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Investigating the genetic association of *HCP5*, *SPATA2*, *TNIP1*, *TNFAIP3* and *COG6* with psoriasis in Chinese population

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Summary

Psoriasis is a chronic inflammatory skin disease occurred under the interaction of genetic and environmental factors. The genes HLA complex P5 (*HCP5*), spermatogenesis associated 2 (*SPATA2*), tumour necrosis factor alpha-induced protein 3 (*TNFAIP3*), TNFAIP3-interacting protein 1 (*TNIP1*) and the component of oligomeric Golgi complex 6 (*COG6*) were reported to be associated with psoriasis in western populations by genome-wide association studies. The aim of this study was to investigate whether the *HCP5*, *TNIP1*, *TNFAIP3*, *SPATA2* and *COG6* genes were genetic risk factors for psoriasis in Chinese population. One single nucleotide polymorphism (SNP) from each gene was evaluated using Chinese patients with psoriasis (n = 201) and controls (n = 300). The results demonstrated that SNPs rs2395029, rs17728338 and rs610604 from the *HCP5*, *TNIP1* and *TNFAIP3* genes, respectively, were associated with psoriasis in the studied population at both genotype level and allelic level ($P < 0.05$). Thus, the data suggested that HCP5, TNIP1 and TNFAIP3 may play a role in common pathogenic of psoriasis in Chinese and confer risk factors for psoriasis in various ethnic populations. These results provide potential makers for diagnosing, treating and preventing the psoriasis.

Introduction

Psoriasis is a chronic inflammatory and hyperproliferative skin disease affected by both genetic and environmental factors. The prevalence of psoriasis varied among populations with different genetic backgrounds and habitats, from 3% in Northern Europe (8.5% in Norway) and 2% in North America (0.91% in United States) and the UK to 0.1–0.3% in American Indians and East Asia (Yip, 1984; Gudjonsson & Elder, 2007; Parisi *et al.*, 2013). Psoriasis is also associated with other immune disease, such as arthritis and Crohn's disease, supporting psoriasis is a heterogeneous disease (Nair *et al.*, 1997).

Genome-wide association studies (GWAS) have revealed that the genes HLA complex P5 (*HCP5*), conserved oligomeric Golgi complex component 6 (*COG6*), spermatogenesis associated 2 (*SPATA2*), tumour necrosis factor alpha-induced protein 3 (*TNFAIP3*) and TNFAIP3-interacting protein 1 (*TNIP1*) were risk factors for psoriasis in western populations (Capon *et al.*, 2008; Liu *et al.*, 2008; Duffin & Krueger, 2009; Elder, 2009; Nair *et al.*, 2009). GWAS results from Chinese patients have revealed considerable heterogeneity of disease susceptibility between the Chinese and western populations (Sun *et al.*, 2010).

In this study, we aimed to determine whether psoriasis is associated with six genes that have been strongly associated with psoriasis in Europeans but not well studied in Chinese. The five included SNPs are rs2395029 in the *HCP5* gene, rs17728338 in *TNIP1*, rs610604 in *TNFAIP3* gene, rs7993214 in *COG6* and rs495337 in the *SPATA2* gene. We concluded that SNP rs2395029 in *HCP5*, rs17728338 in *TNIP1* and rs610604 in *TNFAIP3* were associated with psoriasis in Chinese population. Our results are potentially useful for diagnosing, treating and preventing the psoriasis.

Materials and methods

Study population

A total of 201 patients with psoriasis and 300 healthy controls were recruited in this study. All participants did not suffer from any other diseases and belonged to Han nationality in Yunnan Province, China. The study was performed according to the Helsinki Declaration with approval of the institutional review boards of the Affiliated Yan'an Hospital of Kunming Medical University and the Kunming Institute of Botany.

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Informed consent was obtained from each participant before inclusion in this study.

Determination of genotype

Genomic DNAs were isolated from whole blood using regular phenol/chloroform method. The SNP rs495337 in the *SPATA2* gene was genotyped by the TaqMan allelic discrimination method (Applied Biosystems, Foster City, CA, USA). For the other SNPs, genotypes were determined by PCR-RFLP methods using the forward and reverse primers (mismatch is shown in bold and underlined font) as shown in Table 1. PCR was carried out in a total volume of 20 μL containing 20 ng of genomic DNA, 1× PCR buffer, 1.5 mM of $MgCl_2$, 200 μM of each dNTP, 30 ng of each primer and 1 unit of Taq DNA polymerase (TakaRa, Dalian, Liaoning, China). Samples were denatured at 95°C for 2 min followed by 30 cycles of 94°C for 45 s, anneal temperature (Table 1) for 45 s and 72°C for 45 s and ended with a final extension for 7 min at 72°C. PCR products were digested with 4 U of appropriate restriction endonuclease, electrophoresed on 3% agarose gels and stained with ethidium bromide. The restriction endonucleases, PCR product lengths and restriction patterns are shown in Table 1.

Data analysis

Statistics analysis was performed by SPSS software version 13.0 for windows (SPSS Inc., Chicago, IL, USA). The frequencies of genotypes and alleles for all the studied loci were determined assuming co-dominant inheritance. The statistical significance of the genotype and allele frequency variables between the patients with psoriasis and control group was evaluated by chi-square test with Yates correction for small numbers. Using the chi-square test, Hardy–Weinberg equilibrium was tested for the studied five SNPs in patients with psoriasis and controls using the HWSIM program (http://krunch.med.yale.edu/hwsim/). Relative risk associated with the significant genotype was estimated by the odds ratio (OR). OR with 95% confidence intervals (95% CI) was tested using a chi-square distribution, and the null hypothesis being tested is OR = 1. P values <0.05 were considered as statistically significant.

Results

Polymorphism (minor allele frequency >1%) has been found for all studied SNPs (Table 2). Genotype frequency for the 10 alleles studied fits the Hardy–Weinberg expectations according to chi-square tests using a Monte–Carlo permutation procedure in the patient with psoriasis and control groups ($P > 0.05$). In the studied population, the results demonstrate that SNP rs2395029 in *HCP5*, rs17728338 in *TNIP1* and rs610604 in *TNFAIP3* were associated with psoriasis

Table 1. Primer sequences, PCR product lengths, restriction endonucleases and restriction patterns for the PCR-RFLP analysed single nucleotide polymorphism (SNPs)

SNP	Gene	Position of SNP in genomic sequence	Forward primer (position in genomic sequence)	Reverse primer (position in genomic sequence)[a]	Product length (bp)	Restriction endonucleases	Fragments of frequent allele genotype (bp)	Fragments of heterozygous genotype (bp)	Fragments of rare allele genotype (bp)
rs2395029	*HCP5*	31419230:G/T	AGGATCTATTACCTGTGCCT	GCACTTCTCTCCCAAAACCA	194	Bme1390I	194	62+72+134	62+72
rs17728338	*TNIP1*	150458501:G/A	GTATGTTTTGCACCTAGCACGT	CCATTCGGGAGCCTTTTGC**C**A	197	Nco I	197	23+174+194	23+174
rs610604	*TNFAIP3*	138199417:A/C	GTAAGTTAGCTTCATCCAACCT	TCAGATCATGTTGCGTGAAA**A**AGT**C**T	197	Hpy188I	197	24+173+194	24+173
rs7993214	*COG6*	40350912:C/T	AAATGTTCTGCAGCCCTGTT	AGCTAGATAGTCCCCACTTC	207	Bsp119I	24+183	24+183+207	207

[a]Mismatch is shown in bold and underlined font.

Table 2. Genotyping of six single nucleotide polymorphism (SNPs) in patients with psoriasis (n = 201) and controls (n = 300)

SNP	Gene	Population	Genotype (%)			Minor allele (%)
rs2395029	*HCP5*	–	T/T	T/G	G/G	G
		Controls	295 (98.3)	5 (1.7)	0	5 (0.8)
		Psoriasis*1	186 (92.5)	15 (7.5)	0*[a]	15 (3.7)*[b]
rs17728338	*TNIP1*	–	G/G	G/A	A/A	A
		Controls	260 (87.0)	38 (12.7)	1 (0.3)	39 (6.5)
		Psoriasis*2	150 (74.6)	46 (22.9)	5 (2.5)*[a]	56 (13.9)*[b]
rs610604	*TNFAIP3*	–	A/A	A/C	C/C	C
		Controls	242 (80.7)	58 (19.3)	0	57 (9.7)
		Psoriasis*3	181 (90.5)	20 (9.5)	0*[a]	18 (4.7)*[b]
rs495337	*SPATA2*	–	G/G	G/A	A/A	A
		Controls	117 (39.0)	148 (49.3)	35 (11.7)	218 (36.3)
		Psoriasis	85 (42.3)	94 (46.8)	22 (10.9)	138 (34.3)
rs7993214	*COG6*	–	C/C	C/T	T/T	T
		Controls	155 (51.7)	131 (43.7)	14 (4.6)	159 (26.5)
		Psoriasis	109 (54.2)	82 (40.8)	10 (5.0)	102 (25.4)

The significant differences of genotypes and alleles frequencies are shown by bold font and asterisk, and the significant P values as follow: *[1]:*[a]: $P = 0.0018$; *[b]: $P = 0.0020$; *[2]: *[a]: $P = 0.0005$; *[b]: $P = 0.0002$; *[3]: *[a]: $P = 0.0054$; *[b]: $P = 0.0078$.

in Chinese at both the genotypic level and allelic level ($P < 0.05$). Other two SNPs of the *SPATA2* and *COG6* genes were not associated with psoriasis at either genotypic or allelic level ($P > 0.05$).

No homozygous-minor-allele genotype at the SNPs rs2395029 and rs610604 was found (Table 2). At the SNP rs2395029, the odds ratio (OR) for being psoriasis was 4.76 [95% confidence interval (C.I.) 1.70–13.71] for individuals with heterozygote (T/G) genotype and was 4.61 (95% CI: 1.66–12.79) for individuals with minor allele G (Table 3). An odds ratio of 1.5 indicates that a person with the genotype is 50% more likely to be in the psoriasis group than not. An odds ratio of 0.5 indicates that a person with the genotype is 50% less likely to be in the psoriasis group than not. Therefore, the T/G polymorphism at rs2395029 in the *HCP5* gene was a risk factor of psoriasis at both genotypic and allelic level in the studied population.

As shown in Table 3, at the SNP rs17728338, the OR for being psoriasis was 2.10 (95% CI: 1.31–3.37) for individuals with heterozygote (G/A) genotype, was 8.67 (95% CI: 1.0–74.9) for individuals with genotype (A/A or G/A) and was 2.32 (95% CI: 2.316–2.324) for individuals with minor allele A. Therefore, the minor allele A at rs17728338 in the *TNIP1* gene was a risk factor of psoriasis in the studied population. At the SNP rs610604, the OR for being psoriasis was 0.46 (95% CI: 0.28–0.79) for individuals with heterozygote (A/C) genotype and was 0.4 (95% CI: 0.3–0.7) for individuals with minor allele G (Table 3). Therefore, the minor allele G at rs610604 in the *TNFAIP3* gene was a protective factor of psoriasis in the studied population.

Table 3. Association of the studied single nucleotide polymorphism (SNPs) and psoriasis traits

SNP	Genotype	Psoriasis	Controls	OR (95%CI)	Allele	Psoriasis	Controls	OR (95%CI)
rs2395029	T/T	186	295	–	–	–	–	–
	T/G	15	5	4.76 (1.70–13.71)	T	387	595	–
	G/G	0	0	–	G	15	5	4.61 (1.66–12.79)
rs17728338	G/G	150	260	–	–	–	–	–
	G/A	46	38	2.10 (1.31–3.37)	G	346	558	–
	A/A	5	1	8.67 (1.00–74.91)	A	56	39	2.32 (2.316–2.324)
rs610604	A/A	181	242	–	–	–	–	–
	A/C	20	58	0.46 (0.28–0.79)	A	382	484	–
	C/C	0	0	–	C	20	58	0.44 (0.26–0.74)
rs495337	G/G	85	117	–	–	–	–	–
	G/A	94	148	0.87 (0.59–1.27)	G	264	382	–
	A/A	22	35	0.86 (0.48–1.59)	A	138	218	0.92 (0.71–1.20)
rs7993214	C/C	109	155	–	–	–	–	–
	C/T	82	131	0.89 (0.62–1.29)	C	300	441	–
	T/T	10	14	1.02 (0.44–2.38)	T	102	159	0.94 (0.70–1.25)

Discussion

Psoriasis is a complex genetic disorder caused by the interaction of genes and environments and the interplay among different genes. Recent genetic studies indicate that the location of these genes varies considerably among populations and families. It is interesting to know whether psoriasis is associated with the genes that have been strongly associated with psoriasis in Europeans but not well studied in Chinese.

Our results showed that the *HCP5* gene was associated with psoriasis in Chinese at both genotypic and allelic level, which is in line with the findings from European studies (Capon *et al.*, 1999; Fernandes *et al.*, 2011). Our observation indicates that the *HCP5*-G allele is a risk allele for psoriasis (OR = 4.61). The HLA class I histocompatibility antigen protein P5 (HCP5) is not belonged to MHC class I gene family, but a homologue of human endogenous retroviruses HERV-L and HERV-16 (Kulski & Dawkins, 1999). Together with the allele HLA-B*5701, HCP5 was reported to be associated with low viral loads in untreated HIV patients (Fellay *et al.*, 2007). But HCP5 was susceptibility to Nevirapine-induced Stevens Johnsons Syndrome/Toxic Epidermal Necrolysis (Tse *et al.*, 2011; Borgiani *et al.*, 2014) and abacavir hypersensitivity (Sanchez-Giron *et al.*, 2011). HCV was also associated with HCV-associated hepatocellular carcinoma (Lange *et al.*, 2013). Therefore, HCP5 was associated with strong immunopathogenesis to some antiviral drugs and viral infections. As psoriasis could be triggered by varieties of immunogenic agents, it is possible that certain substances exposed to *HCP5*-C carriers in daily life could trigger a consistent immune reaction and causing excessive inflammation in skin and joints.

The *TNIP1* and *TNFAIP3* genes were associated with psoriasis in the studied population at both the genotypic level and allelic level, which confirmed the previous studies in Chinese populations (Sun *et al.*, 2010; Bowes *et al.*, 2011; Feng *et al.*, 2013; Yang *et al.*, 2013). With single SNP analysis, no association is found between the psoriasis and the *SPATA2*. However, when current data were pooled with the data we published previously (Li *et al.*, 2014), the prevalence of the homozygous of major alleles for the three SNPs from *SPATA2* (G/G at rs495337), *IL13* (C/C at rs1800925) and *IL15* gene (A/A at rs56245420) is significantly lower in patients with psoriasis (15 of 201) than the control group (49 of 300, $P < 0.001$). Thus, the combination of variations from the *SPATA2*, *IL13* and the *IL15* genes showed genetic risk contributions for psoriasis in Chinese.

Therefore, our results, together with other findings, suggest that the *HCP5*, *TNIP1* and *TNFAIP3* genes were the risk genetic factors for psoriasis in various ethnic populations, indicating their role in common pathogenic mechanism of psoriasis. As the population size in this study was relatively small, our results should be further confirmed in larger population size and populations with different genetic backgrounds.

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Conflict of interest

The authors declared that they have no conflict of interests.

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