

ORIGINAL ARTICLE

Association of insertion/deletion polymorphism of the angiotensin-converting enzyme gene with angio-oedema accompanying chronic urticaria but not chronic urticaria without angio-oedema or the autologous serum skin test response

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Keywords

angio-oedema, angiotensin-converting enzyme, chronic urticaria, insertion/deletion polymorphism

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Abstract

Background Chronic urticaria is defined as the daily or almost daily occurrence of weals for more than 6 weeks. The underlying pathophysiology is reported to be mast cell activation, with release of mast cell mediators, predominantly histamine. Substance P is a neuropeptide and has the capacity to provoke histamine release from skin mast cells. Angiotensin-converting enzyme (ACE), widely expressed in skin, is one of the major peptidase for the degradation of substance P. An insertion/deletion polymorphism (I/D) in the ACE gene has been reported to be related to the levels of enzyme.

Objective An increase in substance P levels due to a polymorphism in ACE gene might be related to the pathology. Thus, we aimed to investigate whether there is an association between ACE I/D polymorphism and chronic ordinary urticaria.

Methods Ninety-five patients with chronic ordinary urticaria were recruited and divided into two groups according to autologous serum skin test status and accompanying angio-oedema. One hundred and sixty-one healthy subjects were enrolled as control group. All participants were genotyped for I/D polymorphism in intron 16 of the ACE gene by polymerase chain reaction.

Results A statistically significant association was not found between ACE I/D polymorphism and chronic ordinary urticaria. Further analyses of chronic ordinary urticaria patients showed that ACE I/D polymorphism was not associated with autologous serum skin test status of patients. However, the frequencies of II genotype and I allele were statistically significantly higher in chronic ordinary urticaria patients with accompanying angio-oedema with regard to angio-oedema-negative patients (II genotype: 24% vs. 9%, $P = 0.0002$; I allele: 58% vs. 27%, $P = 0.0001$) and control group (II genotype: 24% vs. 19%, $P = 0.01$; I allele: 58% vs. 41%, $P = 0.03$).

Conclusion The results of this study suggest no evidence of an association between ACE I/D polymorphism and risk of developing chronic ordinary urticaria. However, it can be a contributing factor to susceptibility of angio-oedema in chronic ordinary urticaria.

Introduction

Chronic urticaria (CU) is a common disease. It affects up to 0.1% of the population, including both adults and

children. The underlying molecular mechanisms are still unknown but might have a genetic basis.¹ It is defined as frequent or daily hives persisting for more than 6 weeks. Apart from urticarial vasculitis and physical urticarias, in

the majority of cases, a cause cannot be individuated. This entity has been suggested to be named as chronic ordinary urticaria (COU).²

The pathophysiology is based on the cutaneous mast cell release of mediators, predominantly histamine.³ Human skin mast cells are closely associated with sensory nerve endings that release neuropeptides upon antidromic stimulation by physical and chemical stimuli and stress. Substance P (SP) is a neuropeptide. It seems to be stored in peripheral and central terminals of the same sensory neurones. SP is released from these nerve endings upon various stimuli and has the capacity to provoke histamine release from skin mast cells.⁴ Intradermal injection of SP in normal human skin has been observed to induce typical weal and flare reactions.⁴

Angiotensin-converting enzyme (ACE) is the major proteolytic peptidase for the metabolism of SP. It is expressed in a wide range of tissues including skin and vascular endothelium.^{5,6} An insertion-deletion (I/D) polymorphism, which is based on the presence or absence of a 287-bp element in the non-coding region (intron 16) of the ACE gene on chromosome 17q23, has been reported to be related to the levels of circulating enzyme.^{7,8} The I allele of the ACE gene has been shown to be associated with lower ACE activity.⁹ Thus, an increase in skin SP level due to a polymorphism in ACE gene might be related to the pathology in COU patients. On this basis, the ACE gene might be one of the candidates for COU aetiopathogenesis.

In the light of these results, we aimed to analyse the possible role of I/D polymorphism of ACE gene in susceptibility to COU in the present study.

Patients and methods

Patients

This study comprised 95 patients diagnosed with COU. Patients had a disease duration of more than 6 weeks, occurring at least four times a week. The patients had urticaria at the time of the study. Antihistamines were discontinued 1 week before blood samples were collected, and none of the patients were taking other drugs at the time of the vein puncture. Known causes of CU were ruled out by appropriate investigations. The exclusion criteria were urticarial vasculitis, physically induced urticaria, drug or alcohol abuse, pregnancy, lactation, and C1-esterase inhibitor deficiency. ASST was done and read with the method by Sabroe *et al.*¹⁰ A consecutive series of 161 age matched healthy subjects formed the control group. Both the patients and control subjects were all Caucasian and Turkish descendants. This study was approved by Local Ethics Committee, and a written consent form was obtained from each subject.

Genotyping and statistical analysis

One-millilitre venous blood samples, drawn in EDTA as anticoagulant, were obtained from each subject. Genomic DNA was extracted from leucocytes manually by the method of Miller and Dykes,¹¹ and samples were stored at +4°C until further analysis. The ACE I/D polymorphism was determined with little modifications.¹² First, flanking primers GIIS (5'-CT-CAAGCACGCCCCCTCACAGGACTG-3') and GAS (5'-GATGTGGCCATCACATTCGTCA-GAT-3') were used to amplify intron 16 across the insertion. Amplification was carried out using AmpliTaq DNA polymerase (Fermentas, Vilnius, Lithuania) with an initial incubation of 2 min at 94 °C followed by 15 cycles of 1 min at 94 °C, 1 min at 62 °C, and 1 min at 72 °C in 25 mL buffer containing 2 mM MgCl₂ and 0.5% dimethyl sulfoxide. Second, after cooling the samples briefly at 4 °C, both the primer FYM (5'-ATCAGGAGGTCAGGA-GATCGAGAC-3'), which corresponds to the insertion sequence, and GIIS were added into the tubes, and polymerase chain reaction (PCR) was continued for an additional 15 cycles in the same conditions with a final extension time of 7 min at 72 °C. The extension between GAS and GIIS generated a 561-bp and a 274-bp PCR product for the I and D allele, respectively. The addition of FYM and GIIS in the second step of the PCR created a 376-bp product, which is coexisting only with the I allele and increasing the sensitivity and specificity of insertion amplification. The PCR products were visualized by electrophoresis in a 2% agarose gel with ethidium bromide and documented with a gel documentation system (Vilber-Lourmar, Eberhardzell, Germany).

Expected genotype frequencies were derived by the Hardy-Weinberg equation from single allele frequencies. Comparison of genotype and allele frequencies between different populations was done using Fischer's exact and χ^2 tests. $P < 0.05$ was considered significant. Results were analysed using the GraphPad Prism (version 3.02).

Results

Ninety-five (32 men: mean age $\pm$ SD, 36.81 $\pm$ 13.01 years and 63 women: mean age $\pm$ SD, 40.51 $\pm$ 13.58 years) patients diagnosed with COU and 161 control subjects (81 men: mean age $\pm$ SD, 32.60 $\pm$ 16.36 years and 80 women: mean age $\pm$ SD, 33.75 $\pm$ 16.13) were enrolled in this study.

Allele and genotype frequencies subgrouped by characteristics of patients and control subjects are presented in Table 1.

The results were analysed as allelic and genotypic frequencies of ACE polymorphism. First, the frequencies of ACE alleles were delineated in COU and control subjects. No significant difference was found in frequencies of ACE I/D alleles between COU and control subjects and

Table 1 ACE allele and genotype frequencies subgrouped by characteristics of patients and control subjects

	<i>n</i>	ACE genotypes			ACE alleles	
		DD	ID	II	D	I
Control	161	59 (36%)	72 (45%)	30 (19%)	190 (59%)	132 (41%)
Sex						
Female	80	31 (40%)	34 (43%)	13 (17%)	96 (62%)	60 (38%)
Male	81	28 (34%)	38 (46%)	17 (20%)	94 (57%)	72 (43%)
COU	95	40 (42%)	43 (45%)	12 (13%)	123 (65%)	67 (35%)
Sex						
Female	63	26 (41%)	26 (41%)	11 (18%)	78 (62%)	48 (38%)
Male	32	14 (44%)	17 (53%)	1 (3%)	45 (70%)	19 (30%)
ASST						
Positive	53	24 (45%)	23 (44%)	6 (11%)	71 (67%)	35 (33%)
Negative	42	16 (38%)	20 (48%)	6 (14%)	52 (62%)	32 (38%)
Angio-oedema						
Positive	25	2 (8%)	17 (68%)	6 (24%)	21 (42%)	29 (58%)*†
Negative	70	38 (54%)	26 (37%)	6 (9%)	102 (72%)	38 (27%)‡

n, number of subjects.

CL, confidence limits; OR, odds ratio.

**P* = 0.0001 vs. angio-oedema negative (OR, 3.7; CL, 1.88–7.27).†*P* = 0.03 vs. control (OR, 1.98; CL, 1.08–3.63).‡*P* = 0.004 vs. control (OR, 0.53; CL, 0.34–0.82).

between each ASST groups and control group. In addition, when a comparison was made between the subgroups of COU patients, no correlation was observed for sex and ASST in frequencies of ACE alleles (*P* > 0.05). But the frequency of I allele was found to be statistically significantly higher in COU patients with accompanying angio-oedema with regard to angio-oedema-negative patients and control group (*P* = 0.0001 and *P* = 0.03, respectively). A statistically significant clustering of D allele was found in patients with no angio-oedema.

Next a comparison was made on the homozygous and the heterozygous states of patients. No significant relation in genotype frequencies both between the sex and ASST subgroups of COU and between control and each of the above COU patient subgroups was observed (*P* > 0.05). An over-representation of II genotype was noted in patients with angio-oedema compared with the angio-oedema-negative and control groups (*P* = 0.0002 and *P* = 0.01, respectively).

Discussion

CU is defined as the daily or almost daily occurrence of weals for more than 6 weeks. Degranulation of mast cells with release of histamine is central to the development of its clinic.⁴ Studies of substance P have shown its ability to release histamine from cutaneous mast cells especially when the threshold of mast cell activation is decreased in exacerbation periods of CU.¹³

ACE is the major proteolytic peptidase for the metabolism of substance P. In an *in vitro* model, the inhibition of ACE activity by kaptopril is reported to block the inactivation of substance P¹⁴. Recently, the I allele of the ACE gene has been shown to be associated with lower ACE activity.¹⁰ For this reason, we speculated that ACE activity change due to I/D polymorphism might be one of the factors related to urticarial skin lesions by lower degradation of substance P. But we did not find any relationship between the I/D polymorphism of the ACE gene and COU.

An autoimmune origin is suggested in a significant proportion of patients with COU based on the demonstration that the intradermal injection of autologous serum causes a weal and flare reaction by activating normal donor basophils and mast cells and induce histamine release (autologous serum skin test, ASST).² In most cases, this is because of the circulating autoantibodies against IgE receptors of mast cells and basophils or/and against IgE. Although the mechanism involved in the remaining ASST-positive patients is still undefined, mast cell degranulation is believed to initiate the inflammatory process in autoimmune CU.¹⁵

SP is released from some sensory nerve endings upon various stimuli and has the capacity to provoke histamine release from skin mast cells. In normal human skin, intradermal injection of SP has been observed to induce typical weal and flare reactions.⁴ However, we did not find any relationship between ACE I/D genotype and ASST status of the patients. Thus, when our results are taken together,

an association between COU and/or CIU and the ACE I/D polymorphism could not occur for one of two reasons. First, substance P may not have an important role in the pathogenesis of this inflammatory skin disease. Second, substance P degrading enzymes other than ACE may have a greater role in termination of its bioactivity in skin of these patients.

Up to 40% of patients with either chronic autoimmune or chronic idiopathic urticaria have concomitant angio-oedema, most typically located on the face, lips, tongue, pharynx and extremities.¹⁴ Angio-oedema is a swelling involving the deeper layers of the skin or submucosal tissue and tends to recur chronically.¹⁶ Although clinically similar, angio-oedema can be caused by either mast cell degranulation or activation of kinin formation.¹⁵ ACE, also known as kininase II, is capable of degrading the vasodilating and substance P-inducing oligopeptide bradykinin. There are many reports concerning the induction of angio-oedema by ACE inhibitors, which has been attributed to the ACE inhibitor-induced augmentation of kinin levels in plasma. In the present study, analysis of the genotypes in COU patients with accompanying angio-oedema showed particularly significant excess of II genotype and I allele. However, our results may not suggest that kinins and/or substance P may have a role in the pathogenesis of COU in I allele carrier patients with accompanying angio-oedema because we do not have very good grounds for believing that both contribute to the clinical illness. Conversely, a statistically significant clustering of D allele was found in patients with no angio-oedema. Thus, for the patients with COU, D allele may protect against the development of angio-oedema.

In conclusion, the ACE I/D polymorphism could be involved in molecular pathogenesis of angio-oedema in COU. Further analyses in different ethnic groups will provide evidence whether ACE I/D polymorphism is a general risk factor or not.

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