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Association between Alcohol, Smoking and HLA-DQA1*0201 Genotype in Psoriasis

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Abstract Psoriasis is a chronic skin disease triggered by genetic, environment or other risk factors such as infection, drugs, stress, moisture, alcohol, and smoking. A major psoriasis susceptibility locus at 6p21.3 has been identified. Further studies found that HLA-DQA1*0201 allele was associated with psoriasis. However, there were few data exploring an association between the environmental factors and susceptibility genes. In this study, the samples of 189 patients with psoriasis and 333 healthy controls were collected with their consent and were carried on analysis through polymerase chain reaction sequence-specific primer (PCR-SSP) method. The proportion of male psoriasis patients engaging in the smoking and alcohol was much higher than that of the control group (P<0.005). The HLA-DQA1*0201 allele was present at significantly higher frequency in the patients with psoriasis (OR=4.25, $P<1.0\times10^{-6}$). Association was found between smoking, alcohol and HLA-DQA1*0201 in male patients with psoriasis (OR>6.91, $P<1.0\times10^{-4}$).

Key words psoriasis; HLA-DQA1*0201 allele; smoking; alcohol; association

Psoriasis is a common chronic disease characterized by erythema and scaling plaques. Although the disease is not fatal, it detrimentally affects the life-quality of the sufferer, but no effective curative therapy has been established. Up to now, the pathogenesis of psoriasis still remains elusive. Genetic predisposition is essential to trigger this disease, and exogenous risk factors are required in its developing process [1,2]. It has also been suggested that psoriasis is an autoimmune disease involving interactions among cytokines and various kinds of immunocyte [3]. In the psoriasis lesion tissues, lymphocyte/macrophage infiltration and activated T cells were found [4]. The genome-wide screening results have identified a major psoriasis susceptibility locus at chromosome 6p21.3 [5–10] the interval region of which the human leukocyte antigen (HLA) genes locate in and are called HLA region. The HLA region was first identified by virtue of the fact that it contains genes encoding the major transplantation antigens subsequently shown to play physiological roles in the antigen processing of T lymphocytes. These results strongly support those case-control studies, suggesting that an HLA gene might be the major genetic component to psoriasis. Some studies have found that the HLA-DQA1*0201 allele is closely associated with psoriasis [11–13]. On the other hand, epidemiological studies showed that alcohol and smoking were the main etiological factors for psoriasis [14,15]. In this study, we attempted to explore the relationships among alcohol level, smoking and HLA-DQA1*0201 allele frequency in Chinese psoriasis patients.

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Materials and Methods

Patients and controls

All patients had been diagnosed in 1997–2001 according to the clinical criteria [16,17]. Totally 189 psoriasis

patients 3–73 year old were interviewed, of which 105 (55.56%) were male and 84 (44.44%) were female. During the same period, a total of 333 healthy controls residing in the same geographic regions were chosen from an available pool of 1400 healthy volunteers. They answered an identical structured questionnaire. Oral informed consent was obtained from each subject before the data were collected. In the control group, 175 (52.55%) were male and 158 (47.45%) were female, with age ranging from 3 to 73 years.

All of the patients and healthy controls underwent physical examination and answered questionnaire on demographic factors and personal characteristics and habits, including alcohol intake and smoking. These data were analyzed using the Epi Info 6 software (American Center for Disease Control, Atlanta, USA). The average consumption of alcohol per day was calculated according to the following Formula [18]:

150 ml wine=330 ml beer=30 ml spirits=10–12 g alcohol

10 ml blood sample was obtained from each patient or healthy control for DNA extraction.

Genomic DNA extraction

It is well known that leukocyte plays a significant role in the immune system. Genomic DNA was extracted from leukocyte of peripheral blood by use of the Qiamp blood kit following the protocol recommended by the manufacturer.

PCR conditions

The amplification primers were used as described previously [19]. The size of the PCR product was 170 bp. A 796 bp fragment was obtained when amplified with inner control primers (Table 1).

PCR reaction mixture (10 μl) consisted of 100 ng genomic DNA, 200 μM dATP, dCTP, dGTP and dTTP, 0.30 μmol HLA-DQA1*0201 allele primers, 0.06 μmol inner control primers, 0.5 u *Taq* polymerase (MBI Fragment, Hanover, MD, USA) and PCR buffer [1.5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.3, and 0.001 gelatin (*W*/*V*)]. Preliminary denaturation was performed at 94 °C for 3 min, followed by 35 cycles (denaturation at 94 °C for 30 s, anneal at 60 °C for 30 s, extension at 72 °C for 30 s) and a final extension (72 °C for 5 min). PCR amplification was carried out in a temperature-gradient thermocycler (Biometra German, Whatman, Gottingen, Germany). After reaction, the PCR product was mixed with 2 μl loading buffer [30% glycerol (*V*/*V*) stained with bromophenol blue and xylene cyanol], and detected by 2% agarose gel prestained with 0.5 μg/ml ethidium bromide. The electrophoresis parameters were: 5 V/cm for 30 min in 0.5×TBE buffer (89 mM Tris-base, 89 mM boric acid and 2 mM EDTA, pH 8.0).

Statistical analysis

The allelic frequencies were calculated with the software PopGene [20]. For each variable, we computed the odds ratio (OR) of psoriasis, 95% confidence interval (95%), χ^2 value and *P* value (two-sided).

Results

We detected the HLA-DQA1*0201 allele of each experimental subject by PCR-SSP method (Fig. 1), and found that the frequency of HLA-DQA1*0201 allele was 21.16% in the psoriasis patient group and 7.35% in the control population group, respectively (OR=4.25, $P<1.0\times10^{-6}$).The number and percentage of smokers or drinkers of the same sex in patient or control group are analyzed, and the results were shown in Table 2. Significant differences were found between the male subject of patient group and control group in terms of tobacco use (OR=2.62, *P*=0.0002) and alcohol consumption (OR=2.28, *P*=0.0024), while in females, no differences were found in alcohol consumption (*P*=0.5448) and smoking (*P*=1.0000).

Stratified analysis of smoking (ever versus never) and HLA-DQA1*0201 genotype (with and without) in male subjects was presented in Table 3. Never-smoker without

Table 1 Primers for the amplification of HLA-DQA1*0201 allele and inner-control

	PCR product size (bp)	Primer sequence	
HLA-DQA1*0201 allele	170	Forward	5′-ACGGTCCCTCTGGCCAGTT-3′
		Reverse	5′-CAGGATGTTCAAGTTATGTTTTAG-3′
Inner control	796	Forward	5′-TGCCAAGTGGAGCACCCAA-3′
		Reverse	5′-GCATCTTGCTCTGTGCAGAT-3′

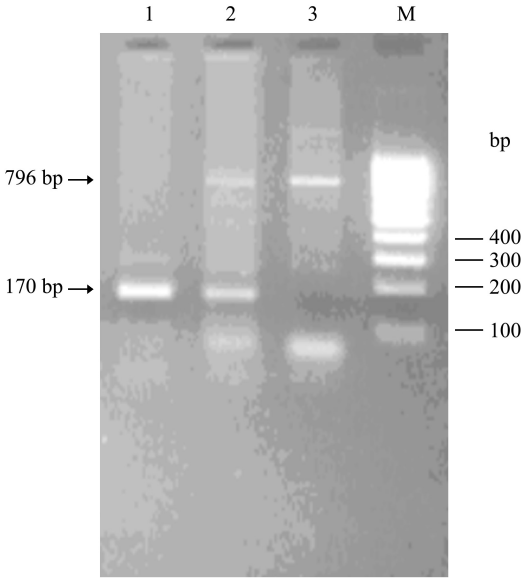

Fig. 1 Electrophoresis of the PCR product

1, standard HLA-DQA1*0201 allele; 2, sample from patient with HLA-DQA1*0201 allele (+); 3, sample from patient without HLA-DQA1*0201 allele (–); M, DNA ladder.

HLA-DQA1*0201 genotype was the referent group for comparison. We found the effect of genotype on never-smokers (OR=3.45, P=0.0009), and the higher risk of those with HLA-DQA1*0201 genotype (OR=6.91, $P<1.0\times10^{-5}$) compared with those without HLA-DQA1*0201 genotype (OR=2.74, P=0.0007) in smoking patients. Subdividing of smokers was done according to the cigarettes smoked per day or by years smoked. A relationship of smoking and HLA-DQA1*0201 genotype was found in patients who consume 1–14 cigarettes per day (OR=16.36, P=0.0001). But the risk did not increase with consumption of cigarettes (OR=4.12, P=0.0051). In patients with HLA-DQA1*0201 genotype, who smoked for over 10 years showed a higher risk (OR=13.09, $P<1.0\times10^{-6}$) than those less than 9 years (OR=1.96, P=0.4008).

Stratified analysis of alcohol consumption (ever versus never) and HLA-DQA1*0201 genotype (with and without) in male subjects was shown in Table 4. Non-drinker without HLA-DQA1*0201 genotype was the referent group for comparison. The effect of genotype alone was found

Table 2 Comparison of smoking and alcohol between the patient and control group

Group		*n*(%)		OR	95%CI	*P* value
		Case	Control			
Male	Smoking	53(50.48)	49(28.00)	2.62	1.53–4.49	0.0002
	Alcohol	39(37.14)	36(20.57)	2.28	1.28–4.06	0.0024
Female	Smoking	0(0.00)	2(1.26)	0.00	0.00–7.71	0.5448
	Alcohol	2(2.38)	3(1.90)	1.26	0.14–9.49	1.0000

n, number of individuals; OR, odds ratio; CI, confidence interval.

Table 3 HLA-DQA1*0201 allele and smoking stratified analysis in male subjects

Smoking group		DQA1*0201[a]	Case (*n*=105)	Control (*n*=175)	OR	95%CI	*P* value
Ever/never	Never	–	33	108	1[b]	–	–
	Never	+	19	18	3.45	1.52–7.86	0.0009
	Ever	–	34	40	2.74	1.46–5.31	0.0007
	Ever	+	19	9	6.91	2.65–18.43	0.0000
Cigarettes	1–14	–	21	14	4.91	2.11–11.55	0.0000
per day	1–14	+	10	2	16.36	3.12–114.33	0.0001
	>=15	–	13	26	1.64	0.70–3.78	0.2096
	>=15	+	9	7	4.12	1.31–13.78	0.0051
Years	1–9	–	16	19	2.76	1.19–6.39	0.0086
	1–9	+	3	5	1.96	0.35–10.16	0.4008
	>=10	–	18	21	2.81	1.26–6.28	0.0054
	>=10	+	16	4	13.09	3.74–50.19	0.0000

[a] –, does not carry HLA-DQA1*0201 allele; +, carries HLA-DQA1*0201 allele. [b] from reference. *n*, number of individuals; OR, odds ratio; CI, confidence interval.

Table 4 HLA-DQA1*0201 allele and alcohol stratified analysis in male subjects

Alcohol group		DQA1*0201[a]	Case (*n*=105)	Control (*n*=175)	OR	95%CI	*P* value
Ever/never	Never	–	45	118	1[b]	–	–
	Never	+	21	21	2.62	1.24–5.57	0.0057
	Ever	–	22	30	1.92	0.96–3.86	0.0468
	Ever	+	17	6	7.43	2.54–22.68	0.0000
Alcohol per day (g)	1–20	–	9	18	1.31	0.50–3.37	0.5423
	1–20	+	4	4	2.62	0.52–13.17	0.2280
	>=21	–	13	12	2.84	1.12–7.26	0.0142
	>=21	+	13	2	17.04	3.45–114.16	0.0000
Years	1–9	–	10	12	2.19	0.81–5.90	0.0864
	1–9	+	8	3	6.99	1.59–35.01	0.0036
	>=10	–	12	18	1.75	0.72–4.20	0.1726
	>=10	+	9	3	7.47	1.74–36.66	0.0019

[a] –, do not carry HLA-DQA1*0201 allele; +, carry HLA-DQA1*0201 allele. [b] from reference. *n*, number of individuals; OR, odds ratio; CI, confidence interval.

(OR=2.62, P=0.0057) among non-drinker. Alcohol drinkers with HLA-DQA1*0201 genotype were more risk (OR=7.43, $P<1.0\times10^{-4}$) compared with those without HLA-DQA1*0201 genotype (OR=1.92, P=0.0468). First, there was no association between alcohol and HLA-DQA1*0201 in patients consuming less than 20 g alcohol per day (OR=2.62, P=0.2280). Second, close relationship between alcohol and DQA1*0201 allele was seen in patients intaking over 20 g alcohol per day (OR=17.04, $P<1.0\times10^{-4}$). Third, there was a higher odds ratio in those with HLA-DQA1*0201 genotype and drinking over 10 years (OR=7.47, P=0.0019) than those drinking no more than 9 years (OR=6.99, P=0.0036).

Discussion

Many epidemiological investigations have confirmed that smoking and drinking were risk factors for psoriasis [14,15]. A detailed study from Northern Italy documented alcohol consumption was a risk factor in male patients with psoriasis [21]. Naldi *et al*. [22] reported that smoking 15 cigarettes per day was one of the risk factors that induced psoriasis. Poikolainen *et al*. [23] suggested that smoking, alcohol, and life events were related with psoriasis, especially for women. Genetic factors also played important roles in the pathogenesis of psoriasis [1]. Schmitt-Egenolf *et al.* [11] first found that HLA-DQA1*0201 allele was a susceptibility gene for psoriasis. Ikaheimo *et al.* [12] studied 64 Finnish psoriasis patients, and the results confirmed the finding of Schmitt-Egenolf.

Accumulating evidences indicate that psoriasis is a multifactorial disorder caused by the concerted action of multiple genes in a single individual, triggered by environmental factors. But there are few data exploring a link between genotype and environmental factors in psoriasis. This study gave a direct evidence of strong association between smoking, drinking and HLA-DQA1*0201 genotype in male patients with psoriasis. Moreover, primary quantitative assay was carried out in our experiment. This study demonstrated that HLA-DQA1*0201 allele was a susceptibility gene for Chinese psoriasis also. In this study, the interaction of smoking and HLA-DQA1*0201 genotype was found in male psoriasis, but the dose-response relationship was not found in men psoriasis. At the same time, it was seen the evidence of interaction between alcohol and HLA-DQA1*0201 genotype and the dose-response relationship in male patients with psoriasis.

Smoking could influence psoriasis via a variety of mechanisms. Polymorphonuclear cells are prominent in the inflammatory infiltrate of psoriasis and its morphologically and functionally were altered by smoking. Keratinocytes possess nicotinic cholinergic receptors that stimulate calcium influx and accelerate cell differentiation [14].

It is well known that psoriasis is abnormal differentiation of keratinocytes, hyerpropliferation of keratinocytes, and the infiltration of inflammatory components into the skin. Protein kinase C (PKC) isoenzymes play an important part in signal transmission regulating cellular growth, differentiation, cytokine production and adhesion molecule expression. In psoriasis, down-regulation of several PKC isoenzymes occurs. *In vitro* studies have shown that

ethanol can either inhibit or enhance PKC activity, depending on the experimental conditions [24]. An increase in the cGMP/cAMP ratio or decreased cAMP levels causes stimulation of cell activity. All these changes may lead to the proliferation of epidermal cells [25,26]. Recently, a new study shows that ethanol and acetone enhance the pro-liferation of HaCaT cells [27]. HaCaT cells are commonly used as a model system to study hyperproliferative skin diseases such as psoriasis [28,29]. The maximum increase in the number of viable cells and the maximum proliferative response was observed with 4.28 mM ethanol and 13.6 mM acetone. These results also indicate that the effects of ethanol and acetone are partly the elevation of transcripts characteristic of proliferating keratinocytes such as α5 integrin, Keratinocyte growth factor receptor (KGFR) and cyclin D1 [27]. The high α5 integrin levels in non-lesional psoriatic skin may result in the hyperresponsiveness of psoriatic keratinocytes to proliferation signals provided by lymphokines produced by T lymphocytes [30]. It is possible that the effect of ethanol and acetone on α5 integrin expression of HaCaT cells increases their proliferating capacity.

In conclusion, smoking and alcohol would have a significant effect on psoriasis. There are strong evidences of association between smoking, alcohol and HLA-DQA1*0201 allele in psoriasis. Further investigation of the mechanism for gene-environmental interaction between smoking, alcohol and HLA-DQA1*0201 is needed.

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