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Letter to the Editor

Genetic variants rs2393903 at 10q21.2 and rs6010620 at 20q13.33 are associated with clinical features of atopic dermatitis in the Chinese Han population



Atopic dermatitis (AD) is a chronic inflammatory skin disorder with a doubled or tripled increasing prevalence during the past three decades in industrialized countries; 15–30% of children and 2–10% of adults are affected [1,2]. The clinical phenotype of AD is complex and the disease pathogenesis remains unclear so far [3].

Our previous GWAS provided confidential association evidence for rs7701890 within 5q22.1, rs2393903 within 10q21.2, rs6010620 within 20q13.33 [4]. In the present study, the association of these SNPs with subphenotype of AD was investigated.

Study subjects were selected from our previous AD GWAS in the Chinese Han population from hospitals located in multiple cities in central and northern China [4]. Generally, 4538 cases and 13,412 controls being available for detailed clinical information in terms of gender, age, age onset, family history, concomitant diseases (asthma and allergic rhinitis) and disease severity were used in this study. All the samples were recruited with written consent. The study was approved by the Institutional Ethical Committee of Anhui Medical University and was conducted according to the Declaration of Helsinki principles.

We compared the genotype distribution of rs7701890, rs2393903 and rs6010620 between cases and controls by using

Chi squared test on 2 × 2 contingency table (Table 1). When the AA genotype was used as the reference for rs7701890, rs2393903 and rs6010620, for SNP rs7701890 ($P_{GA} = 2.00E-07$, OR = 1.23); for SNP rs2393903 ($P_{GG+GA} = 1.21E-12$, OR = 1.40); for SNP rs6010620 ($P_{GG} = 1.83E-07$, OR = 1.40).

We also observed SNPs rs2393903 and rs6010620 showed significant protective effect on clinical types of AD (Table 2) (AD with and without asthma for SNP rs2393903 $P_{genotype} = 0.02$, $P_{Bonferroni} = 0.012$; OR = 0.73; familial and sporadic AD for SNP rs6010620 $P_{genotype} = 0.01$, $P_{Bonferroni} = 0.006$; OR = 0.60). However, SNP rs7701890 showed no either risk or protective effect on any investigated subphenotype of AD in the current study (data not shown). There was also no other statistical significant association for any other clinical features of AD in terms of patients with and without allergic rhinitis, disease severity and family history atopic disease of asthma, allergic rhinitis and AD for SNPs rs2393903 and rs6010620 ($P_{Bonferroni} > 0.05$) (Table 2).

This study might help to investigate whether there are phenotype specific genetic factors for AD, which should give us new insights into the etiology and pathogenesis of AD. Currently, few relationships between AD subphenotypes and individual risk alleles have been reported such as association of the toll-like receptor 2 A-16934T promoter polymorphism with severe AD [5], flaggrin polymorphism P478S conferring susceptibility to the development of AD and modified by IgE levels [6]. These studies implied that AD is an extremely

Table 1
Distribution of genotypes of SNP rs2393903, rs6010620 and rs7701890 in patients and controls.

SNP ID	Genotype	Cases (%)	Controls (%)	P_{value}	OR(95%CI)
rs2393903	AA	1105(25.1%)	2481(29.4%)	NA	1
	GA	2235(50.7%)	4196(49.8%)	6.09E-05	1.20(1.10–1.30)
	GG	1070(24.2%)	1750(20.8%)	2.21E-09	1.37(1.24–1.52)
	GA+GG	3305(74.9%)	5496(70.6%)	1.21E-12	1.40(1.24–1.47)
rs6010620	AA	2274(50.4%)	7155(54.4%)	NA	1
	GA	1828(40.5%)	5065(38.5%)	4.73E-04	1.14(1.06–1.22)
	GG	412(9.1%)	930(7.1%)	1.83E-07	1.40(1.23–1.58)
	GA+GG	2240(49.6%)	5995(45.6%)	2.76E-06	1.175(1.10–1.26)
rs7701890	AA	3246(71.6%)	9749(75.6%)	NA	1
	GA	1194(26.3%)	2915(22.6%)	2.00E-07	1.23(1.14–1.33)
	GG	95(2.1%)	232(1.8%)	9.30E-02	1.22(0.97–1.59)
	GA+GG	1289(28.4%)	3147(24.4%)	2.76E-06	1.18(1.14–1.33)

For rs2393903, " $P_{value} = 1.19E-08$ " for genotype using a 3 × 2 contingency table.

For rs6010620, " $P_{value} = 9.71E-08$ " for genotype using a 3 × 2 contingency table.

For rs7701890, " $P_{value} = 6.21E-07$ " for genotype using a 3 × 2 contingency table.

Table 2
Distribution of genotypes of rs2393903 and rs6010620 polymorphisms in clinical features of AD.

Phenotype	Genotype			<i>P</i> _{value} *	Combined genotypes		<i>P</i> _{combined}	OR(95%CI)
	AA (%)	GA (%)	GG (%)		AA (%)	GA+GG (%)		
rs2393903								
Family history of atopic disease								
AD positive	174(25.1%)	325(47.0%)	193(27.9%)	0.04	174(25.1%)	499(74.9%)	0.02	1.24(1.03–1.49)
AD negative	909(24.8%)	1880(51.4%)	869(23.8%)		909(24.8%)	2789(75.2%)		
Asthma positive	149(21.0%)	383(54.0%)	177(25.0%)	0.03	149(21.0%)	532(79.0%)	0.70	1.04(0.86–1.25)
Asthma negative	936(25.7%)	1822(50.0%)	885(24.3%)		936(25.7%)	2758(74.3%)		
Allergic rhinitis positive	512(24.9%)	1032(50.2%)	512(24.9%)	0.75	512(24.9%)	1544(75.1%)	0.47	1.05(0.92–1.21)
Allergic rhinitis negative	573(25.0%)	1173(51.1%)	550(23.9%)		573(25.0%)	1746(75.0%)		
Disease severity								
Mild 0–14	212(24.5%)	427(49.2%)	228(26.3%)	0.49	212(24.5%)	639(75.5%)	0.31	
Moderate 15–40	721(24.8%)	1490(51.3%)	691(23.9%)		721(24.8%)	2211(75.2%)		
Severity > 40	168(26.7%)	311(49.4%)	150(23.8%)		168(26.7%)	482(73.2%)		
AD with asthma	157(26.1%)	325(54.1%)	119(19.8%)	0.02	157(26.1%)	482(73.9%)	0.004	0.73(0.59–0.91)
AD without asthma	924(24.7%)	1874(50.1%)	941(25.2%)		924(24.7%)	2798(75.3%)		
AD with allergic rhinitis	337(23.5%)	746(52.1%)	349(24.4%)	0.29	337(23.5%)	1083(76.5%)	0.93	0.99(0.86–1.15)
AD without allergic rhinitis	743(25.6%)	1452(49.9%)	712(24.5%)		743(25.6%)	2195(74.4%)		
rs6010620								
Family history of atopic disease								
AD positive	368(51.9%)	298(42.0%)	43(6.1%)	0.01	368(51.9%)	666(48.1%)	0.002	0.60(0.43–0.83)
AD negative	1874(50.0%)	1508(40.3%)	363(9.7%)		1874(50.0%)	3382(50.0%)		
Asthma positive	357(50.0%)	288(40.3%)	69(9.7%)	0.87	357(50.0%)	645(50.0%)	0.59	1.08(0.82–1.41)
Asthma negative	1886(50.4%)	1518(40.6%)	338(9.0%)		1886(50.4%)	3404(49.6%)		
Allergic rhinitis positive	1040(49.4%)	863(40.9%)	204(9.7%)	0.35	1040(49.4%)	1903(50.6%)	0.25	1.13(0.92–1.38)
Allergic rhinitis negative	1202(51.2%)	943(40.1%)	204(8.7%)		1202(51.2%)	2145(48.8%)		
Disease severity								
Mild 0–14	452(51.1%)	347(39.3%)	85(9.6%)	0.91	452(51.1%)	799(48.9%)	0.81	
Moderate 15–40	1495(50.3%)	1207(40.6%)	270(9.1%)		1495(50.3%)	2702(49.7%)		
Severity > 40	323(49.9%)	268(41.4%)	56(8.7%)		323(49.9%)	591(50.1%)		
AD with asthma	303(49.0%)	267(43.1%)	49(7.9%)	0.26	303(49.0%)	570(51.0%)	0.24	0.83(0.61–1.13)
AD without asthma	1932(50.5%)	1533(40.1%)	359(9.4%)		1932(50.5%)	3465(49.5%)		
AD with allergic rhinitis	728(49.7%)	600(40.9%)	138(9.4%)	0.82	728(49.7%)	1328(50.3%)	0.71	1.04(0.84–1.29)
AD without allergic rhinitis	1506(50.6%)	1200(40.3%)	270(9.1%)		1506(50.6%)	2706(49.4%)		

Bold values indicate $P_{\text{value}} < 0.05/2 = 0.025$.

* P_{value} for genotype using a 3×2 contingency table.

heterogeneous disease and will advance our understanding on the etiology of AD.

In the current study, we found that 10q21.2 (rs2393903 is located in intron of *ZNF365*) and 20q13.33 (rs6010620 is located in intron of *RTEL1*) showed protective effect for AD without asthma and sporadic AD, respectively. At 10q21.2, the association signal refers to a single gene *ZNF365*, which encodes the zinc finger protein 365. Disruption of normal *ZNF365* function causes mitotic failure, possibly because of centrosome defects or incomplete cytokinesis. *ZNF365* has also been associated with Crohn's disease [7]. Thus, our results strengthen the hypothesis of joint barrier disease genes and further functional study on *ZNF365* will help us to explore its potential role in the pathogenesis of AD without asthma.

At 20q13.33, multiple genes were implicated. Expression quantitative trait loci analysis suggested that the risk-associated SNP rs6010620 might be associated with *TNFRSF6B* and *ZGPAT* expression ($P = 0.024$ and $P = 0.039$, respectively) [4]. *TNFRSF6B* has a critical role in adaptive immune responses, which acts on T-cell, dendritic cell and macrophage responses. *ZGPAT* is involved in the epidermal growth factor receptor (*EGFR*) pathway [8]. *EGFR* is overexpressed in lesional skin of individuals with AD, and blocking *EGFR* induces dysregulated chemokine expression in keratinocytes and leads to enhanced skin inflammation [9]. Taken together, these results indicate that *TNFRSF6B* and *ZGPAT* are plausible candidate genes for AD and therefore predispose to sporadic AD. However, further studies will be required to investigate their underlying role in the development of sporadic AD.

In summary, our study not only implied that AD is an extremely heterogeneous disease with various features, but also indicated that 10q21.2 and 20q13.33 showed protective effect on AD without asthma and sporadic AD. It further suggested that these two loci might contribute to different subphenotypes of AD and showed that complex genetic factors are involved in the disease mechanisms. Therefore, understanding the relationships between AD genotype and phenotype of the disease might help to further elucidate the pathogenesis of AD and seek better clinical evaluation and therapy.

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Letter to the Editor

Reagents inducing epidermal proliferation also induce pigmentation: Induction of keratinocyte proliferation as a novel strategy for the treatment of vitiligo*



To the Editor,

Vitiligo is an acquired disorder characterized by depigmentation of the skin and hair. Histochemical studies have shown a lack of dopa-positive melanocytes in the basal layer of the epidermis in this condition. Electron microscopic studies have confirmed the loss of melanocytes in vitiligo skin. Moreover, the epidermis of the areas around the margins of vitiligo lesions shows keratinocyte abnormalities and degenerating melanocytes [1]. Our previous study demonstrated that the epidermis of vitiligo lesions is thin and flat, and rete ridge recovery and keratinocyte proliferation occurred at sites of repigmentation (Wu et al., submitted). We hypothesized that epidermal keratinocytes could be the target of vitiligo treatment, and that reagents that induce keratinocyte proliferation may also induce repigmentation.

Since the above-mentioned results also indicate that epidermal keratinocyte proliferation is accompanied by pigmentation, we attempted to induce skin pigmentation in guinea pigs by topical application of various pharmaceutical agents that can induce epidermal keratinocyte proliferation. We applied these reagents once a day for 28 days on the back of the animals. After 28 days, the skin was biopsied and examined histopathologically with hematoxylin and eosin (H&E) and Fontana-Masson staining. As shown in Fig. 1A and B, some of the topically applied reagents induced skin pigmentation and epidermal acanthosis. Representative data of tretinoin tocoferil are shown in Fig. 2. Tretinoin tocoferil-treated skin showed marked pigmentation (Fig. 2A) and epidermal acanthosis with slight lymphoid cell infiltration (Fig. 2B) compared with the control (Fig. 2C). Fontana-Masson staining showed that the skin pigmentation induced by tretinoin tocoferil is in fact due to increased melanin pigments (Fig. 2D) compared with the control

(Fig. 2E). A very strong correlation was found between epidermal acanthosis and skin pigmentation (Fig. 1C, R2: 0.80).

Tretinoin tocoferil ointment was applied on human vitiligo skin, for the patients being treated with narrowband-ultraviolet (UV)B or steroid ointment, and the additive effect was evaluated. Fifteen vitiligo patients were enrolled after informed consent, and tretinoin tocoferil ointment was applied twice daily for 12 weeks. Ten patients showed more than “slightly effective”, which showed repigmentation in 25–50% of the lesion, and 3 patients showed “very effective”, which showed repigmentation in more than 75% of the lesion and needed no further treatment (Kikuchi K, personal communication).

These results suggest that induction of epidermal thickness induced skin pigmentation.

Although the effect of cytokines produced by keratinocytes in our setting needs further clarification, we examined the relation between keratinocyte proliferation and pigmentation by topically applying pharmaceutical reagents that can induce epidermal keratinocyte proliferation in guinea pig skin. Thereby, we succeeded in inducing skin pigmentation in the animals. Skin pigmentation was due to melanin deposition, as confirmed by Fontana-Masson staining. It is possible that activated keratinocytes caused activation of melanocytes, resulting in pigmentation. It is also possible that these pharmacological reagents worked directly on melanocytes. However, the common nature of these compounds is that they induce keratinocyte proliferation, and our study demonstrated that the proliferation-inducing potential correlated to the degree of pigmentation. Thus, this study suggests a novel strategy for treating vitiligo by stimulating keratinocytes. Interestingly, tacrolimus, which is reported to be effective for vitiligo [2], induced not only skin pigmentation but also epidermal acanthosis.

Although the precise mechanisms for these processes are yet to be revealed, and our experiment strategy is limited and mainly done in guinea pig normal skin, this study suggests that epidermal keratinocytes could be a novel target for the treatment of vitiligo patients.

Male Weiser-Maples guinea pigs (Kwl:WM, 5 weeks old) were used in this study. The animals were maintained under a specific

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