ADAM 33 gene V4 C/G rs2787094 polymorphism in psoriasis

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ABSTRACT

AIMS: Psoriasis is a common chronic inflammatory disease. A disintegrin and metalloproteinase 33 (ADAM33) gene is the first novel susceptibility gene for asthma. The aim of this study was to investigate the relationship of ADAM 33 gene V4 C/G rs2787094 polymorphism with the risk of psoriasis in the Turkish population.

METHODS: ADAM33 gene polymorphism (V4 C/G rs2787094) was analyzed in 97 psoriasis patients and 50 healthy control subjects. This study was performed by polymerase chain reaction–based restriction fragment length polymorphism (PCR-RFLP) analysis.

RESULTS: There was no significant difference in ADAM33 genotype and allele distributions between psoriasis and control groups (p > 0.05).

CONCLUSIONS: ADAM33 V4 C/G rs2787094 polymorphism was not associated with psoriasis risk in the Turkish population. Larger studies with different ethnicities are needed to determine the impact of ADAM33 polymorphism on the risk of developing psoriasis.

KEY WORDS: ADAM 33, V4 C/G gene polymorphism, psoriasis.

Introduction

Psoriasis is a chronic inflammatory skin disorder characterized by epidermal hyperproliferation and dermal infiltration of inflammatory cells (1, 2) and the most common autoimmune disorder that affects around 1–2% of population (3, 4). Although, the disease has many different clinical features, the most common form is characterized by papulosquamous lesions covering elbows, knees and lumbosacral area or in most severe cases, larger parts of body (4).

A disintegrin and metalloprotease 33 (ADAM33) gene is a member of ADAM family (5), which has been extensively studied as a susceptibility gene in asthma. ADAM33 protein is a zinc-dependent endopeptidase, with pro-domain, catalytic, disintegrin-like, cysteine-rich and epidermal growth factor-like domain (5). ADAM33 is a complex molecule, the expression of which is largely restricted to mesenchymal cells, including fibroblasts and smooth muscle cells, and codes for a protein important for cellular fusion, adhesion, and signaling, as well as for proteolysis (6). Recently, several single nucleotide polymorphisms (SNPs) in ADAM33 gene have been shown to be significantly associated with the development of lung dysfunctions as asthma, bronchial hyper-responsiveness, and atopy (7, 8).

There are limited data in the literature on the association between ADAM 33 polymorphism and occurrence of psoriasis. The purpose of this study was to evaluate the distribution of ADAM 33 V4 C/G rs2787094 polymorphism in Turkish patients with psoriasis and to determine whether this polymorphism is a risk factor for the development of psoriasis.

Methods and methods

Study subjects

The case-control study consisted of 97 individuals with psoriasis, and 50 healthy individuals with no history of psoriasis or any other systemic disease who served as controls. Psoriasis patients with diagnosis confirmed by dermatologists of the Department of Dermatology of Dumlupinar University in Kütahya, Turkey. All individuals, both in case and control groups signed the informed consent. The protocol of the study was approved by the Clinical Studies Ethics Committee of Dumlupinar University, and the study was conducted in accordance with the Helsinki Declaration.

DNA extraction and genotyping

Genomic DNA was extracted from 5 ml of frozen whole peripheral blood collected from psoriasis patients and control subjects using a GeneJET DNA Extraction Kit (Thermo, Cat No: # K0722, Lithuania) according to the manufacturer’s protocol and following the manufacturers’ instructions. The amount and purity of DNA was measured at 260 nm using a Maestro Nano Micro-Volume spectrophotometer (Maestrogen Inc., Las Vegas, NV). DNA samples were stored at −20 °C until analysis. ADAM 33 gene V4 C/G rs2787094 polymorphism was analyzed using polymerase chain reaction and restriction fragment length polymorphism (PCR–RFLP) methodology in accord with that previously described by Zihilif et al (9).
ADAM 33 gene V4 C/G rs2787094 polymorphism

The PCR of ADAM 33 gene V4 C/G rs2787094 polymorphism was performed in a total volume of 50 μl, using 1 μg of genomic DNA with 100 pmol primers each (F: 5’-ACACA-CAGAATGCGGGAGGAG-3’, R: 5’-CCAGAAGCAAGGTCACACA-3’), 5 μl dNTP (2 mM), 5μl reaction buffer (X10), 3 μl MgCl2 (25 mM), and 2 U TaqDNA polymerase (abm, Canada). The cycling conditions performed in a SensoQuest Labcycler PCR System (Göttingen, Germany) were as follows: amplification consisted of a five-minute denaturation step at 94 °C; 35 cycles, 30 s at 94 °C, 30 s at 53 °C and 30 s at 72 °C; and five-minute final extension at 72 °C, followed by cooling to 4 °C.

Genotype analysis was conducted using RFLP. PCR products were digested with a specific restriction enzyme: PstI (Thermo, Cat No: #ER0612, Lithuania) according to the manufacturer’s protocol. The digested PCR products mixing with 2 μl loading buffer for ADAM 33 gene V4 C/G were separated and subjected to electrophoresis in 5 % ethidium bromide-stained 2% agarose gel at 100 mV for 1 h in 100 mL TBE buffer. The size of the restriction fragments for RFLP reactions products were determined using 100 bp DNA ladder (Invitrogen, USA). The differences in polymorphic allele were directly typed under ultraviolet light. The PCR products were of 374 bp for C allele and 168, 206 bp for G allele. Genotyping primers (Genscript, Piscataway, USA) sequences, PCR conditions, restriction enzymes, and length of the PCR: 374 bp

Tab. 1. Primers, PCR conditions, restriction enzymes and restriction fragment sizes used for genotyping analysis of ADAM33 V4C/G

| SNP         | Primers | PCR conditions | Restriction enzymes | PCR product and restriction fragment sizes |
|-------------|---------|----------------|---------------------|------------------------------------------|
| ADAM33 V4C/G| F: 5’-ACACA...-3’ | 94°C 5 min; 35 cycles, 94°C 30 s, 53°C 30 s, 72°C 30 s; 72°C 5 min | PstI | 374 bp |
| rs2787094   | R: 5’-CCAGAAGCAAGGTCACACA-3’ |                         |                       | G allele: 168 and 206 bp               |

Statistical analysis

Statistical analyses were done by SPSS (Statistical Package for Social Sciences, Chicago, IL, USA) 16.0 package program. All data are given as mean ± standard error of the mean (SEM). We tested for the Hardy–Weinberg equilibrium (HWE) among cases and controls separately. Comparisons of the distributions of the allele and genotype frequencies were performed using the chi-square test. The relative risk associated with rare alleles was estimated as an odds ratio (OR) with a 95 % confidence interval (CI). All p values ≤ 0.05 were accepted as statistically significant.

Results

The frequency of ADAM 33 gene V4 C/G rs2787094 polymorphism genotypes in control and psoriasis groups were showed a significant deviation from Hardy–Weinberg equilibrium (p ≤ 0.05) (Tab. 2). Three different genotypes and allele frequencies of ADAM 33 gene V4 C/G rs2787094 polymorphism were CC, GC and GG genotypes and C and G alleles, respectively. Allele frequencies and genotypes of ADAM 33 gene V4 C/G rs2787094 gene polymorphism in psoriasis patients and healthy controls are shown in Table 3. As with ADAM 33 gene V4 C/G, allele frequencies were found as follows: C-34 and G-66 % for the patient group, and C-37.1 and G-62.9 % for the control group. The distribution of alleles was similar in both groups (χ² = 0.296; df = 1; p = 0.586). The observed frequencies of ADAM 33 gene V4 C/G, CC, GC and GG genotypes were 5.2, 57.7 and 37.1 % for the psoriasis patients; for healthy subjects they were 1.7, 70.7 and 27.6 %, respectively. Genotype distribution or carrier frequencies of ADAM 33 gene V4 C/G polymorphism were not significantly different between patients with psoriasis and healthy controls (χ² = 3.059; df = 2; p = 0.217) (Tab. 3). No significant association between ADAM 33 gene V4 C/G polymorphism [GC/CC: OR = 0.27, 95%CI (0.03–2.42), p = 0.24; GG/CC: OR = 0.45, 95%CI (0.04–4.17), p = 0.48; G/C: OR = 0.87, 95%CI (0.54–1.41), p = 0.58]. There was no significant difference in the genotype or allele frequencies of ADAM 33 gene V4 C/G gene polymorphism between the psoriasis patients and controls and this polymorphism was not associated with psoriasis.

Fig. 1. PstI digestion of the polymerase chain reaction (PCR) products (374 bp) amplified by primer pair ADAM33 V4C/G, showing a homozygote CC (374 bp), heterozygote GC (168 + 206 + 374 bp), and homozygote GG (168 + 206 bp) after separation in 2% agarose gel electrophoresis. (M; markers 100 bp).

Tab. 2. Allele/Genotype frequencies and test of Hardy–Weinberg (HW) equilibrium.

|            | Controls | Psoriasis |
|------------|----------|-----------|
| O | E | O | E |
| CC | 1 | 7.97 | 5 | 11.2 |
| GC | 41 | 27.0 | 56 | 43.5 |
| GG | 16 | 22.9 | 36 | 42.2 |
| χ² | 15.3 | df=2; p = 0.00008 | χ² | 7.93; df=2; p = 0.004 |
| f(C) | 0.370 | 0.340 |
| f(G) | 0.630 | 0.660 |

f = observed frequency of each allele (C or G); O = observed genotype numbers; E = expected genotype numbers under a Hardy–Weinberg (HW) equilibrium assumption; χ² = Chi-square values; p = probability of difference.
Discussion

ADAM 33 is a transmembrane metalloproteinase belonging to a subgroup of a zinc-dependent metalloproteinase superfamily comprising over 30 members, which are structurally very complex (10). It has been identified as an asthma susceptibility gene in ethnically diverse populations (11, 12, 13). The gene is expressed by lung fibroblasts and bronchial smooth muscle cells and is involved in cellular adhesion, fusion, and signaling, as well as in proteolysis, by releasing various factors (14). Psoriasis is a chronic disorder in which T-cell-mediated inflammation causes the thickening of the skin (15). Lesueur et al (16) have found that common biological pathways of ADAM33 and psoriasis may be involved in the etiology of psoriasis and other clinically distinct immune-mediated diseases (16). In the current study, we investigated the relation between single nucleotide polymorphism of ADAM 33 gene V4 C/G rs2787094 genotypes in Turkish familial psoriasis patients compared to age-matched Turkish controls, while V4 [rs2787094] was significantly associated with psoriasis in heterozygotes but not in homozygotes for the minor allele. This decrease in risk observed in heterozygotes only may result from a more complex underlying model involving interactions between SNPs at a given locus or with other factors (20).

ADAM33 signaling may have an important role in the physiopathology of many immune-mediated disorders such as asthma, psoriasis, type 2 diabetes, and obesity, although their effects and functions are still unclear. Although ADAM33 gene polymorphism has been studied in association with psoriasis, the physiological and functional effects of ADAM33 gene is not yet well known. However due to the limited size of the sample, this association of this gene with psoriasis needs to be further testified. In conclusions, our results show that ADAM 33 gene V4 C/G rs2787094 polymorphism has no significant effect on psoriasis susceptibility in Turkish patients. Therefore, we suggest that ADAM 33 gene V4 C/G rs2787094 polymorphism is not a genetic risk factor of psoriasis sensibility. This is the first report in respect of ADAM 33 gene V4 C/G rs2787094 polymorphism in Turkish familial psoriasis patients. However, the difference in the ADAM 33 promoter polymorphism in psoriasis patients and its associations with disease manifestations may be of ethnic origin. Thus, further studies are necessary to confirm our results. In view of these data, the involvement of other genetic and/or environmental factors seems to be required while being more important in psoriasis. Further analysis of other ADAM 33 gene polymorphisms and extended samples may clarify the current findings, eventually resulting in a better understanding of the genetic components of psoriasis. However, there is still a need for further research on this topic, including screening for etiological relationships between other functional polymorphic sites in the ADAM 33 gene locus and sensitivity on psoriasis.

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