

## Association between the -1438A/G polymorphism of the serotonin 2A receptor gene and late-onset psoriasis in a Thai population

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**ABSTRACT.** Expression of serotonin 2A receptor (*5-HTR2A*) is known to increase in psoriasis, a chronic inflammatory skin disease. We investigated a possible association between the -1438A/G single nucleotide polymorphism (rs6311) in the promoter region of *5-HTR2A* gene and psoriasis in a Thai population. One hundred and twelve psoriatic patients and 151 unrelated healthy controls were included in our study. Genotyping was performed using the polymerase chain reaction and restriction fragment length polymorphism techniques. We found no overall differences in genotype distributions and allele frequencies when comparing between the two groups. When we analyzed a subset of psoriatic patients classified by onset and severity, only the -1438A allele was significantly increased in patients with late-onset psoriasis when compared with the healthy control group ( $\chi^2 = 4.77$ , d.f. = 1,  $P = 0.029$ , odds ratio = 2.298 [95% confidence interval =

1.126-4.691]). This single nucleotide polymorphism may be involved in late-onset psoriasis in this Thai population.

**Key words:** Psoriasis; Psoriasis subtype; Association study; Serotonin 2A receptor; Single nucleotide polymorphism; Thai population

## INTRODUCTION

Psoriasis, a chronic inflammatory skin disease affecting about 2-4% of the population worldwide, is thought to be a multifactorial disease with both genetic and immunogenic backgrounds (Schön and Boehncke, 2005; Lowes et al., 2007). Since psoriasis occurs in connection with stress and mood disorders (Peters et al., 2000; Griffiths and Richards, 2001) and is apparently induced in patients who have been treated with antidepressants (Barth and Baker, 1986; Osborne et al., 2002; Tan Pei Lin and Kwek, 2009), the neuro-immuno-cutaneous-endocrine (NICE) model appears to fit the mode of action of this disease (O'Sullivan et al., 1998; Locala, 2009). Mediators, such as serotonin (5-hydroxytryptamine, 5-HT), may play a central role in this NICE network.

The serotonergic system, which consists of serotonin-producing cells, serotonin receptors and serotonin transporters, may play a significant role in psoriasis. In support of this hypothesis, serotonin expression has been reported to be significantly stronger in prickle, sweat gland, and sebaceous gland cells, as well as in the hair roots of lesions that appear during the progressive stage of psoriasis than in the static stage; serotonin is not expressed in normal skin (Huang et al., 2004). In our previous study, we observed similar serotonin levels in the serum of psoriatic patients and healthy controls; however, platelet serotonin concentrations were significantly decreased in the psoriatic patients (Tencomnao et al., 2007). We also found that expression of serotonin 2A receptor (5-HTR2A), a mediator of the downstream effects of serotonin, was significantly greater in involved and noninvolved psoriatic skin than in normal skin; symptomatic psoriatic skin had the highest 5-HTR2A levels (Nordlind et al., 2006); this demonstrates the molecular role of 5-HTR2A in promoting cell proliferation (Azmitia, 2001). It is also present in activated T cells (Stefulj et al., 2000; León-Ponte et al., 2007). Consequently, the molecular involvement of the 5-*HTR2A* gene in susceptibility to inflammatory disorders such as psoriasis has received much attention.

The human 5-*HTR2A* gene is located on chromosome 13q14-21 (Sparkes et al., 1991). It consists of three exons, separated by two introns, and spans over 63 kb. Two single nucleotide polymorphisms (SNPs) of this gene have been frequently found in psychiatric disorders: -1438A/G (rs6311) in the promoter region (Collier et al., 1997) and 102T/C (rs6313) in exon 1 (Warren Jr. et al., 1993). Functional studies have recently sought to identify the molecular mechanisms underlying certain genetic diseases and have focused on these two SNPs. Since they are in complete linkage disequilibrium (Ohara et al., 1999; Ono et al., 2001), study of one of these polymorphisms generally provides information about the other. The latter SNP is a synonymous change and may not directly influence the transcription of this gene (Bray et al., 2004). In contrast, the former SNP has been shown to transcriptionally modulate 5-*HTR2A* gene expression (Parsons et al., 2004; Myers et al., 2007). In addition, *in silico* analysis of -1438A/G allelic variants has revealed the A allele, which contains a consensus binding site for transcription factor Th1/E47; also an electrophoretic mobility shift assay successfully

demonstrated allele-specific binding to support the bioinformatics predictions (Smith et al., 2008). Differences in disease susceptibility may be due to altered density of the receptor crucial for neurotransmitter mechanisms, making this SNP a promising candidate for an association study. Consequently, we made a case-control study design to investigate the possible involvement of the -1438A/G SNP of the *5-HTR2A* gene in psoriasis. Genetic association was also analyzed in a subset of patients with psoriasis classified by onset and severity.

## MATERIAL AND METHODS

### Subjects

We successfully genotyped genomic DNA samples obtained from 112 Thai patients who had psoriasis diagnosed clinically by experienced dermatologists at King Chulalongkorn Memorial Hospital, Bangkok, and Sappasitprasong Hospital, Ubon Ratchathani, and from 151 unrelated healthy Thai volunteers without a family history of psoriasis recruited from the National Blood Center, Thai Red Cross Society and the Faculty of Allied Health Sciences, Chulalongkorn University. In the patient group, each individual was classified according to onset of disease: early-onset (<40 years of age) or late-onset ( $\geq$ 40 years of age). The severity of psoriasis was classified according to the Psoriasis Area and Severity Index (<10 = mild, 10-20 = moderate, >20 = severe). The Ethics Committee of Medical Experiments on Human Subjects (Faculty of Medicine, Chulalongkorn University) approved the study. Informed consents were obtained from all participants prior to inclusion in the study. The characteristics of the subjects are summarized in Table 1.

**Table 1.** Characteristics of the Thai psoriatic patients and control subjects.

	Psoriasis*	Early-onset psoriasis	Late-onset psoriasis	Mild psoriasis	Moderate psoriasis	Severe psoriasis	Healthy controls
Total number of subjects	112	68	38	61	34	13	151
Males	67 (59.8%)	37 (54.4%)	25 (65.8%)	33 (54.1%)	25 (73.53%)	6 (46.15%)	81 (53.64%)
Female	45 (40.2%)	31 (45.6%)	13 (34.2%)	28 (45.9%)	9 (26.47%)	7 (53.85%)	70 (46.36%)
Age at enrolment (years)	47.62 $\pm$ 14.7	40.04 $\pm$ 11.2	60.50 $\pm$ 11.3	46.92 $\pm$ 15.6	48.03 $\pm$ 14.56	48.38 $\pm$ 11.95	34.54 $\pm$ 11.51
Age at onset of symptoms (years)	37.01 $\pm$ 15.6	27.72 $\pm$ 8.38	53.60 $\pm$ 10.9	38.97 $\pm$ 15.7	37.03 $\pm$ 14.67	27.92 $\pm$ 14.75	-

Data are reported as number with percent in parentheses or as means  $\pm$  SD. \*Clinical data concerning onset and severity of disease was not available for some of the psoriatic patients.

### DNA extraction

DNA was isolated from leukocytes collected with ethylenediaminetetraacetic acid as an anticoagulant, using either the salting-out method (Miller et al., 1988) or the FlexiGene DNA kit (Qiagen GmbH, Hilden, Germany).

### Determination of -1438A/G *5-HTR2A* SNP

Genotyping of the -1438A/G *5-HTR2A* SNP was performed by polymerase chain reaction, using specific primers (forward primer 5'- AAC CAA CTT AIT TCC TAC CAC -3' and reverse

primer 5'- AAG CTG CAA GGT AGC AAC AGC -3') and subsequent restriction fragment length polymorphism with *MspI*, as previously described (Collier et al., 1997). The fragments were resolved by 3% agarose gel electrophoresis and visualized by ethidium bromide staining. Fragments containing the uncut A allele had a \ 468-bp band; fragments containing the G allele had two bands of 244 and 224 bp. Randomly selected DNA samples were subjected to direct sequencing to verify the genotypes.

### Statistical analysis

A goodness-of-fit test was employed to test whether the genotype distribution was in Hardy-Weinberg equilibrium. Using the chi-square ( $\chi^2$ ) test with Yates correction, allele and genotype frequencies were compared between the group of patients with psoriasis and healthy controls. In addition, comparisons were made between the subset of psoriatic patients by onset and severity of disease and the healthy control group. A P value of <0.05 was considered to be significant. Odds ratios (OR) and the 95% confidence interval (CI) were calculated using EpiCalc 2000 version 1.02 (<http://www.brixtonhealth.com/epicalc.html>).

### RESULTS

The genotype distributions and allele frequencies of -1438A/G 5-*HTR2A* SNP were determined in 263 Thai subjects, including 151 healthy controls and 112 psoriatic patients (Table 2). The most common genotype in this Thai population was the AA homozygote; the number of GG homozygotes was small. The genotype distributions of the -1438A/G SNP of 5-*HTR2A* were in Hardy-Weinberg equilibrium for both psoriatic subjects ( $\chi^2 = 0.59$ , d.f. = 1, P = 0.444) and healthy controls ( $\chi^2 = 0.15$ , d.f. = 1, P = 0.695).

**Table 2.** Genotype distributions and allele frequencies of the -1438A/G 5-*HTR2A* single nucleotide polymorphism in the psoriatic patients, classified by onset and severity of disease, and healthy controls.

Variables	Genotype (%)					Allele				
	AA	AG	GG	$\chi^2$ , d.f. = 2	P	A	G	$\chi^2$ , d.f. = 1	P	OR (95%CI)
Psoriasis (N = 112)	68	27	5	4.14	0.126	82	18	3.74	0.053	1.554 (1.015-2.379)
Early-onset (N = 68)	62	34	4	1.05	0.591	79	21	0.80	0.371	1.285 (0.791-2.086)
Late-onset (N = 38)	76	21	3	5.51	0.064	87	13	4.77	0.029	2.298 (1.126-4.691)
Mild (N = 61)	62	34	4	1.56	0.459	80	20	1.07	0.301	1.351 (0.812-2.249)
Moderate (N = 34)	68	23	9	2.25	0.324	79	21	0.56	0.455	1.343 (0.707-2.552)
Severe (N = 13)	85	15	0	4.28	0.118	92	8	3.34	0.068	4.179 (0.965-18.100)
Control (N = 151)	56	37	7	-	-	74	26	-	-	-

d.f. = degrees of freedom; OR = odds ratio; CI = confidence interval.

There was no significant difference in the genotype distributions between the group of psoriatic patients and healthy controls. Although comparison of allele frequencies revealed an excess of the A allele in psoriatic patients, we did not find any significant difference in the allele frequencies between the two groups. In an analysis of subsets of psoriatic patients classified by onset and severity of disease, only the -1438A allele was significantly increased in patients with late-onset psoriasis when compared with the healthy control group ( $\chi^2 = 4.77$ , d.f. = 1, P = 0.029, OR = 2.298 [95% CI = 1.126-4.691]); there was no significant association between severity of disease with any specific genotype or allele (Table 2).

## DISCUSSION

Owing to the importance of -1438A/G allelic variants in the regulation of *5-HTR2A* gene expression, this SNP has attracted much attention for not only genetic association studies, but also for pharmacogenetics studies involving various neuropsychiatric diseases, such as schizophrenia, mood and anxiety disorders (Serretti et al., 2007). It has been reported that 5-HTR2A regulates signal transduction by activating the phospholipase C second messenger pathway (Conn et al., 1986; Hoyer et al., 2002) and triggering the tyrosine phosphorylation of Jak2 kinase in response to serotonin (Guillet-Deniau et al., 1997).

Psoriasis appears to be characterized by stress-induced chronic inflammation, and it has been well documented that stress and stress-related hormones are associated with the serotonergic system. We carried out this case-control study on the genotype distributions and allele frequencies of -1438A/G *5-HTR2A* SNP in order to determine if there is an association between this polymorphism and psoriasis. More than 70% of the patients enrolled in our study were diagnosed to have chronic plaque-type psoriasis. To the best of our knowledge, this is the first study to investigate a potential association between a functionally relevant genetic variation in the promoter of the gene encoding the serotonin receptor and an inflammatory skin disorder. We had previously demonstrated associations of certain SNPs in the vascular endothelial growth factor gene and interleukin-10 gene with early- and late-onset psoriasis, respectively (Wongpiyabovorn et al., 2008a,b). In this current, most recent investigation, we found no overall differences in genotype distributions and allele frequencies when patients with psoriasis were compared with healthy controls. Since the overall allele frequencies of -1438A were higher in patients than in healthy controls, a possible association of this SNP with a subset of psoriatic patients classified by age at onset and severity of disease was subsequently addressed. Association between this SNP and late-onset psoriasis was evident as the -1438A allele was significantly more frequent than in the psoriasis patients than in the control group. This is similar to what has been reported for SNPs in terms of predisposition to psoriasis and for disease subtype (Reich et al., 2002; Nedoszytko et al., 2007; Wongpiyabovorn et al., 2008a,b; Wu et al., 2009). The -1438A allele may contribute to disease susceptibility because it is associated with increased 5-HTR2A expression (Parsons et al., 2004); this is also supported by bioinformatics studies (Smith et al., 2008). However, the molecular impact of this particular allele has been demonstrated to be modulated by another SNP of this gene, -783A/G (Myers et al., 2007). In addition, the -1438A allele has been shown to be associated with increased 5-HTR2A receptor binding (Turecki et al., 1999). Taken together, these lines of evidence suggest that patients suffering from late-onset psoriasis have these symptoms because the -1438A allele leads to an increase in *5-HTR2A* gene expression and receptor binding, thus activating relevant signaling cascades.

It is known that the frequency of -1438A/G alleles differs among ethnic groups. The -1438A allele was found to be the major allele in Thais. This was also found for Chinese populations (Zhang et al., 2008; Ying et al., 2009). However, we found a high percentage of the -1438A allele (74%) in healthy Thai subjects, suggesting that this SNP is not very polymorphic in this population. A larger sample size would help determine if our conclusions are correct. However, laboratory investigations involving DNA genotyping need to be carried out carefully, since there have been cases of misgenotyping data on major neurotransmitter system genes, specifically dopamine receptor D1 gene -48A/G polymorphism (Tencomnao and Boonmalert, 2009).

Recently, genetic variations in the 5-HTR2A gene have been found to be associated with rheumatoid arthritis, a chronic and systemic autoimmune disorder (Kling et al., 2008), highlighting the role of SNPs in the serotonergic system in chronic inflammatory disorders. The hypothesis that genetic polymorphisms in serotonergic genes play a role in psoriasis susceptibility has been examined by our group and by other researchers. No association was found between serotonin transporter gene-linked polymorphic region and psoriasis in German Caucasian samples (Mössner et al., 2009). However, notably, they found that 20% of the patients with psoriasis suffered from at least mild depression.

In addition to this SNP, other functionally relevant genetic variations of the serotonergic system may play a part in the etiology of psoriasis; exploring their functional significance would be useful. In clinical practice, developing drugs influencing the serotonergic system might be a simpler mission because these types of agents, such as selective serotonin reuptake inhibitors, are commercially available for therapeutic purposes.

In conclusion, we found evidence that the -1438A allele is associated with an increased risk for late-onset psoriasis in a Thai population. This association should be checked in other populations.

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