# Interleukin 17 Receptor a Haplotype Analysis in Chronic Spontaneous Urticaria

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**Research**

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# Abstract

### Background:

Chronic spontaneous urticaria (CSU) is a distressing skin disease. Family clustering and heterogeneity in the onset and progression indicate that susceptibility to CSU is a complex trait. In this study, we performed haplotype analysis for one of the key player gene, *IL17RA*, for CSU to test the association with disease susceptibility and severity.

### Methods

The study included 70 CSU patients and 30 healthy controls. The severity of the disease was evaluated by autologous serum skin test (ASST) and urticaria activity score (UAS). ASST test was done and quality of life was assessed using a questionnaire. Allelic discrimination analysis for rs4819554 and rs879577 was performed using Real-Time Polymerase Chain Reaction technology.

### Results:

Carriers of rs4819554*G were more prone to develop CSU than its counterpart ($p$ = 0.039), while rs4819554*A allele displayed more severe phenotype in the form of more prolonged disease duration ($p$ = 0.040), concurrent angioedema ($p$ < 0.001), higher level of treatment ($p$ < 0.001), and higher score of quality of life ($p$ < 0.001). Additionally, homozygote patients with rs879577*CC were associated with angioedema ($p$ < 0.001). Haplotype analysis revealed that cohorts with both rs4819554*A and rs879577*T conferred protection against developing CSU (OR = 0.07, 95%CI = 0.01 - 0.32, $p$ = 0.001).

### Conclusions:

Our results showed that *IL17RA* gene polymorphisms might contribute to the increased susceptibility to CSU.


# Background

Chronic spontaneous urticaria is a distressing skin disease characterized by recurrent itching and hives with or without angioedema, with symptoms persisting for more than 6 weeks without a known cause [1]. However, the exact initiative mechanisms of chronic spontaneous urticaria are still poorly understood [1]. CSU shows both autoimmune and allergic disease characteristics. Histamine-releasing autoantibodies directed against FcεRI or IgE were detected in only one-third of CSU patients, suggesting that other circulating mediators, including cytokines, may be involved in its pathophysiology. The determination of the dominant cytokine pattern and T lymphocyte subgroup in CSU patients could be the key to explaining this complicated immunopathogenesis [2].

Interleukin-17 (IL-17) is produced by T helper 17 (Th17) subsets of CD4 + T cell. IL-17 plays an important role in the development and progression of inflammatory and autoimmune diseases [3]. Cytokines

produced from T lymphocytes and natural killer (NK) cells form a cascade of reactions that might contribute significantly to the pathogenesis of CSU [4]. Increased levels of proinflammatory cytokines including IL-17 family members have also been found in the circulation of patients with CSU, atopic dermatitis, psoriasis, and bullous skin diseases [5–9]. Blocking the action of IL-17 appeared to be favorable in the treatment of these categories of disorders [4].

*IL17RA* gene is highly polymorphic (www.ensemble.org). Several common single nucleotide polymorphisms (SNPs) in this gene have been linked to increased susceptibility for developing cancer, autoimmune diseases, and inflammatory conditions [10, 11]. One common variant of *IL17RA* existed upstream in the gene (c.-947G > A) is rs4819554 SNP. Being within the promoter region and closely related to the binding site of transcription factors as Ikaros (IK) family, which is related to Th17 cell differentiation [12], we proposed the possibility to have a functional consequence on the transcription rate of the gene. This SNP was reported to be associated with autoimmune diseases and cancer [13]. Another common missense variant rs879577 is caused by a C to T substitution (c.1100C > T) in exon 13 leading to an amino acid change from alanine to valine at codon 367, which in turn might have altered function.

The role of IL-17RA gene polymorphism in the pathogenesis of CSU has not been studied yet, therefore we hypothesized that IL-17RA gene polymorphism may be associated with CSU like other autoimmune diseases. The present study aimed to determine the association of single nucleotide polymorphisms (rs4819554 and rs879577) of IL-17RA gene with CSU susceptibility and severity.

## Methods

# Study population

Seventy CSU patients (29 males and 41 females) were enrolled in the study (November 2014 and April 2015). The control group comprised of 30 sex- and age-matched healthy subjects with negative ASST results. Ethical approval by the Regional Ethics Committee of the Faculty of Medicine, Suez Canal University Hospital was obtained (#2747).

# Clinical assessment

Demographic and clinical data were collected. These included a skin prick test for food and inhalant allergens; and provocation tests for physical urticaria. Early onset was considered if the age of CSU onset was below or equal to 40 years. Familial CSU was considered positive if there was at least one first- or second-degree affected relative. Urticaria activity score (UAS) Urticaria Patient Daily Diary (UPDD) was applied for disease severity assessment [14, 15]. Health-related quality of life (HRQoL) was assessed using the Dermatology Life Quality Index (DLQI) [16]. An autologous serum skin test (ASST) was performed to detect basophil histamine-releasing activity. Sedating antihistamines were stopped at 7 days, and non-sedating antihistamines were stopped at 3 days before blood samples were collected. The control group underwent the same laboratory and dermatological tests as the patients to exclude any inflammatory or autoimmune diseases.

# Biochemical analysis

Liver function tests, complete blood count (CBC), erythrocyte sedimentation rate, a parasite stool test, and complete urine analysis were performed in the clinical pathology department laboratories.

Allelic discrimination analysis by real-time PCR

DNA extraction from blood leukocytes was done using QIAamp DNA Blood Mini kits (*Qiagen, Clinilab Co., Egypt, Catalog no. 51104)*, according to the manufacturer's instructions. Genotyping of the promoter variant (rs4819554, c.-947G > A) and the missense mutation (rs879577, c.1100C > T; p.A367E or p. 367V) was performed using real-time polymerase chain reaction (RT-PCR) using TaqMan® genotyping assay (*Applied Biosystem, assay ID C__337392_30 and C__2666446_20*) as previously described [17].

# Statistical analysis

Statistical analysis was carried out using SPSS software, version 26.0. The test for normality was performed using the Shapiro–Wilk test. Descriptive statistics were represented as mean ± standard deviation (SD) for quantitative variables and percentages for qualitative variables. For qualitative variables, the Chi-square and Fisher's Exact tests were used. For quantitative variables, the student's t and Kruskal-Wallis tests were used for non-parametric variables. A p-value < 0.05 was considered statistically significant, and all statistical tests were two-sided.

The genotype and allele frequencies and Hardy–Weinberg equilibrium (HWE) were calculated [18]. The associations of the genotypes and susceptibility to urticaria disease were estimated by computing odds ratios (ORs) and their 95% confidence intervals (CIs) from binary logistic regression analysis (Enter method) after adjustment for possible confounders. Five genetic association models were computed [19]. Linkage disequilibrium statistics and haplotype frequency estimation were performed in SNPStats software (https://www.snpstats.net/).

Data exploration by multivariate analysis (MVA) was performed using Bray-Curtis Ordination analysis and Two-way hierarchical cluster analysis to identify similarities and dissimilarities between urticaria patients based on their characteristics and genotypes. PC-ORD version 5.0 software was employed for analysis. The following setup for 70 strands and 17 factors was adjusted for MVA: Distance method: Sorensen, Endpoint selection method: Variance-regression, group linkage method: Flexible beta (0.75), and clustering factor: relative by factor maximum, axis projection geometry: Euclidean, calculation of residual distance: Euclidean, and calculated scores for factor by weighted averaging.

## Results

# Baseline characteristics of the study population

The demographic and baseline characteristics of the study population are demonstrated in Table 1. A total of 70 patients, including 41 (58.6%) females and 29 (41.4%) males, participated in the study. The

mean age of the patients was 28.2 ± 11.9 years (range: 14–56). Thirty-five patients had positive ASST results, and 35 had negative results. Among patients, only three (4.3%) had a family history of CSU while 67 (95.7%) did not have a positive family history. The majority (53.4 of CSU, 74.3%) were employed. On the other hand, the control group (30 individuals) included 13 (43.3%) males and 17 (56.7%) females with a mean age of 32.3 ± 12.3 years (range:13–63). All control subjects had negative ASST results.

# Disease characteristics of urticaria patients

The mean disease duration of patients was 8.71 ± 1.37 weeks. Thirty-four patients (48.6%) experienced urticaria disease for less than or equal 8 weeks, 31 patients (44.3%) had lesions for less than or equal 10 weeks, while 5 patients (7.1%) showed further prolonged features for ≤ 12 weeks. Regarding disease course, almost all patients had recurrent disease. Lesions were mostly distributed in the abdomen and arms. Less than a quarter the patients had concomitant angioedema, Fig. 1. Assessment of patients via AAS) was employed to estimate the level of five key factors related to angioedema symptoms. All CSU patients with angioedema had a total daily score of more than 8. According to the ASST, thirty-five (50%) patients had positive ASST results, while the other half had negative results. Regarding patients' treatment, 60% of CSU patients were treated with Step 1 medications including non-sedating second-generation antihistamines, while 40% of patients received Step 2 medications with doses up to four times those recommended on the label of non-sedating, second-generation antihistamines. The second-generation antihistamines provided moderate-to-good control for 50% of patients with CSU.

Disease severity was assessed using the UAS system, Table 2. Half patients, accounting for 45.7%, had a moderate number of wheals ranging from 11 to 50, while about a quarter (25.7%) exhibited a large number of wheals. Moreover, 20% of patients displayed wheals with > 3 cm in size. Three-quarters of CSU cases experienced moderate to severe itching. Also, urticaria lesions of more than half of the patients (55.7%) disappeared by 12 hours.

Stratified analysis by gender revealed a non-significant difference in all UAS symptoms between males and females, Table 2. However, stratification by angioedema and ASST demonstrated that patients with angioedema [16 (22.8%)] had more wheals ($p < 0.001$), a wider distribution ($p < 0.001$), higher wheal score ($p < 0.001$), intense itching ($p < 0.001$), and more prolonged duration ($p < 0.001$) than patients without angioedema, Fig. 2 **(A-E)**. Similar findings were encountered in patients with positive ASST, Fig. 2 **(F-J)**. Dermatological quality of life was assessed using six parameters. The mean QoL score was 12.9 ± 6.13 in urticaria patients with non-significant differences between genders, Table 3.

# Molecular analysis of IL17RA gene polymorphism

A total of 70 patients and 30 controls were genotyped. The distribution of *IL17RA* genotype among patients and controls were found following those expected by the Hardy Weinberg equilibrium ($p > 0.05$). SNP analysis of the promoter variant rs4819554 (c.-947G > A) showed that the frequencies of G and A alleles in controls were 0.47 and 0.53, respectively. As depicted in Table 4, comparing between urticaria patients and controls, GG was significantly the most prevalent genotype among patients (42.9% in

patients *versus* 16.7% in controls), while GA genotype was predominant in the healthy control group (38.6% in patients *versus* 60% in controls), $p = 0.039$. Consistently, the frequency of A allele was significantly lower in urticaria patients compared to controls (37.1% *versus* 53.3%; $p = 0.042$). Carriers for A variants were less likely to develop urticaria than their counterpart under homozygote comparison (OR = 0.18, 95%CI = 0.03–0.89), heterozygote comparison (OR = 0.24, 95%CI = 0.06–0.91), dominant model (OR = 0.24, 95%CI = 0.06–0.91), and allelic model (OR = 0.40, 95%CI = 0.66–0.91).

In contrast, SNP analysis of rs879577 (c.1100C > T) showed that the frequencies of C and T alleles in the whole study population were 0.53 and 0.47, respectively. On comparing urticaria patients and controls, CC was significantly the most prevalent genotype among patients (34.3% in patients versus 13.3% in controls), while the TT genotype was predominant in the healthy control group (18.6% in patients versus 33.3% in controls) (p = 0.022). Consistently, the frequency of T allele was significantly lower in urticaria patients compared to controls (42.9% versus 60.0%; $p = 0.020$). Carriers for T variants were less likely to develop urticaria than their counterparts under homozygote, dominant model, and allelic model, but after adjustment results were insignificant, Table 4.

# Haplotype analysis

Though there was poor linkage equilibrium between the two variants (r = 0.007, $p = 0.92$), patients with genotype combination of rs4819554*A and rs879577*T conferred protection against developing CSU, Table 5.

# Association between IL17RA genotypes and features of CSU patients

On the comparison between CSU patients with different *IL17RA* rs4819554 genotypes (GG, GA, and AA), there was no significant association between genotypes of rs481995 promoter variant and age, sex, family history, or occupation. Though the rs4819554*G variant was showed earlier to be the risky allele in CSU, the other allele (rs4819554*A) displayed more severe features. A/A genotype was associated with more prolonged disease duration (p = 0.044), concurrent angioedema (p < 0.001), and positive ASST status (p < 0.001). A/A genotype also demonstrated more frequent lesions in abdomen (p = 0.049) and legs (p = 0.042). In addition, the same variant was associated with a higher level of treatment step (p < 0.001). CSU patients carrying A/A genotype was associated with the worst score in each UAS domain. A/A patients displayed a greater number of wheals (> 50) (p < 0.001), higher prevalence of wheals > 3 cm (p < 0.001), a greater score of wheals (p < 0.001), more intense itching (p < 0.001), and prolonged persistence of wheals > 12 hours (p = 0.004). Also, A/A genotype was associated with higher scores for quality of life assessment (p < 0.001). Regarding IL17RA rs879577 genotypes (CC, CT, and TT), CC genotype was associated with CSU lesions in legs (p = 0.002) and concurrent angioedema (p < 0.001). Patients with the same genotype also reported poor symptoms in all QoL parameters (p < 0.001), Table 6.

# Multivariate analysis of clinical data

Principal component analysis of CSU patients using molecular and clinical variables scattered patients in a high dimensional space. Classifying patients according to their *IL17RA* promoter genotype at rs4819554 (c.-947G > A) polymorphism is demonstrated in Fig. 3. Ordination revealed that a part of one patient, clear demarcation of CSU patients with A/A genotype was observed. The clustering of this group was highly influenced by prolonged disease duration, concomitant angioedema, in addition to higher AAS, UAS, and QoL scores in these patients. Thus, supporting the results of the univariate analysis in the previous tables.

# Discussion

The present study is, to the best of our knowledge, the first to investigate the potential influence of IL-17RA in urticaria patients. Here, we tried to shed light on the role of analyzing IL-17RA gene polymorphism as a diagnostic and prognostic biomarker for the CSU phenotype. Genotype profiling of *IL17RA* rs4819554 (G/A) and rs879577(C/T) variants was carried out in a sample of an Egyptian population. Our results demonstrated rs4819554*G allele to be a putative marker for disease risk while rs4819554* an allele was associated with a severe phenotype. On the other hand, rs879577*C was associated with angioedema.

In our study population, genotypes of patients and controls of rs4819554 SNP were in accordance with Hardy-Weinberg equilibrium. Allelic discrimination detected G and A alleles with frequencies of 0.47 and 0.53 in the control group. In this SNP, the minor allele (G) are reported to be 0.01 in Africans, 0.41 in Americans, 0.42 in East Asians, 0.24 in South Asians, and 0.21 in Europe (www.ensemble.org). On a comparison between the study groups, GG was the most frequent genotype among CSU patients, while the GA genotype was more prevalent in controls. Additionally, the rs4819554*G variant was the risky allele under heterozygote and homozygote comparisons a well as dominant and allelic models. In agreement with our results, *IL17RA* rs4819554 variant was related to susceptibility to psoriasis in Spanish patients. G carriers were more predominant among patients [20]. In contrast, this promoter variant did not show a significant association with alopecia areata risk in a Korean population [21]. Nevertheless, the rs4819554*A allele was more frequent in patients with papillary thyroid cancer than in control patients [22] and was associated with an increased risk of developing end-stage renal disease[(23)]. At the rs4819554 site in the *IL17RA* gene, the genomic sequence with G allele displayed an SP1 transcription factor consensus sequence; however, this consensus sequence was absent in the sequence with A variant [22].

Another main finding in our results, patients carrying rs4819554*AA genotype was associated with more prolonged disease duration, concomitant angioedema, and positive ASST status, advanced stage in treatment, and worst quality of life score in CSU. Similarly, the homozygosity of AA was associated with more deterioration of renal function [23] and higher primary graft dysfunction [24]. The A allele of *IL17RA* gene also showed a significant difference between the early onset AA and late-onset AA in a Korean population [21]. After analyzing several SNPs in coding and regulatory regions in two Spanish cohorts with ankylosing spondylitis (AS), results indicated the genetic role of our promoter variant in the

development of severe forms of AS [14]. Interestingly, rs4819554* A allele was linked to an increased expression of the IL-17RA protein and higher levels of Th17 cell subsets [23], which might explain the increased severity of immune-related diseases.

The missense mutation rs879577 (c.1100C > T) showed allele frequencies of 0.53 and 0.47 for C and T alleles in the whole study population. In population genetics projects as the 1000 Genome Project Phase 3, similar frequencies (53 and 47% respectively) were reported. In contrast, minor allele frequencies (T allele) accounted for 9%, 26%, and 28% in Asia, Europe, and America respectively (www.ensembl.org). CC genotype was significantly the most prevalent among patients, while the TT genotype was predominant in the healthy control group. Similarly, the frequency of the T allele was significantly lower in urticaria patients compared to controls. Haplotype analysis showed that patients with genotype combination of rs4819554*A and rs879577*T conferred protection against developing CSU. Carriers of the rs879577*C allele, which was the predominant variant in CSU patients was also associated with poor clinical markers in CSU. CC genotype was associated with concurrent angioedema, lesions in legs, and low quality of life domains. A putative explanation for this is depicted in the following structural analysis of the point mutation; in the cytoplasmic domain of IL-17Ra protein, the nucleotide substitution from cytosine to thymine at the position 22:17108319 causes a missense mutation; changing from alanine to more branched valine residue (p.A367V) with the same preserved length of the protein. This amino acid change is located between two beta-strands (297–301 and 379–383), is away from active or binding sites, and is predicted by computational tools as SIFT and PolyPhen to be a tolerable mutation.

The pathogenesis of CSU is probably characterized by a multiplicity of mechanisms, including autoimmunity, autoallergy, and coagulation, each of which may carry different weight in each patient. We have demonstrated for the first time that *IL17RA* polymorphisms have the possibility of determining the pathophysiology of CSU phenotype. Our data supported the biological role of the IL-*17RA* gene in the development and progression of CSU disease. The promoter variant could be a putative marker for disease risk and severity in a sample of the Egyptian population. However, some limitations warrant to be mentioned. First, the small sample size was enrolled in the study, replication in larger cohorts are warranted to confirm the genetic association of rs4819554 and rs879577 to CSU and study the functional impact of the variant on disease progression. Second, the survey/questionnaire format and subjectivity of data might impose a putative bias effect. In addition, adding another positive control group with an inflammatory dermatosis condition would enhance the study. The functional consequence of the polymorphism was not studied in the lab, correlation analysis between the expression level of IL-17RA and polymorphism in the blood of CSU patients would have added more value to the study outcomes.

In conclusion, we combined clinical and molecular analysis to illustrate the influence of the promoter polymorphism (rs4819554) and a missense mutation (rs879577) on CSU development and progression. We found individuals with rs4819554*GG homozygosity were associated with disease risk, while those with rs4819554*AA genotype displayed a more severe phenotype, while rs879577*CC was associated with angioedema. Whether it directly contributes to disease susceptibility or in linkage disequilibrium with other true-causing polymorphisms needs further investigation. Screening CSU patients for *IL17RA* genetic

determinants during the patient's assessment would predict the course of the disease and guide the best medication choice in each patient, which may ultimately enhance care and improve health outcomes.

## Abbreviations

ASST
autologous serum skin test, CAU:chronic autoimmune urticaria, CI:95% confidence intervals, CSU:chronic spontaneous urticaria, DLQI:Dermatology Life Quality Index, HRQoL:health-related quality of life, IL-17:Interleukin-17, MVA:multivariate analysis, NTC:no-template controls, OR:odds ratios, QoL:quality of life, SNP:single nucleotide polymorphisms, $T_h$17:T helper 17, UAS:urticaria activity score, UPDD:Urticaria Patient Daily Diary.

## Declarations

# Ethics approval and consent to participate

Ethical approval by the Regional Ethics Committee of the Faculty of Medicine, Suez Canal University Hospital was obtained (#2747). Written informed consent was taken from all participant before taking part.

### Consent for publication

Not applicable

### Availability of data and materials

All data generated or analysed during this study are included in this published article.

### Competing interests

The authors declare that they have no competing interests

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None.

# Authors' contributions

HN, RI, OA, MA analyzed the clinical data, RH, RI, OA, AF, ET perform laboratory testing, RI, OA, ET did statistical analysis, HN, RH, RI, OA, ET wrote the first draft. All authors contributed to editing the manuscript and approved the final version.

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# Tables

| Table 1. Demographic characteristics of the urticaria patients (n=70) and controls (n=30). | | | | | |
|---|---|---|---|---|---|
| **Demographic data** | | **Controls** | **Patients** | **P value** | **OR (95%CI)** |
| **Total number** | | 30 | 70 | | |
| **Age, years** | Mean ± SD | 32.3 ± 12.3 | 28.2 ± 11.9 | | |
| **Age categories, %** | ≤30 years | 20 (66.7) | 36 (51.4) | 0.191 | *Reference* |
| | >30 years | 10 (33.3) | 34 (48.6) | | 1.88 (0.77-4.61) |
| **Gender** | Female | 17 (56.7) | 41 (58.6) | 0.860 | *Reference* |
| | Male | 13 (43.3) | 29 (41.4) | | 0.92 (0.39-2.19) |
| **FH urticaria** | Negative | 29 (96.7) | 67 (95.7) | 0.824 | *Reference* |
| | Positive | 1 (3.3) | 3 (4.3) | | 1.29 (0.13-13.0) |
| **Occupation** | Housewife | 10 (33.3) | 16 (22.9) | **0.048** | *Reference* |
| | Student | 4 (13.3) | 2 (2.9) | | 0.31 (0.04-2.03) |
| | Working | 16 (53.4) | 52 (74.3) | | 2.03 (0.77-5.35) |
| Data is shown as number (percentage) or mean ± standard deviation. FH; family history. Chi-square, Fisher's exact, and student-t tests were used. Bold values are statistically significant at p<0.05. | | | | | |

**Table 2. Domains of urticaria activity score (UAS) to assess disease severity in patient group (n=70).**

| Characteristics | | Total | Female | Male | P value |
|---|---|---|---|---|---|
| **Number of wheals** | ≤10 | 20 (28.6%) | 14 (34.1%) | 6 (20.7%) | 0.43 |
| | 11 – 50 | 32 (45.7%) | 18 (43.9%) | 14 (48.3%) | |
| | > 50 | 18 (25.7%) | 9 (22.0%) | 9 (31.0%) | |
| **Size of wheals (cm)** | < 1 cm | 23 (32.9%) | 15 (36.6%) | 8 (27.6%) | 0.65 |
| | 1 – 3 cm | 33 (47.1%) | 19 (46.3%) | 14 (48.3%) | |
| | ≥ 3 cm | 14 (20.0%) | 7 (17.1%) | 7 (24.1%) | |
| **Scores of wheals** | Mild | 19 (27.1%) | 12 (29.3%) | 7 (24.1%) | 0.8 |
| | Moderate | 32 (45.1%) | 19 (46.3%) | 13 (44.8%) | |
| | Severe | 19 (27.1%) | 10 (24.4%) | 9 (31.0%) | |
| **Itching intensity** | Mild | 19 (27.1%) | 13 (31.7%) | 6 (20.7%) | 0.50 |
| | Moderate | 31 (44.3%) | 18 (43.9%) | 13 (44.8%) | |
| | Severe | 20 (28.6%) | 10 (24.4%) | 10 (34.5%) | |
| **Duration of wheals** | 1 – 12 hr | 39 (55.7%) | 26 (63.4%) | 13 (44.8%) | 0.15 |
| | > 12 hr | 31 (44.3%) | 15 (36.6%) | 16 (55.2%) | |
| **Frequencies of appearance** | Daily | 70 (100%) | 41 (100%) | 29 (100%) | NA |
| Data is shown as number (percentage). Chi-square or Fisher's exact tests were used. | | | | | |

**Table 3. Assessment of QoL among patient group (n=70).**

| QoL domain | Total | Female | Male | P value |
|---|---|---|---|---|
| **Pruritus** | 1.2 ± 0.4 | 1.2 ±0.4 | 1.3 ±0.4 | 0.25 |
| **Swelling** | 1.2 ± 1.9 | 1.2 ±0.4 | 1.3 ±0.4 | 0.25 |
| **Impact on life activities** | 2.8 ± 1.9 | 25 ±1.9 | 3.2 ±1.9 | 0.16 |
| **Sleep problems** | 2.8 ± 1.3 | 26 ±1.3 | 3.1 ±1.3 | 0.14 |
| **Looks** | 2.8 ± 1.3 | 2.5 ±1.3 | 3.2 ±1.2 | 0.06 |
| **Limit** | 1.7 ± 0.8 | 1.7 ±0.8 | 1.8 ±0.8 | 0.51 |
| **Total CSU-QoL** | 12.9 ± 6.13 | 12.0 ±6.1 | 14.1 ±5.9 | 0.15 |
| Data is shown as mean ± standard deviation. QoL; quality of life. Student-t test was used. | | | | |

**Table 4. Genetic association models for IL-17RA (rs4819554 and rs879577) polymorphisms and risk of urticaria disease.**

| Genetic model | Genotype | Controls (n=30) | Patients (n=70) | *P* value | Crude OR (95% CI) | Adjusted [a] OR (95% CI) |
|---|---|---|---|---|---|---|
| **rs4819554** | | | | | | |
| **HWE P** | | 0.26 | 0.13 | | | |
| **Co-dominant model** [b] | **GG** | 5 (16.7) | 30 (42.9) | **0.039** | 1.0 | 1.0 |
| | **GA** | 18 (60.0) | 27 (38.6) | | **0.25 (0.07-0.75)** | **0.24 (0.06-0.91)** |
| | **AA** | 7 (23.3) | 13 (18.6) | | 0.31 (0.07-1.21) | **0.18 (0.03-0.89)** |
| **Dominant model** | **GG** | 5 (16.7) | 30 (42.9) | **0.011** | 1 | 1 |
| | **GA+AA** | 25 (83.3) | 40 (57.1) | | **0.27 (0.08-0.76)** | **0.24 (0.06-0.91)** |
| **Recessive model** | **GG+GA** | 23 (76.7) | 57 (81.4) | 0.585 | 1 | 1 |
| | **AA** | 7 (23.3) | 13 (18.6) | | 0.75 (0.26-2.24) | 0.75 (0.18-3.15) |
| **Allelic model** | **G** | 28 (46.7) | 87 (62.1) | **0.042** | **1** | 1.32 (0.31-5.51) |
| | **A** | 32 (53.3) | 53 (37.8) | | **0.53 (0.28-0.98)** | **0.4 (0.66-0.91)** |
| **rs879577** | | | | | | |
| **HWE P** | | 0.54 | 0.92 | | | |
| **Co-dominant model** | **CC** | 4 (13.3%) | 24 (34.3%) | **0.022** | 1 | 1 |
| | **CT** | 16 (53.3%) | 33 (47.1%) | | 0.34 (0.08-1.13) | 0.34 (0.06-1.73) |
| | **TT** | 10 (33.3%) | 13 (18.6%) | | **0.22 (0.05-0.84)** | 0.26 (0.05-1.31) |
| **Dominant model** | **CC** | 4 (13.3%) | 24 (34.3%) | **0.03** | 1 | 1 |
| | **CT+TT** | 26 (86.7%) | 46 (65.7%) | | **0.29 (0.08-0.91)** | 0.34 (0.06-1.73) |
| **Recessive model** | **CC+CT** | 20 (66.7%) | 57 (81.4%) | 0.11 | 1 | 1 |
| | **TT** | 10 (33.3%) | 13 (18.6%) | | 0.46 (0.17-1.24) | 0.75 (0.24-2.34) |
| **Allelic model** | **C** | 24 | 81 | **0.02** | 1 | 1.32 (0.42- |

| | | | | | |
|---|---|---|---|---|---|
| | | (40.0%) | (57.8%) | | 4.10) |
| | **T** | 36 (60.0%) | 59 (42.2%) | **0.48 (0.26-0.90)** | 0.34 (0.06-1.73) |

Values are shown as number (%). HWE P; p value of Hardy-Weinberg equilibrium. Chi square test was used. OR (95% CI), odds ratio and confidence interval. ([a]) adjusted for confounding factors (age, gender, FH, and occupation). ([b]) represented both heterozygote and homozygote comparison models. There was a statistical significance increase in frequency of GG genotype and G Allele among patient group compared to control group (P<0.05).

**Table 5. Haplotype analysis of IL-17RA (rs4819554 and rs879577) polymorphisms and risk of urticaria disease.**

| | rs4819554 | rs879577 | Total | Controls | Cases | OR (95%CI) | P value |
|---|---|---|---|---|---|---|---|
| 1 | A | C | 0.3036 | 0.3615 | 0.2906 | 1.00 | --- |
| 2 | G | T | 0.2714 | 0.1052 | 0.3309 | 5.59 (0.71 - 26.70) | 0.303 |
| 3 | G | C | 0.2214 | 0.0385 | 0.288 | 5.52 (0.64 - 47.32) | 0.12 |
| 4 | A | T | 0.2036 | 0.4948 | 0.0906 | **0.07 (0.01 - 0.32)** | 0.0011 |

Data is shown as number (percentage). Chi-squaretest was used.

**Table 6. Demographic features of urticaria patients, according to IL-17RA genotypes.**

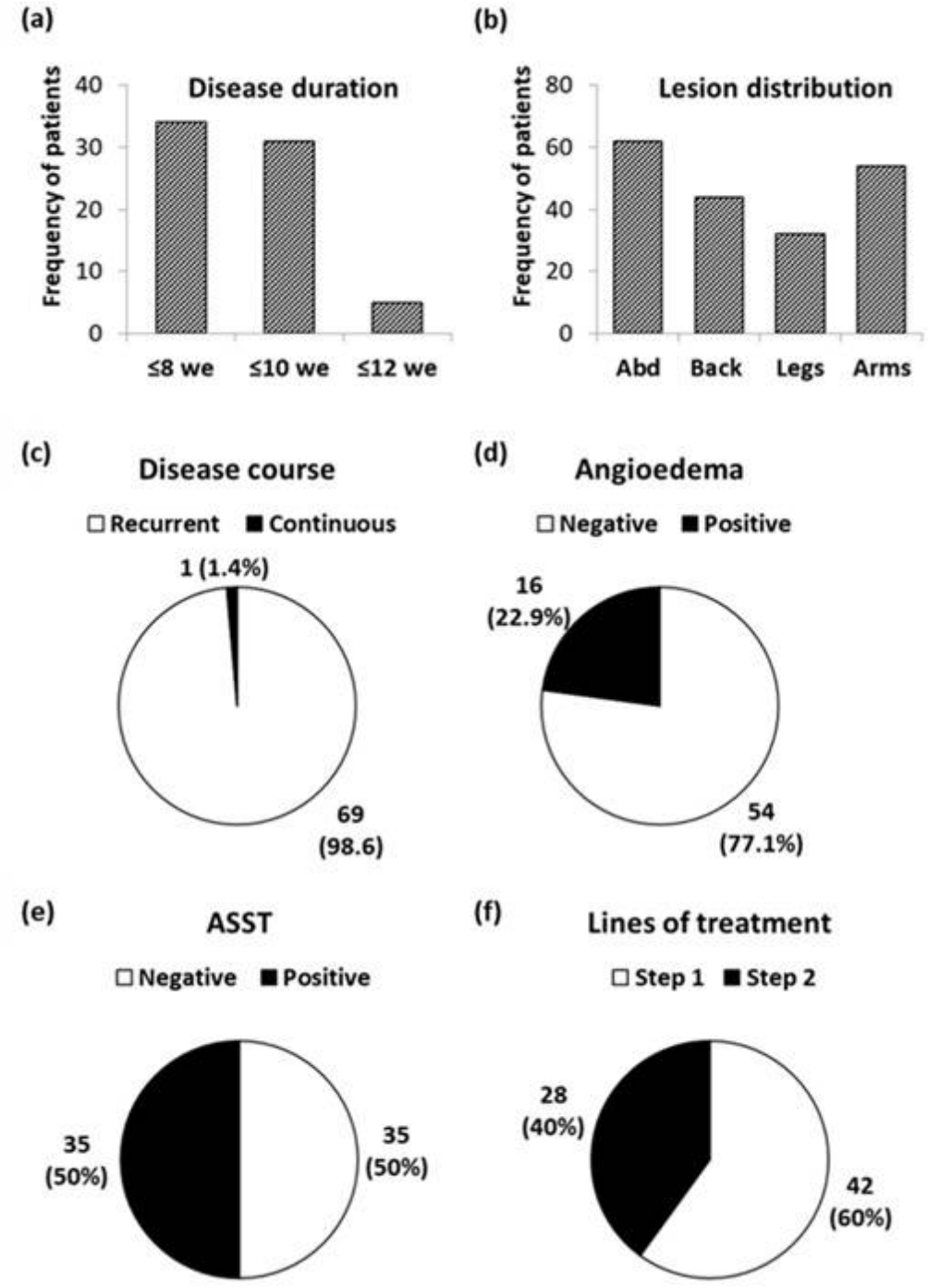
| Characteristics | Patient | rs4819554 genotypes | | | P value | rs879577 genotypes | | | P value |
|---|---|---|---|---|---|---|---|---|---|
| | (n = 70) | GG | GA | AA | | CC | CT | TT | |
| | | (n = 30) | (n = 27) | (n = 13) | | (n = 24) | (n = 33) | (n = 13) | |
| **Age, years** | | | | | | | | | |
| ≤30 | 36 (51.4) | 16 (53.3) | 13 (48.1) | 7 (53.8) | 0.91 | 13 (54.2) | 17 (51.5) | 6 (46.2) | 0.90 |
| >30 | 34 (48.6) | 14 (46.7) | 14 (51.9) | 6 (46.2) | | 11 (45.8) | 16 (48.5) | 7 (53.8) | |
| **Sex** | | | | | | | | | |
| Female | 41 (58.6) | 21 (70.0) | 14 (51.9) | 6 (46.2) | 0.23 | 14 (58.3) | 20 (60.6) | 7 (53.8) | 0.92 |
| Male | 29 (41.4) | 9 (30.0) | 13 (48.1) | 7 (53.8) | | 10 (41.7) | 13 (39.4) | 6 (46.2) | |
| **FH Urticaria** | | | | | | | | | |
| Negative | 67 (95.7) | 29 (96.7) | 26 (96.3) | 12 (92.3) | 0.8 | 22 (91.7) | 33 (100) | 12 (92.3) | 0.25 |
| Positive | 3 (4.3) | 1 (3.3) | 1 (3.7) | 1 (7.7) | | 2 (8.3) | 0 (0.0) | 1 (7.7) | |
| **Occupation** | | | | | | | | | |
| Housewife | 16 (22.9) | 8 (26.7) | 7 (25.9) | 1 (7.7) | 0.44 | 2 (8.3) | 9 (27.3) | 5 (38.5) | 0.25 |
| Student | 2 (2.9) | 1 (3.3) | 0 (0.0) | 1 (7.7) | | 1 (4.2) | 1 (3.0) | 0 (0.0) | |
| Working | 52 (4.3) | 21 (70.0) | 20 (74.1) | 11 (84.6) | | 21 (87.5) | 23 (69.7) | 8 (61.5) | |
| **Duration** | | | | | | | | | |
| ≤8 weeks | 34 (48.6) | 14 (46.7) | 17 (63.0) | 3 (23.1) | **0.04** | 8 (33.3) | 20 (60.6) | 6 (46.2) | 0.10 |
| ≤10 weeks | 31 (44.3) | 15 (50.0) | 9 (33.3) | 7 (53.8) | | 12 (50.0) | 12 (36.4) | 7 (53.8) | |
| ≤12 weeks | 5 (7.1) | 1 (3.3) | 1 (3.7) | 3 (23.1) | | 4 (16.7) | 1 (3.0) | 0 (0.0) | |
| **Distribution** | | | | | | | | | |
| Abdominal | 8 | 5 | 0 | 3 | **0.05** | 3 | 4 | 1 | 0.9 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | (11.4) | (16.7) | (0.0) | (23.1) | | (12.5) | (12.1) | (7.7) | |
| Back | 26 (37.1) | 9 (30.0) | 10 (37.0) | 7 (53.8) | 0.33 | 7 (29.2) | 12 (36.4) | 7 (53.8) | 0.33 |
| Legs | 38 (54.3) | 13 (43.3) | 14 (51.9) | 11 (84.6) | **0.04** | 20 (83.3) | 13 (39.4) | 5 (38.5) | **0.002** |
| Arms | 16 (22.9) | 7 (23.3) | 5 (18.5) | 4 (30.8) | 0.69 | 7 (29.2) | 7 (21.2) | 2 (15.4) | 0.61 |
| **Angioedema** | | | | | | | | | |
| Negative | 54 (77.1) | 29 (96.7) | 24 (88.9) | 1 (7.7) | **<0.001** | 11 (45.8) | 30 (90.9) | 13 (100) | **<0.001** |
| Positive | 16 (22.9) | 1 (3.3) | 3 (11.1) | 12 (92.3) | | 13 (54.2) | 3 (9.1) | 0 (0.0) | |
| **ASST status** | | | | | | | | | |
| Negative | 35 (50.0) | 21 (70.0) | 14 (51.9) | 0 (0.0) | **<0.001** | 9 (37.5) | 19 (57.6) | 7 (53.8) | 0.31 |
| Positive | 35 (50.0) | 9 (30.0) | 13 (48.1) | 13 (100) | | 15 (62.5) | 14 (42.4) | 6 (46.2) | |
| **Treatment** | | | | | | | | | |
| Step 1 | 42 (60.0) | 24 (80.0) | 17 (63.0) | 1 (7.7) | **<0.001** | 10 (41.7) | 23 (69.7) | 9 (69.2) | 0.08 |
| Step 2 | 28 (40.0) | 6 (20.0) | 10 (37.0) | 12 (92.3) | | 14 (58.3) | 10 (30.3) | 4 (30.8) | |
| **Number of wheals** | | | | | | | | | |
| ≤10 | 20 (28.6) | 14 (46.7) | 6 (22.2) | 0 (0.0) | **<0.001** | 6 (25.0) | 10 (30.3) | 4 (30.8) | 0.89 |
| 11-50 | 32 (45.7) | 13 (43.3) | 17 (63.0) | 2 (15.4) | | 13 (54.2) | 14 (42.4) | 5 (38.5) | |
| >50 | 18 (25.7) | 3 (10.0) | 4 (14.8) | 11 (84.6) | | 5 (20.8) | 9 (27.3) | 4 (30.8) | |
| **Size of wheals** | | | | | | | | | |
| <1 | 23 (32.9) | 14 (467) | 9 (33.3) | 0 (0.0) | **<0.001** | 9 (37.5) | 10 (30.3) | 4 (30.8) | 0.86 |
| 1-32 | 33 (47.1) | 14 (46.7) | 15 (55.6) | 4 (30.8) | | 12 (50.0) | 15 (45.5) | 6 (46.2) | |
| >3 | 14 (20.0) | 2 (6.7) | 3 (11.1) | 9 (69.2) | | 3 (12.5) | 8 (24.2) | 3 (23.1) | |
| **Score of** | | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **wheals** | | | | | | | | | |
| Mild | 19 (27.1) | 12 (40.0) | 7 (25.9) | 0 (0.0) | **<0.001** | 6 (25.0) | 9 (27.3) | 4 (30.8) | 0.72 |
| Moderate | 32 (45.7) | 15 (50.0) | 16 (59.3) | 1 (7.7) | | 13 (54.2) | 15 (45.5) | 4 (30.8) | |
| Severe | 19 (27.1) | 3 (10.0) | 4 (14.8) | 12 (92.3) | | 5 (20.8) | 9 (27.3) | 5 (38.5) | |
| **Itching intensity** | | | | | | | | | |
| Mild | 19 (27.1) | 13 (43.3) | 6 (22.2) | 0 (0.0) | **<0.001** | 6 (25.0) | 9 (27.3) | 4 (30.8) | 0.84 |
| Moderate | 31 (44.3) | 14 (46.7) | 16 (59.3) | 1 (7.7) | | 12 (50.0) | 15 (45.5) | 4 (30.8) | |
| Severe | 20 (28.6) | 3 (10.0) | 5 (18.5) | 12 (92.3) | | 6 (25.0) | 9 (27.3) | 5 (38.5) | |
| **Duration of wheals** | | | | | | | | | |
| 1-12 hours | 39 (55.7) | 21 (70.0) | 16 (59.3) | 2 (15.4) | **0.004** | 13 (54.2) | 20 (60.6) | 6 (46.2) | 0.66 |
| >12 hours | 31 (44.3) | 9 (30.0) | 11 (40.7) | 11 (84.6) | | 11 (45.8) | 13 (39.4) | 7 (53.8) | |

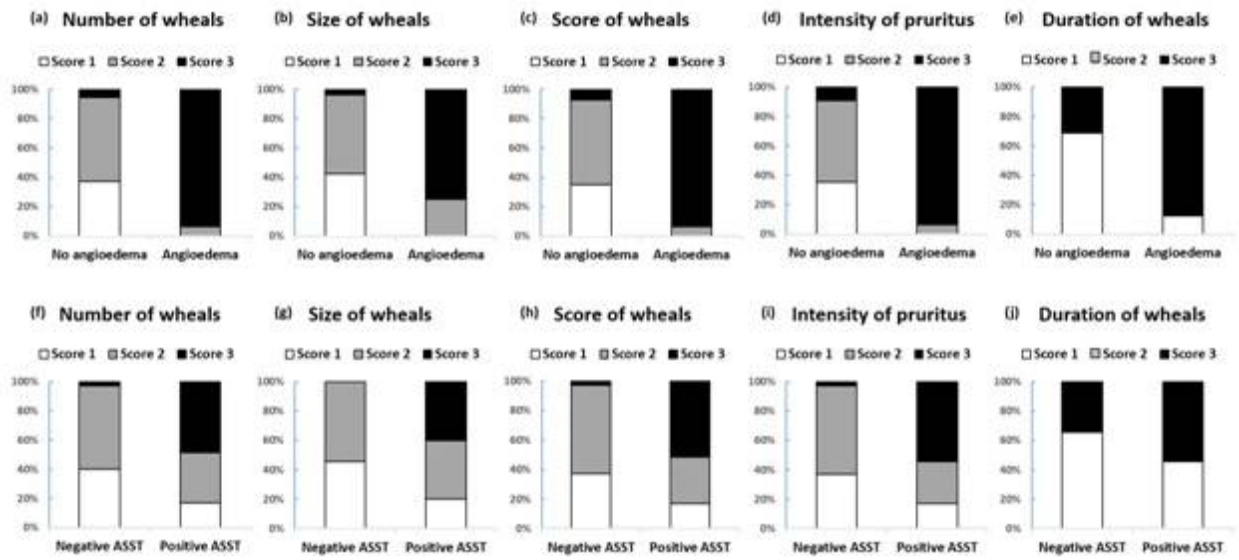
Values are shown as a number (percentage). Chi-square test was used. Statistical significance at P<0.05.

# Figures



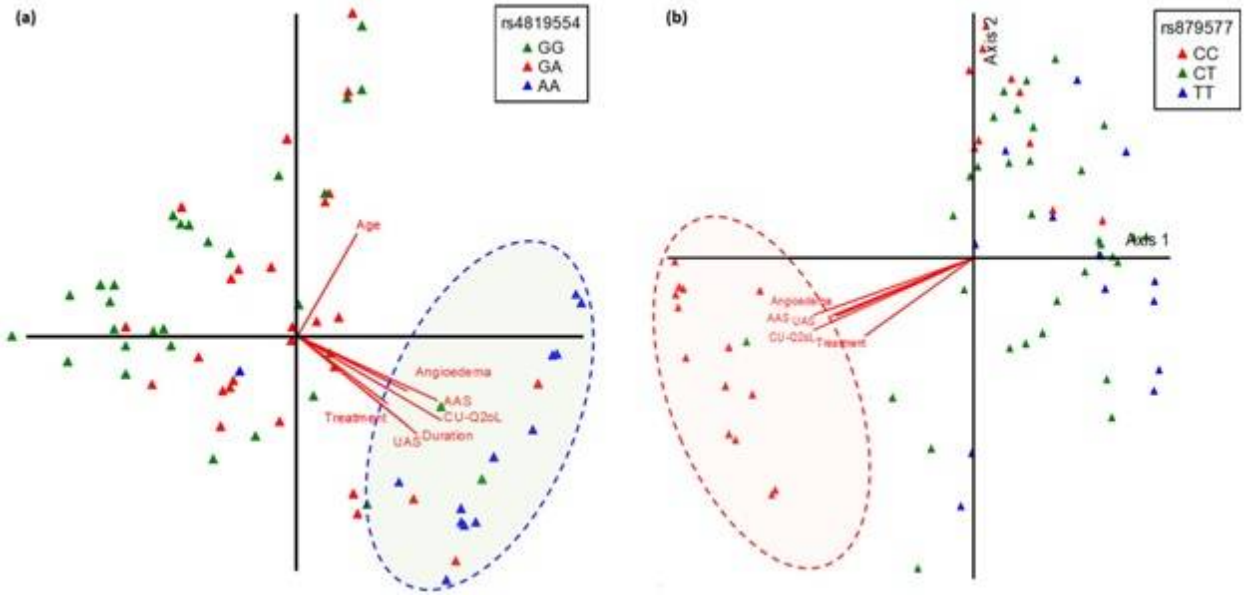

## Figure 1

Disease characteristics of urticaria patients. (a) Duration of urticaria disease, (b) Site of lesions, (c) Presence of associated angioedema, (d) Course of urticaria disease, (e) Results of autologous serum skin test (ASST), (f) Therapeutic modalities received by CSU patients based on antihistamines medication levels.




## Figure 2

Stratification analysis.Stratificationof urticaria activity score (UAS) by angioedema and autologous serum skin test in the patient group (n=70).




## Figure 3

Ordination plot for multivariate analysis. Based on clinical parameters, CSU patients with various IL-17RA rs4819554*AA and rs879577*CC genotypes were associated with angioedema, higher AAS, UAS, and prolonged duration. Thus, showed some clustering.