly increased during infection, acute injury and autoimmune-related diseases^[17]. CRP is regarded as an acute phase protein (APP) in the course of mast cell degranulation and complement system activation in the coagulation system^[18-21]. CRP is correlated with the disease severity of CSU^[22]. Recently, studies showed that *CRP* polymorphisms affected the serum CRP level, and there was a positive association between *CRP* polymorphisms and disease disorders, such as HBV-related liver cancer and stroke^[23, 24]. Interestingly, in our previous study, serum CRP protein level in CSU patients is significantly correlated with the severity of CSU^[25]. However, the association between *CRP* gene polymorphisms and the risk of CSU has not been studied. Thus, we aimed to investigate whether *CRP* polymorphisms have impacts on susceptibility to CSU.

The second generation nonsedating H1 antihistamines are the first choice for the treatment of CSU^[26, 27]. Nonsedating H1 antihistamines drugs Mizolastine, desloratadine and fexofenadine are widely used as H1-receptor antagonist. They can

stabilize mast cell membranes and have anti-inflammatory effects, such as inhibiting the secretion of Th2 cytokines and the production of leukotriene^[28]. It is reported that gene variants also have an impact on treatment outcomes^[29, 30]. According to our previous studies, both *C5AR* rs11673309 and *ORAI1* rs3741595 were associated with the efficacy of the nonsedating H1 antihistamine desloratadine^[31, 32]. Among patients treated with levocetirizine, researchers observed that the elevated CRP level was associated with poor response and *CRP* polymorphisms affected the serum CRP level^[33, 34]. Based on these facts, we hope to investigate whether *CRP* polymorphisms may influence the therapeutic efficacy of nonsedating H1 antihistamines through affecting the level of CRP.

2 MATERIALS AND METHODS

2.1 Patient enrolment

All patients enrolled in our study were outpatients of Xiangya Hospital from 2012 to 2013, diagnosed independently by two dermatologists according to the 2009 EAACI/GA(2)LEN guidelines. All the patients were treated with nonsedating H1 antihistamine monotherapy. The inclusion and exclusion criteria for enrolment were the same as in our previous studies^[32, 35]. This study was approved by the Ethics Committee of Xiangya Hospital and was registered on the Chinese Clinical Trial Registry online (the registration number is ChiCTR-OCH-14004518). Written informed consent was obtained from each patient.

All patients were treated with nonsedating H1 antihistamines monotherapy for four weeks (mizolastine 10 mg/d, desloratadine 5 mg/d, and fexofenadine 120mg/d). The Dermatology Life Quality Index (DLQI) was used to assess the quality of life in CSU patients. The efficacy of the antihistamines was evaluated using the weekly urticaria activity score (UAS7) by two professional dermatologists independently, both at baseline and after antihistamine monotherapy for four weeks. Responders were defined as those patients who had an improvement in UAS7 scores above 50% after the 4-week treatment^[31, 32, 35].

2.2 SNPs genotyping

Single nucleotide polymorphisms (SNPs) rs3093059 (-757C/T) and rs2794521 (-717G/A) locate in the promoter region of *CRP* gene and have already been discovered in the Asian population (http://asia.ensembl.org/Homo_sapiens/Transcript/Exons?db=core;g=ENSG00000132693;r=1:159712289-159714589;t=ENST00000255030). Three web-based analysis software programs were used to predict the functional damage of the SNPs rs3093059(-757C/T) and rs2794521(-717G/A).(<http://genome.ucsc.edu/cgi-bin/hgVai>; <http://asia.ensembl.org/Tools/VEP>; http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3). Both of the SNPs rs3093059 and rs2794521 were located in the promoter region of the *CRP* gene which may be the transcription factor binding site for C/EBP α , Cutl1, and POU1F1a.

Five milliliters of venous blood was obtained by venipuncture from chronic spontaneous urticaria patients. Genome DNA was extracted from the whole blood sample using a genome DNA isolation kit (Qiagen, CO) and then stored at -80°C for further analysis. All the samples were genotyped by the method of allele-specific matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Sequenom, Inc., CA, USA). Only when a 95% positive rate of a genotype matching a SNP was certified.

2.3 Detection of serum levels of IL-6 and TNF- α by ELISA

We detected the serum levels of cytokines, including IL-6 and TNF- α levels, in different rs3093059T/C and rs2794521G/A genotype carriers, according to the instructions of the ELISA kits (BD Biosciences, CA, USA).

2.4 Cells culture

HEK 293T and RBL-2H3 cells (ATCC, VA, USA) were cultured in DMEM (Biological Industries, Israel) with 10% foetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin at 37°C with 5% CO₂ in a humidified atmosphere.

2.5 Cloning of expression plasmids, Luciferase reporter assay

The pGL3 vectors expressing *CRP* rs3093059T and rs3093059C alleles were constructed by PCR amplification using human genomic DNA as a template. The product size was 1128 bp, and the primers are shown in Table S1 (see supplementary).

Each of the PCR products of the rs3093059 wild-type T allele and mutant type C allele vector was sub-cloned separately into the KpnI–XhoI site of the pGL3-Basic luciferase reporter vector (Promega, Madison, WI). The expression plasmids were transfected into HEK 293T using Turbofect Reagent (Thermo Scientific, USA). Then, the constructs (0.4 µg) were transfected into each well with pRL-TK (0.1 µg/well) plasmid for normalization of transfection efficiency differences in HEK 293T cells. After 24-hours of incubation, the cells were lysed by 1×PLB lysis and then stored at -80 °C before use. The relative reporter gene level was analyzed in a dual luciferase reporter assay according to the manufacturer's procedures (Promega Corporation, Madison, WI) and using a microplate system.

2.6 β-Hexosaminidase and cytokine release assay

We indirectly evaluated the mast cell degranulation level via β-Hexosaminidase release assay as previously described ^[36]. RBL-2H3 cells were pre-planted into 24-well plates, and recombinant CRP at different concentrations (0, 1, or 10 µg/ml) with or without mizolastine (10^{-6} mol/L) (Xi'an Janssen Pharmaceutical, Ltd) was added for 6 h. After washing three times with HBSS, phorbol-12-myristate-13-acetate (PMA) (100 nM, Sigma) and ionomycin (1 µM, Sigma) were used to stimulate cells degranulation for 4 h. Subsequently, cells and supernatant were collected to evaluate the β-hexosaminidase release ratio by microplate spectrophotometer at 405 nm, and the data were analyzed by comparing the supernatant release rate to the total

β -hexosaminidase ^[31]. Meanwhile, cytokines such as IL-6 and TNF- α in the supernatant were detected via ELISA according to the manufacturer's instructions (BD Biosciences, CA, USA).

2.7 Statistical analysis

The data were all analyzed using SPSS version 22.0 (IBM SPSS, Chicago), and the frequencies of alleles and genotypes and Hardy–Weinberg equilibrium were determined by χ^2 test. The clinical data of two groups were analyzed by independent *t*-test, and the difference of CRP concentration between different genotypes were analyzed by analysis of variance (ANOVA). Bonferroni correction was used to correct the significance after multiple-testing, and the corrected *p* values less than 0.025(0.05/2) were considered significant in this study.

The power of the sample size was calculated by G power (3.1.9.2 version). In the responder and non-responder group treated with mizolastine, the power was analyzed with the effect size being 0.7697, the α error being 0.05, the total sample size being 64; then, the estimated power was 0.9998. Clinical characteristics, such as CRP and the baseline UAS7, were also analyzed. The effect size ranged was 1.7356 in ANOVA test, the α error was 0.05, the total sample size was 145, and the power was 1.000.

3 RESULTS

3.1 Clinical characteristics of CSU patients

We detected the clinical features and *CRP* rs3093059 and rs2794521 genotypes of 145 enrolled CSU patients (55 males and 90 females with ages ranging from 18 to 66 years). The frequencies of *CRP* rs3093059 and rs2794521 genotypes in 145 CSU patients were in Hardy–Weinberg equilibrium ($p=0.400$ and 0.709 , respectively), and the rate in groups treated with mizolastine and desloratadine were also in Hardy–Weinberg equilibrium ($p=0.252$ and 0.977 , respectively). There were no significant differences of age, duration of disease, or baseline DLQI score among rs3093059TT, rs3093059CT and rs3093059CC genotypes ($P>0.05$) (Table S2). However, the serum CRP level was significantly higher in rs3093059CT heterozygotes and rs3093059CC homozygotes when compared with rs3093059TT homozygotes (4.9 ± 6.9 mg/dl vs. 11.1 ± 12.5 mg/dl vs. 2.5 ± 3.4 mg/dl, $p=0.001$). We found significantly different levels of baseline UAS7, gender ratio among different rs3093059T/C genotypes ($p=0.011$, 0.016 , respectively). The same trend could be seen among rs2794521GG, rs2794521GA and rs2794521AA genotypes (Table S2). The detailed demographic information is shown in supplementary. No significant difference of the rs3093059 and rs2794521 alleles' frequencies was found between the enrolled CSU patients and the data of South Han Chinese from the 1000 Genomes Project regarding different (Table S3).

3.2 Anti-histamine and anti-inflammatory effects of nonsedating H1 antihistamine monotherapy on CSU patients with different genotypes

Among all the 145 patients, 98 (67.59%) of the enrolled CSU patients responded well, while the other 47 (32.41%) patients were non-responders. Among all the treated patients, 76 of 145 patients were treated with desloratadine (55 patients were effective and 21 patients were no response), 64 were treated with mizolastine (40 were effective and 24 with no response), and 5 were treated with fexofenadine (3 were effective and 2 with no response). There was no significant difference between the responder group and non-responder group regarding age, gender, duration of disease, CRP, and baseline UAS7 and DLQI scores, even in the mizolastine and desloratadine-therapy group (Table S4). However, we found the whole nonsedating H1 antihistamine efficacy was different between rs3093059TT homozygotes and rs3093059CT/CC genotypes (Table 1, OR=2.58, $p=0.011$). We also observed a higher serum CRP level in rs3093059CT heterozygotes and rs3093059CC homozygotes than rs3093059TT genotype ($p=0.001$). Serum IL-6 and TNF- α levels were significantly higher in rs3093059CC and CT carriers compared with those in rs3093059TT carriers (Figure 1A, 1B).

Meanwhile, we observed a different efficacy between rs3093059TT homozygotes and rs3093059CC/CT genotypes in mizolastine treated group (Table 1). The rs3093059CT heterozygotes and rs3093059CC homozygotes manifested a weaker response than rs3093059TT homozygotes after mizolastine therapy (OR=4.20, $p=0.015$), but this was not seen in patients treated with desloratadine

monotherapy (Table 1). Additionally, patients carrying the C allele showed a poor response than those carrying the T allele ($p=0.012$). The data of CSU patients treated with fexofenadine could not be analyzed due to the small sample size. For rs2794521G/A SNP showed no influence on the nonsedating H1 antihistamines efficacy ($p=0.040$, Table 1), thus we didn't explore its further mechanisms.

3.3 Relative luciferase activity of different rs3093059 alleles

To investigate the mechanism of rs3093059T/C on the impact of nonsedating antihistamines efficacy, the luciferase reporter assay was tested in *in vitro* experiments. We constructed luciferase reporter plasmids expressing rs3093059C, rs3093059T and transfected them into HEK293T cells. Constructs that expressing the rs3093059C allele exhibited significantly higher luciferase activity compared with rs3093059T allele ($P < 0.001$, Figure 2A). This result suggests that the *CRP* gene with the rs3093059C allele is more active than the rs3093059T, respectively.

3.4 β -Hexosaminidase release assay after stimulation with recombinant CRP

After incubation with mizolastine and recombinant CRP at different concentrations, PMA and ionomycin-induced β -hexosaminidase release was measured. We found that the degranulation level in RBL-2H3 cells decreased with the mizolastine incubation ($P < 0.001$, Figure 2B) and this inhibition was attenuated by recombinant CRP at a higher dose on β -hexosaminidase release (Figure 2B).

3.5 Cytokine secretion from mizolastine-treated RBL-2H3 cells stimulated by recombinant CRP at different concentrations

In RBL-2H3 cells, the degranulation level is elevated after the incubation of CRP alone and declined when added mizolastine. The degranulation of mast cell is accompanied with the release of cytokines and chemokines. Just like the degranulation inhibition, we found that the cytokines IL-6 and TNF- α secreted in the supernatant were also inhibited by mizolastine, and this inhibition was weakened by a higher concentration of recombinant CRP in RBL-2H3 cells. (Figure 2C, 2D).

4 DISCUSSION

Our results suggest that CRP SNP rs3093059T may predict a good efficacy of non-sedating H1 antihistamines among CSU patients, especially mizolastine. Rs3093059T allele carriers took weaker promoter activity, and lower TNF- α and IL-6 level. The inhibition ability of the mast cell degranulation by mizolastine is weakened with a higher CRP level.

Studies indicated that not all CSU patients respond well to the H1 antihistamines, even at high doses^[7]. Nearly 20% patients prescribing with up-dosing fexofenadine still responded poorly^[12]. The mechanisms underlying the inter-individual differences are still unclear. It is suggested that gene polymorphisms may have an impact on individual responsiveness to drugs. In a Korean study, the *CRTH2* gene mutant influenced the cAMP level and resulted in an elevation in calcium influx thus affecting

the desloratadine doses in CSU patients^[30]. CRP is secreted by liver cells and CRP can influence mast cell degranulation. Previous studies showed that CRP might predict non-sedating H1 antihistamine responsiveness, in which non-responders have a higher serum CRP level^[37, 38]. A Tunisian CSU study showed there was a CRP high concentration remission after desloratadine therapy^[39]. Meanwhile, articles on the study of *CRP* gene polymorphisms indicate that *CRP* genetic variants (such as rs1800947, rs1205, rs3093059) are associated with disease, including stroke, Takayasu arteritis and HBV-related liver cancer^[40-42]. Thus, we investigate whether CRP gene polymorphisms influence drug therapy and disease risk. Our in vitro data and results suggest that the CRP gene polymorphism rs3093059T/C may be associated with the therapeutic efficacy of non-sedating H1 antihistamines in CSU patients. As we known, this is the first study to illustrate the influence of CRP genetic polymorphisms on the development of CSU and even the non-sedating H1 antihistamines efficacy.

Among the CSU patients, we observed that patients carrying the rs3093059C allele had weaker responses to mizolastine than those harboring the rs3093059T allele. Rs3093059C allele carriers had higher serum CRP, IL-6, and TNF- α levels compared with rs3093059T carriers. TNF- α could mediate pro-inflammatory cytokines and IL-6 was representative Th2 cytokine^[43]. According to previous studies, IL-6 can induce the release of CRP and then aggravate cytokine release and augment humoral immune responses, which may suggest that the rs3093059T allele may improve the therapeutic efficacy of mizolastine by decreasing CRP levels^[19]. We

suggest that CRP rs3093059T/C as well as serum CRP level would be taken as predictive markers of mizolastine efficacy.

As nonsedating antihistamines including mizolastine could inhibit histamine release and cytokines secretion^[28]. To further investigate the mechanism that why patients carrying rs3093059C allele lack more response to standard dose mizolastine, we conducted the in vitro study with β -HEX release assay which indirectly represent the level of mast cell degranulation. We found that the β -HEX release ratio showed a decrease when adding mizolastine. Furthermore, CRP dose-dependently attenuated the inhibiting effects of mizolastine on mast cell activation through increasing the release of β -HEX. Taken together, our findings suggested that patients carrying rs3093059C allele responded poorly to mizolastine may because of the weakened inhibition of mast cell activation with higher CRP concentration.

Additionally, our previous studies have determined the genetic polymorphisms that are associated with the risks of CSU susceptibility. For instance, *FCER1A* rs2298805G, *ORAI1* rs3741596A, and rs12313209T increased the risks to CSU susceptibility^[31, 35]. CRP acted as an acute phase protein in inflammation^[44, 45]. In CSU, CRP may play a role in the complement system and coagulation, thus affecting tissue factor release and mediating mast cell activation^[19]. However, we did not observe a correlation between rs3093059T/C and CSU susceptibility in our study, although the serum CRP level was different among different rs3093059 T/C genotypes. The association between rs2794521G/A and CSU susceptibility was also not significant. Since there is no report on the relationship between CRP gene

polymorphisms and CSU susceptibility at home and abroad, we are temporarily unable to determine whether CRP polymorphisms are really associated with the onset of CSU. We need to expand the sample study to add more evidence to prove the relationship between CRP gene polymorphisms and CSU susceptibility.

Our present study was first to investigate the association of *CRP* SNPs rs3093059, rs2794521 in CSU susceptibility and even nonsedating H1 antihistamine responses. Although we did not find any association between the *CRP* rs3093059T/C polymorphism and the risk of CSU, we found a relationship between this SNP and nonsedating H1 antihistamine mizolastine efficacy. However, due to the limitations of our sample size and single-center study, our findings need to be further confirmed.

Therefore, further multi-center and large sample size studies should be undertaken to confirm this hypothesis, and functional studies should be undertaken to explore the mechanism of nonsedating H1 antihistamines in inter-individual differences in CSU.

In conclusion, *CRP* rs3093059T/C may be associated with the response to nonsedating H1 antihistamine mizolastine monotherapy. Patients carrying the rs3093059C allele may respond poorly to mizolastine with elevated serum CRP, TNF- α and IL-6 levels. These results may guide dermatologists in choosing individualized CSU patient therapy.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Siyu Yan, Wangqing Chen performed the research; Xiang Chen, Jie Li and Wu Zhu contributed to the research study; Wei Zhang ,Qingling Li and Zhaoqian Liu contributed essential reagents or tools; Cong Peng, Mingliang Chen Jiangling Zhang , Fangfang Li and Juan Su contributed to data collecting and analysing; Siyu Yan, Jie Li and Wangqing Chen wrote and revised the manuscript.

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TABLES

Table 1 CRP rs3093059 and rs2794521 polymorphisms of different genotypes on nonsedating H1 antihistamines therapy in CSU patients (n=145)

	Effectiveness	rs3093059		<i>P</i> value [†]	OR(95%CI)	rs2794521		<i>P</i> value [†]	OR(95%CI)
		TT	CC+CT			AA	GG+GA		
Total	Responders(n,%)	68(69.39)	3+27(30.61)	0.011	2.58 (1.26-5.27)	60(61.23)	38(38.77)	0.04	0.43 (0.19-0.96)
	Non-responders(n,%)	22(46.81)	1+24(53.19)			37(78.72)	10(21.28)		
Mizolastine	Responders(n,%)	30(75.00)	0+10(25.00)	0.015	4.20 (1.42-12.39)	28(70.00)	1+11(30.00)	0.372	0.47 (0.13-1.66)
	Non-responders(n,%)	10(41.67)	1+13(58.33)			20(83.33)	0+4(16.67)		
Desloratadine	Responders(n,%)	37(67.27)	3+15(32.72)	0.433	1.54 (0.55-4.33)	31(56.36)	2+22(43.64)	0.063	0.30 (0.09-1.02)
	Non-responders(n,%)	12(57.14)	0+9(42.86)			17(80.95)	0+4(19.05)		

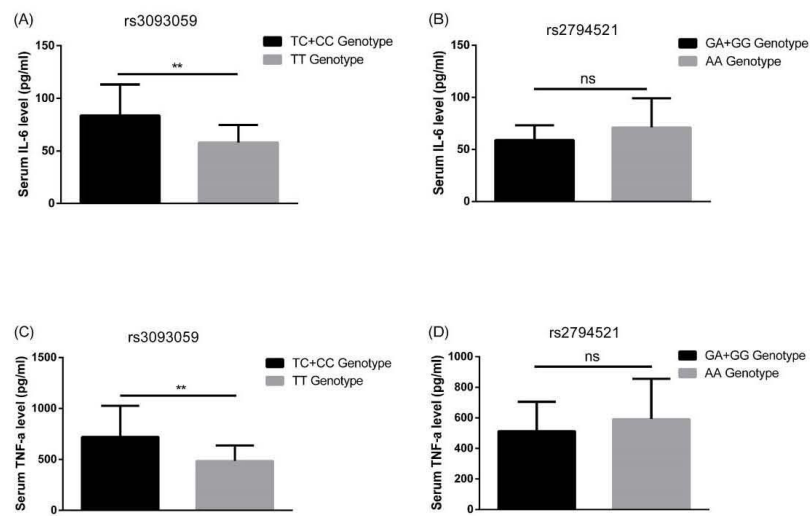
[†] Corrected P value was 0.025.

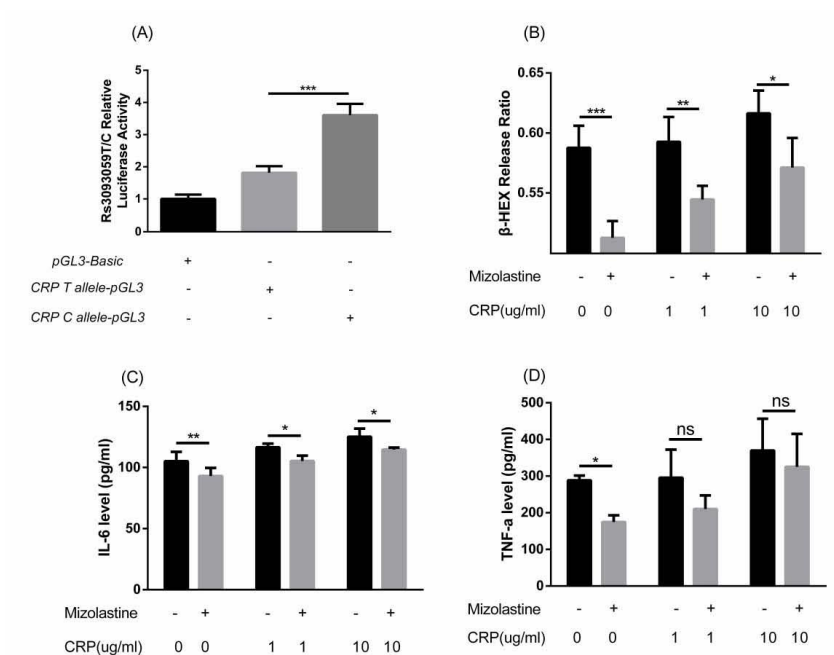
‡ Because of the limitation of sample size in fexofenadine group(n=5), we did not analyze the data in the above table.

Figure legends

Figure 1. Human serum IL-6 and TNF- α levels detected by ELISA in rs3093059 and rs2794521 genotypes (A),(C), serum IL-6 and TNF- α levels in rs3093059TT homozygotes and rs3093059TC heterozygous plus rs3093059 CC homozygotes; (B),(D), serum IL-6 and TNF- α levels between rs2794521AA homozygotes and rs2794521AG heterozygous plus rs2794521GG homozygotes; Data were presented as mean $\pm$ SD. **p< 0.01;

Figure 2. CRP relative luciferase activity in different allele constructs and mast cell activity after CRP stimulation on RBL-2H3 cells. (A), CRP dual luciferase activity was tested after transfection with rs3093059T or rs3093059C allele into HEK 293T cell for 24h; (B), (C),(D), β -hexosaminidase and cytokines release from RBL-2H3 cells after treated with recombinant CRP at different concentrations. Data were presented as mean $\pm$ SD with 4 replicates. *p< 0.05; **p< 0.01; ***p< 0.001.





SUPPLEMENTARY TABLES

Table S1 The primers used in the luciferase reporter assay

Name	Primers
CRP-PGL3-F1	5'GC GGTACCT GAGGAGCAAGGAGAAGGAG-3'
CRP-mutant-R1	5'-CTCAGCCGATTGAGTACACT-3'
CRP-mutant-F2	5'-AGTGTACTCAATCGGCTGAG-3'
CRP-PGL3-R2	5'- GCCTCGAG GCCTGAATTCACCTCCTTTGG-3'

Table S2 Demographic data of 145 CSU patients carrying different rs3093059 and rs2794521 genotypes

	Age(year)	Male	Female	Duration (month)	CRP(mg/dl)	Baseline UAS7	Baseline DLQI	After-treatme nt UAS7	After-treatme nt DLQI
rs2794521	38.2±12.3	38	59(60.8%)	23.5±40.6	3.7±4.8	29.9±10.9	7.5±5.8	12.1±13.8	3.4±4.5
AA (n=97)		(39.2%)							
GA (n=44)	33.7±11.3	14(31.8%)	30(68.2%)	22.8±40.9	3.6±6.8	25.3±11.9	5.9±4.05	7.6±9.5	3.5±4.0
GG (n=4)	37.8±19.9	3(75.0%)	1(25.0%)	5.9±4.4	1.3±0.3	20.0±6.9	8.3±4.9	7.7±10.8	3.5±3.5
<i>P</i> value [†]	0.123	0.213		0.693	0.706	0.025	0.230	0.022*	0.884
rs3093059	37.0±12.7	36(39.1%)	56(60.9%)	23.9±43.0	2.5±3.4	29.4±11.4	7.2±5.4	10.2±12.7	3.4±3.8
TT(n=92)									
CT(n=49)	36.4±11.8	31(63.3%)	18(36.7%)	22.3±35.8	4.9±6.9	25.1±10.9	6.8±5.5	11.5±12.8	3.6±3.5
CC(n=4)	38.5±12.1	1(25.0%)	3(75.0%)	3.5±1.3	11.1±12.5	40.0±3.4	7.5±2.4	6.0±7.1	3.4±4.3
<i>P</i> value [†]	0.917	0.016*		0.608	0.001**	0.011*	0.915	0.377	0.748

[†] Corrected P value was 0.025.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table S3 *CRP* rs3093059T/C allele and rs2794521G/A allele distribution between CSU patients and healthy controls

Alleles	CSU	Healthy	<i>P</i>	OR(95%CI)
	Patients N	Controls	value	
	(%)	N(%) [†]		
rs3093059				
C allele	57(17.38)	41(19.52)	0.971	1.01(0.65-1.58)
T allele	233(82.62)	169(80.48)		
rs2794521				
A allele	238(82.07)	170(80.95)	0.815	1.04(0.81-1.35)
G allele	52(17.93)	40(19.05)		

[†] Healthy control(n=105): the 1000 Genomes Project Phase 3.

	Age (year)	Male	Female	Duration	CRP	Baseline UAS7	Baseline DLQI	After-treatm ent UAS7	After-trea tment
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					(month)	(mg/dl)				
Total	Response (n=98)	36.76±12.57	38 (38.8%)	60 (61.2%)	21.03±40.54	2.59±2.53	28.66±11.46	6.83±4.65	3.71±5.50	2.16±2.93
	Nonresponse (n=47)	37.02±11.79	17 (36.2%)	30 (63.8%)	26.51±39.25	3.18±3.13	27.36 ± 11.25	7.59±6.58	25.89±10.28	6.45±5.46
	<i>P</i> value [†]	0.903	0.856		0.446	0.247	0.521	0.427	0.000 ^{***}	0.000 ^{***}
Mizolastine	Response (n=40)	38.6±13.1	17 (42.5%)	23 (57.5%)	21.6±42.7	2.8±3.6	29.5±10.5	6.9±3.7	2.3±4.4	1.5±2.0
	Nonresponse (n=24)	37.1±12.7	7 (29.2%)	17 (70.8%)	21.0±37.5	3.2± 3.7	25.3± 11.3	6.6±5.6	24.2±9.3	6.4±6.2
	<i>P</i> value [†]	0.673	0.424		0.957	0.688	0.140	0.825	0.000 ^{***}	0.012 [*]
Desloratadine	Response (n=55)	35.1±11.7	19 (34.5%)	36 (65.5%)	21.1±39.9	2.5±2.3	28.2±12.4	7.0±5.3	4.6±6.1	2.5±3.3
	Nonresponse (n=21)	37.4±11.4	9 (42.9%)	12 (57.1%)	30.1±38.3	2.4 ± 1.7	29.1 ± 11.6	8.3±7.8	29.1±9.8	6.4±4.3
	<i>P</i> value [†]	0.45	0.597		0.386	0.819	0.764	0.413	0.000 ^{***}	0.001 ^{**}

Table S4 Clinical characteristics between response group and nonresponse group

[†] Corrected *P* value was 0.025. **p*< 0.05; ***p*< 0.01; ****p*< 0.001.

‡ Because of the limitation of sample size in fexofenadine group(n=5), we did not analyze the data in the above table.