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CLINICAL STUDY

# 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 (*Tab. 3, Fig. 1, Ref. 16)*. Text in PDF *www.elis.sk.*
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).

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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 Kütayha, 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 Zihlif et al (9).

**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 rs2787094 | F: 5'-ACACACAGAATGGGGGAGAG-3' R: 5'-CCAGAAGCAAAGGTCACACA-3' | 94°C 5 min; 35 cycles, 94°C 30 s, 53°C 30 s, 72°C 30 s; 72°C 5 min | PstI | PCR: 374 bp G allele: 168 and 206 bp C allele: 374 bp |

*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 ml, using 1 µg of genomic DNA with 100 pmol primers each (F: 5'-ACACACAGAATGGGGGAGAG-3', R: 5'- CCAGAAGCAAAGGTCACACA-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 digested fragment are shown in Table 1. Electrophoresis picture samples are given in Figure 1.

*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.

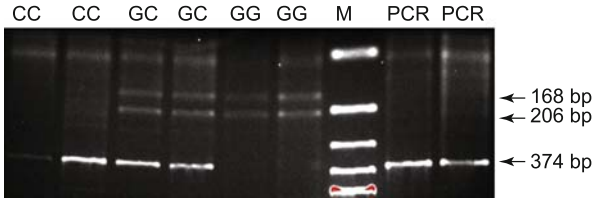


**Fig. 1. PstI digestion of the polymerase chain reaction (PCR) products (374 bp) amplified by primer pair ADAM33 V4C/G, showing a homozygote CC (374 bp), heterozygote GC (168 + 206 + 374 bp), and homozygote GG (168 + 206 bp) after separation in 2% agarose gel electrophoresis. (M; markers 100 bp).**

**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 |
| | $\chi^2$=15.3, df=2, p = 0.00008 | | $\chi^2$=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; $\chi^2$= Chi-square values; p = probability of difference

## 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 ($p \leq 0.05$) (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 ($\chi^2 = 0.296$; 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 ($\chi^2 = 3.059$; 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.

**Tab. 3. The frequencies of allele and genotypes in ADAM33.**

| SNP | Genotypes | Frequency (%) | | OR and CI (95 % CI) | | p |
|---|---|---|---|---|---|---|
| | | Psoriasis (n = 97) | Control (n = 58) | | | |
| rs2787094 (V4) | CC | 5 (5.2) | 1 (1.7) | 3.09 (0.35–27.1) | Reference | – |
| | GC | 56 (57.7) | 41 (70.7) | 0.56 (0.28–1.13) | 0.27 (0.03–2.42) | 0.24 |
| | GG | 36 (37.1) | 16 (27.6) | 1.54 (0.76–3.14) | 0.45 (0.04–4.17) | 0.48 |
| | $\chi^2$=3.059 df=2 p=0.217 | | | | | |
| | Allele | | | | | |
| | C | 66 (34) | 43 (37.1) | 0.87 (0.54–1.41) | Reference | – |
| | G | 128 (66) | 73 (62.9) | 1.14 (0.70–1.84) | 0.87 (0.54–1.41) | 0.58 |
| | $\chi^2$=0.296 df=2 p=0.586 | | | | | |

## 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 and psoriasis risk in Turkish familial psoriasis patients compared to age-matched Turkish controls. To the best of our knowledge, this is the first study among Turkish familial psoriasis patients assessing the interaction of V4 C/G rs2787094 genetic polymorphism and psoriasis.

Our study revealed that there was no statistically significant difference between psoriasis patients and control subjects for ADAM 33 gene V4 C/G rs2787094 genotypes in Turkish patients. We obtained CC genotype only in five cases, GC genotype in fifty six cases, and GG genotype was found in thirty-six cases of psoriasis. Our results showed that the V4 C/G rs2787094 polymorphism was not significantly associated with psoriasis. At present, the mechanistic roles of the disease-associated ADAM 33 gene V4 C/G rs2787094 SNP have yet to be elucidated, especially in the context of the pathophysiology of psoriasis.

Deng et al (17) observed a decreased frequency in CG and GG genotypes of ADAM33 rs2787094 (V4) in cases compared with controls, while V4 [rs2787094] was significantly associated with psoriasis in the Chinese population (17). Han et al (18) showed that both V4[rs2787094] and S2[rs528557] SNPs were significantly associated with psoriasis in a northeastern Chinese population, although V4[rs2787094] SNP was not significantly associated with psoriasis in a French family-based study (16). A study reported that (19) ADAM33 rs597980 is associated with psoriasis in white North Americans. In addition, Siroux et al (20) showed that ADAM33 rs512625 was associated with psoriasis and that rs628977 was specifically associated with the early-onset psoriasis in the French population. In this same study, the estimates of the odds-ratios for rs512625 showed a protective effect on 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.

## References

**1. Bhalerao J, Bowcock AM.** The genetics of psoriasis: a complex disorder of the skin and immune system. Hum Mol Genet 1998; 7: 1537–1545.

**2. Wagner EF, Schonthaler HB, Guinea-Viniegra J, Tschachler E.** Psoriasis: what we have learned from mouse models. Nat Rev Rheumatol 2010; 6: 704–714.

**3. Rivas Bejarano JJ, Valdecantos WC.** Psoriasis as autoinflammatory disease. Dermatol Clin 2013; 31: 445–460.

**4. De Andrade Lima E, de Andrade Lima M.** Reviewing concepts in the immunopathogenesis of psoriasis. An Bras Dermatol 2011; 86: 1151–1158.

**5. Fotis A, Alexander K, Dimosthenis L, Kiriakos K, Kostas S.** DNA sequence variations of metalloproteinases: their role in asthma and COPD. Postgrad Med J 2007; 83: 244–250.

**6. Postma DS, Howard T.** ADAM33 gene: confirming a gene without linkage. Clin Exp Allergy 2004; 34, 1–3.

**7. Wang X, Li L, Xiao J, Jin C, Huang K, Kang X, Wu X, Lv F.** Association of ADAM33 gene polymorphisms with COPD in a northeastern Chinese population. BMC Med Genet 2009; 10: 10–132.

**8. Howard TD, Postma DS, Jongepier H, Moore WC, Koppelman GH, Zheng SL, Xu J, Bleecker ER, Meyers DA.** Association of a disintegrin and metalloprotease 33 (ADAM33) gene with asthma in ethnically diverse populations. J Allergy Clin Immunol 2003; 112: 717–722.

**9. Zihlif M, Mahafza T, Obeidat NM, Froukh T, Shaban M, Al-Akhras FM, Zihlif N, Naffa R.** Frequency of genetic polymorphisms of ADAM33 and their association with allergic rhinitis among Jordanians. Gene 2013; 1; 531 (2): 462–466.

**10. Edwards DR, Handsley MM, Pennington CJ.** The ADAM metalloproteinases. Mol Aspects Med 2008; 29: 258–289.

**11. Hirota T, Hasegawa K, Obara K, Matsuda A, Akahoshi M, Nakashima K, Shirakawa T, Doi S, Fujita K, Suzuki Y, Nakamura Y, Tamari M.** Association between ADAM33 polymorphisms and adult asthma in the Japanese population. Clin Exp Allergy 2006; 36: 884–891.

**12. Bijanzadeh M, Ramachandra NB, Mahesh PA, Mysore RS, Kumar P, Manjunath BS, Jayaraj BS.** Association of IL–4 and ADAM33 gene polymorphisms with asthma in an Indian population. Lung 2010; 188: 415–422.

**13. Su D, Zhang X, Sui H, Lü F, Jin L, Zhang J.** Association of ADAM33 gene polymorphisms with adult allergic asthma and rhinitis in a Chinese Han population. BMC Med Genet 2008; 9 (9): 82.

**14. Holloway JW, Laxton RC, Rose-Zerilli MJ, Holloway JA, Andrews AL, Riaz Z, Wilson SJ, Simpson IA, Ye S.** ADAM33 expression in atherosclerotic lesions and relationship of ADAM33 gene variation with atherosclerosis. Atherosclerosis 2010; 211: 224–230.

**15. Bowcock AM, Cookson WO.** The genetics of psoriasis, psoriatic arthritis and atopic dermatitis. Hum Mol Genet 2004; 1: R43–55.

**16. Lesueur F, Oudot T, Heath S, Foglio M, Lathrop M, Prud'homme JF, Fischer J.** ADAM33, a new candidate for psoriasis susceptibility. PLoS One 2007; 19 (2): e906.

**17. Deng W, Liang WB, Gao LB, Wang YY, Lv ML, Zhu Y, Zhang L.** Association of ADAM33 polymorphisms and susceptibility to psoriasis. DNA Cell Biol 2010; 29 (8): 435–439.

**18. Han J, Xiao J, Wu Q, Hao L.** Association of ADAM33 gene polymorphisms with psoriasis in a northeastern Chinese population. Mol Biol Rep 2014; 41 (6): 4001–4005.

**19. Li Y, Liao W, Chang M, Schrodi SJ, Bui N, Catanese JJ, Poon A, Matsunami N, Callis-Duffin KP, Leppert MF, Bowcock AM, Kwok PY, Krueger GG, Begovich AB.** Further genetic evidence for three psoriasis-risk genes: ADAM33, CDKAL1, and PTPN22. J Invest Dermatol 2009; 129: 629–634.

**20. Siroux V, Bouzigon E, Dizier MH, Pin I, Demenais F, Kauffmann F.** Replication of association between ADAM33 polymorphisms and psoriasis. PLoS One 2008; 3: e2448.

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