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Raja Hussain Ali, Sabba Mahmood
Syed Irfan Raza
Abdul Aziz
Irfanullah
Syed Kamran-ul-Hassan Naqvi
Naveed Wasif
Muhammad Ansar
Wasim Ahmad*
Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan

Sayed Hajan Shah
Center for Human Genetics and Molecular Medicine, Sindh Institute of Urology and Transplantation (SIUT), Karachi, Pakistan

Bakht Tarin Khan[a,b]
[a]Department of Zoology, University of Peshawar, KPK, Pakistan,
[b]Department of Zoology, Abdul Wali Khan University, Mardan, KPK, Pakistan

Qaiser Zaman
Department of Zoology, University of Peshawar, KPK, Pakistan

Ajab Gul, Abdul Wali
Department of Biotechnology and Informatics, BUITEMS, Quetta 87100, Pakistan

Ghazanfar Ali
Department of Biotechnology, Azad Jammu & Kashmir, Pakistan

Saadulah Khan
Department of Biotechnology & Genetic Engineering, Kohat University of Science & Technology, Kohat, Khyber Pakhtunkhwa, Pakistan

Muhammad Khisroon
Department of Zoology, University of Peshawar, KPK, Pakistan

Sulman Basit
Center for Genetics and Inherited Diseases, Taibah University Almadinah Almunawarah, Saudi Arabia

* Corresponding author. Fax: +92 51 90643003.
E-mail address: wahmad@qau.edu.pk (W. Ahmad).

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Letter to the Editor

Association analysis of allergic sensitization susceptibility loci with atopic dermatitis in Chinese population

Keywords
Atopic dermatitis
Allergic sensitization
Susceptibility

Atopic dermatitis is a chronically recurrent disorder involving disturbed skin barrier functions with inflammatory hypersensitivity. It was often accompanied with other atopic manifestations, elevated serum immunoglobulin E [1]. The prevalence was about 10–20% in children and 1–3% in adults [2]. The etiology has not been fully elucidated which combined with genetic and environmental factors. Previous genetic studies had identified amount of susceptibility genes/loci that associated with AD [3]. However, these findings did not fully explain the risk of AD, suggesting additional genetic factors remain need to be discovered. Recent meta-analysis had established several susceptibility loci for allergic sensitization [4]. Due to the similar features between allergic sensitization and AD, they may share common genetic components in the etiology of these two diseases. In order to identify the overlapping susceptibility loci and enhance understanding their relationship, we performed the association analysis of allergic sensitization related loci with AD in Chinese population.

A total of 2205 cases (1359 men and 846 women, mean age of 4.10 ± 1.41) and 2208 controls (1162 men and 1046 women, mean age of 25.01 ± 15.23) were enrolled. The diagnosis of AD according to standard criteria [5], demographic and clinical information, such as accompanying symptoms, serum IgE level, age of onset, SCORAD and family history were collected. The study was approved by the Ethical Committee and was conducted according to Declaration of Helsinki principles. We selected 8 SNPs (Table 1) in non-HLA region which reached the genome-wide significance threshold of $P \leq 5 \times 10^{-8}$ [4], and genotyped by Sequenom Mass Array system. P-values, ORs and 95% CIs were estimated using PLINK 1.07 software. The level of associated significance was assigned at $P < 0.006$ (0.05/8) after Bonferroni correction. The genetic statistical power for SNPs was estimated using CaTS-Power Calculator.

We only found rs10056340 ($P = 0.003$, OR = 1.19) and rs2155219 ($P = 0.004$, OR = 0.88) significantly associated with AD (Table 1). For rs10056340, risk allele G was the minor allele in Han population, which was higher in AD than controls (16.6% vs. 14.4%). Compared to the additive model ($P = 0.006$, OR = 1.12), the dominant model provided best fit for rs10056340 associated with AD in genetic model analysis ($P = 0.002$, OR = 1.24) (Table A.1). OR of the G allele for rs2155219 was 0.88 (95% CI: 0.81–0.96), which suggested a protective effect relative to the T allele with regards to susceptibility to AD. Genetic model analysis showed

Abbreviations: AD, atopic dermatitis; GWAS, genome-wide association study; OR, odds ratio; CI, confidence interval; SCORAD, scoring of atopic dermatitis; C11orf30, chromosome 11 open reading frame 30; SLC25A46, solute carrier family 25 member 46.

Table 1
The results of 8 SNPs replicated in Han Chinese AD cases and controls.

SNP	Chr	Alleles	Nearest gene	MAF		P value	OR (95% CI)	Statistical power
				Controls	Case			
rs10056340	5q22.1	G/T	*SLC25A46*	0.144	0.166	3.00E-03	1.188 (1.058-1.335)	89%
rs2155219	11q13.5	G/T	*C11orf30*	0.44	0.409	4.00E-03	0.884 (0.812-0.962)	79%
rs17454584	4q27	G/A	*IL2/ADAD1*	0.12	0.131	1.08E-01	1.110 (0.978-1.260)	27%
rs9865818	3q28	G/A	*LPP*	0.297	0.311	1.56E-01	1.068 (0.975-1.170)	17%
rs4410871	8q24.21	T/C	*MYC/PVT1*	0.343	0.356	2.29E-01	1.055 (0.967-1.152)	11%
rs1059513	12q13.3	G/A	*STAT6*	0.074	0.07	4.67E-01	0.941 (0.800-1.108)	3%
rs17616434	4p14	T/C	*TLR1/6/10*	0.353	0.359	6.14E-01	1.023 (0.937-1.116)	2%
rs3771175	2q12.1	A/T	*IL1RL1/IL18R1*	0.086	0.087	9.61E-01	0.996 (0.859-1.156)	1%

rs2155219 was suited to the additive model ($P = 0.004$) compared to the recessive and dominant model (Table A.1) in AD. When TT genotype was used as reference, the combined genotype GG + GT were associated with a higher risk of AD (Table A.1). The other 6 SNPs did not reached the statistical significance, which might result from low statistical power (1 ~ 27%) (Table 1).

We also performed a stratified analysis to determine which subtype of AD was associated with these two SNPs. For rs10056340, there was no significant results for each of the subtype of AD in case-only analysis, except for the genotype test showed a marginally association with age onset ($P = 0.057$) (Table A.2). The risk allele of rs10056340 SNP was significantly associated with early age onset of disease, high SCORAD and with xeroderma syndrome (Table A.2). However, as for the rs2155219, there was a potential difference between the high and low IgE level in the case only analysis ($P = 0.035$) (Table 2). Besides, SNP rs2155219 was significantly associated with high and low level of IgE phenotypes in sub-phenotype-control analyses.

The main effort is to reveal shared susceptibility loci between these two diseases in Chinese population, an alternative approach is to take susceptibility loci of closely related physiological mechanism phenotype to replicate in AD. Through this method, we confirmed that rs2155219 at 11q13.5 and rs10056340 at 5q22.1 were the overlapping susceptibility loci in AD and allergic sensitization. Both of these two SNPs were not contained in our GWAS data of AD. Although the 11q13.5 locus has been identified in allergic sensitization and atopic disease [6,7], it was first confirmed in Chinese population. In previous study [8], we identified SNP rs7936562 at 11q13.5 (Pcombined = 2.98×10^{-4}, OR = 0.91) only with suggestive evidence. The SNP rs2155219 ($P = 0.004$ after correction) showed moderate correlated with rs7936562 ($D' = 0.86$, $r^2 = 0.58$), which further support the role of 11q13.5 in AD. In 5q22.1 region, although SNP rs10056340 was only weakly correlated with the 4 SNPs in Chinese population ($D' = 0.69$, $r^2 = 0.30$ for rs7701890; $D' = 0.70$, $r^2 = 0.28$ for rs10067777; $D' = 0.73$, $r^2 = 0.34$ for rs13360927; $D' = 0.73$, $r^2 = 0.34$ for rs13361382 based

Table 2
Associations between rs2155219 and AD in subphenotype control- and case-only analyses.

	Genotype			$P_{genotype}$ value	Allele		$P_{subgroup}$ vs controls	Combined genotypes		$P_{combined}$ value	OR (95% CI)
	TT	GT	GG		T	G		TT	GG+GT		
Age at onset											
≤1 years	655(34.6)	933(49.2)	307(16.2)	4.22E-01	2243(59.2)	1547(40.8)	5.00E-03	655(34.6)	1240(65.4)	6.70E-01	0.95(0.73-1.23)
>1 years	104(35.9)	132(45.5)	54(18.6)		340(58.6)	240(41.4)	2.50E-01	104(35.9)	186(64.1)		
AD with diseases											
Yes	323(36.0)	426(47.4)	149(16.6)	5.98E-01	1072(59.7)	724(40.3)	8.00E-03	323(36.0)	575(64.0)	3.70E-01	1.09(0.98-1.30)
No	423(34.1)	615(49.6)	203(16.4)		1461(58.9)	1021(41.1)	2.50E-02	423(34.1)	818(65.9)		
IgE level											
High	115(40.1)	137(47.7)	35(12.2)	3.50E-02	367(63.9)	207(36.1)	1.00E-04	115(40.1)	172(59.9)	4.00E-02	1.31(1.01-1.68)
Low	635(33.9)	915(48.8)	324(17.3)		2185(58.3)	1563(41.7)	3.00E-02	635(33.9)	1239(66.1)		
AD with xeroderma											
Yes	550(34.3)	780(48.7)	273(17.0)	7.20E-01	1880(58.2)	1348(41.8)	2.60E-02	550(34.3)	1053(65.7)	5.70E-01	0.94(0.77-1.16)
No	189(35.7)	258(48.7)	83(15.7)		636(60.0)	424(40.0)	1.00E-02	189(35.7)	341(64.3)		
SCORAD											
<25	149(32.8)	224(49.3)	81(17.8)	2.70E-01	522(57.5)	386(42.5)	5.80E-01	149(32.8)	305(67.2)	3.00E-01	0.89(0.71-1.11)
≥25	581(35.4)	794(48.4)	264(16.1)		1956(59.7)	1322(40.3)	1.60E-03	581(35.4)	1058(64.6)		
Familial history											
Positive	87(32.8)	128(48.3)	50(18.9)	4.67E-01	302(57.0)	228(43.0)	6.40E-01	87(32.8)	178(67.2)	6.20E-01	0.93(0.71-1.23)
Negative	672(34.4)	973(49.7)	311(15.9)		2317(59.2)	1595(40.8)	2.40E-03	672(34.4)	1284(65.6)		
AD with Keratosis pilaris											
Yes	111(38.7)	133(46.3)	43(15.0)	2.95E-01	355(61.8)	219(38.2)	7.30E-03	111(38.7)	176(61.3)	1.30E-01	1.22(0.94-1.58)
No	620(34.1)	890(48.9)	309(17.0)		2130(58.5)	1508(41.5)	2.60E-02	620(34.1)	1199(65.9)		
AD with ichthyosis											
Yes	97(30.7)	164(51.9)	55(17.4)	2.57E-01	358(56.6)	274(43.4)	7.50E-01	97(30.7)	219(69.3)	1.00E-01	0.81(0.62-1.04)
No	635(35.5)	859(48.0)	297(16.6)		2129(59.4)	1453(40.6)	2.60E-03	635(35.5)	1156(64.5)		
AD with Palm disease											
Yes	171(38.4)	204(45.8)	70(15.7)	1.84E-01	546(61.3)	344(38.7)	3.30E-03	171(38.4)	274(61.6)	6.60E-02	1.22(0.99-1.52)
No	561(33.8)	819(49.3)	282(17.0)		1941(58.4)	1383(41.6)	4.30E-02	561(33.8)	1101(66.2)		

HapMap data in CHB), we could not be sure whether this SNP represented independent risk variant associated with AD in current findings. Bioinformatics analysis of rs10056340 by HaploReg v3 showed that this SNP was located within a strong enhancer in epidermal keratinocytes. Continue in-depth studies are warranted to investigate whether there are multiple independent risk variants within 5q22.1 region.

For rs2155219, located at 35 kb 3′ of *C11orf30* gene which regulated chromatin states in endothelial cells, B-lymphocyte, lung fibroblasts and encoded the EMSY protein, bind the epithelium-derived cancer [9]. The potential involvement of *C11orf30* in multiple inflammatory and malignant epithelial diseases strongly suggests its role in epithelial immunity, growth or differentiation. SNP rs10056340, located at 89 kb of *SLC25A46* gene at 5q22.1, regulated chromatin states in epithelial cells and epidermal keratinocytes, and encoded mitochondrial carrier proteins [10]. Genotype–phenotype analysis help to clarify whether these 2 loci were associated with specific disease sub-phenotypes. Our findings only indicated that the level of IgE might be related to rs2155219 at 11q13.5, and its biological implications might be involved in generating different AD phenotypes. In conclusion, the 11q13.5 and 5q22.1 have pleiotropic effects of importance in the development of allergic related diseases, which shared between these two disease in Han Chinese population. Further fine mapping and gene functional studies are warranted to identify and characterize the causal gene(s) within these loci.

Conflict of interest

The authors declare no conflict of interests.

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URLs. CaTS-Power Calculator, <http://csg.sph.umich.edu//abecasis/CaTS/>.

HaploReg v3, <http://www.broadinstitute.org/mammals/haploreg/haploreg_v3.php>.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdermsci.2015.09.009.

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Jinping Gao[a,b,1]
[a]Institute of Dermatology and Department of Dermatology, No. 1 Hospital, Anhui Medical University, 81 Meishan Road, Hefei, Anhui 230032, China, [b]State Key Lab Incubation of Dermatology, Ministry of Science and Technology, Hefei, Anhui 230032, China

Yuemei Ma[1]
Department of Allergy and Department of Dermatology, The Second Affiliated Hospital of Harbin Medical University, 246 Xuefu Road, Harbin, Heilongjiang 150086, China

Yujun Sheng[a,b,1]
[a]Institute of Dermatology and Department of Dermatology, No. 1 Hospital, Anhui Medical University, 81 Meishan Road, Hefei, Anhui 230032, China, [b]State Key Lab Incubation of Dermatology, Ministry of Science and Technology, Hefei, Anhui 230032, China

Xianbo Zuo[a,b]
[a]Institute of Dermatology and Department of Dermatology, No. 1 Hospital, Anhui Medical University, 81 Meishan Road, Hefei, Anhui 230032, China, [b]State Key Lab Incubation of Dermatology, Ministry of Science and Technology, Hefei, Anhui 230032, China

Wenjun Wang[a,b]
[a]Institute of Dermatology and Department of Dermatology, No. 1 Hospital, Anhui Medical University, 81 Meishan Road, Hefei, Anhui 230032, China, [b]State Key Lab Incubation of Dermatology, Ministry of Science and Technology, Hefei, Anhui 230032, China

Xiaodong Zheng[a,b]
[a]Institute of Dermatology and Department of Dermatology, No. 1 Hospital, Anhui Medical University, 81 Meishan Road, Hefei, Anhui 230032, China, [b]State Key Lab Incubation of Dermatology, Ministry of Science and Technology, Hefei, Anhui 230032, China

Huayang Tang[a,b]
[a]Institute of Dermatology and Department of Dermatology, No. 1 Hospital, Anhui Medical University, 81 Meishan Road, Hefei, Anhui 230032, China, [b]State Key Lab Incubation of Dermatology, Ministry of Science and Technology, Hefei, Anhui 230032, China

Xianfa Tang[a,b]
[a]Institute of Dermatology and Department of Dermatology, No. 1 Hospital, Anhui Medical University, 81 Meishan Road, Hefei, Anhui 230032, China, [b]State Key Lab Incubation of Dermatology, Ministry of Science and Technology, Hefei, Anhui 230032, China

Fusheng Zhou[a,b]
[a]Institute of Dermatology and Department of Dermatology, No. 1 Hospital, Anhui Medical University, 81 Meishan Road, Hefei, Anhui 230032, China, [b]State Key Lab Incubation of Dermatology, Ministry of Science and Technology, Hefei, Anhui 230032, China

Sen Yang[a,b]
[a]Institute of Dermatology and Department of Dermatology, No. 1 Hospital, Anhui Medical University, 81 Meishan Road, Hefei, Anhui 230032, China, [b]State Key Lab Incubation of Dermatology, Ministry of Science and Technology, Hefei, Anhui 230032, China

Xuejun Zhang[a,b]
[a]Institute of Dermatology and Department of Dermatology, No. 1 Hospital, Anhui Medical University, 81 Meishan Road, Hefei, Anhui 230032, China, [b]State Key Lab Incubation of Dermatology, Ministry of Science and Technology, Hefei, Anhui 230032, China

Liangdan Sun[a,b,*]
[a]Institute of Dermatology and Department of Dermatology, No. 1 Hospital, Anhui Medical University, 81 Meishan Road, Hefei, Anhui 230032, China, [b]State Key Lab Incubation of Dermatology, Ministry of Science and Technology, Hefei, Anhui 230032, China
[1]These authors contributed equally to this study.

* Corresponding author at: Institute of Dermatology & Department of Dermatology, the First Affiliated Hospital in Anhui Medical University, Hefei 230032, China.
E-mail address: ahmusld@163.com (L. Sun).

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Letter to the Editor

Japanese recurrent mutation c.6216 + 5G > T in *COL7A1* leads to a mild phenotype of dystrophic epidermolysis bullosa

Keywords
Recurrent mutation
Splice site mutation
Type VII collagen
Skin blistering
Genodermatosis

Dystrophic epidermolysis bullosa (DEB) is a group of inherited skin-blistering diseases [1], and both dominant and recessive inheritance patterns are known. Both patterns are caused by mutations in *COL7A1*, encoding type VII collagen. Although *COL7A1* mutations show very high diversity and most are known to be family-specific, some mutations are reported to be recurrent mutations [2], including global recurrent mutations and ethnicity-specific recurrent mutations [3]. To date, three Japanese-specific recurrent mutations have been reported, which lead to a premature termination codon (PTC) and result in recessive DEB [4]. Here, we identified a Japanese recurrent mutation leading to a mild phenotype of recessive DEB.

Enrolled in this study were 35 Japanese probands (70 alleles) diagnosed with recessive DEB, referred to the Department of Dermatology, Keio University Hospital and subjected to mutational searches from 1995 to 2013. This study was approved by the institutional review board in accordance with the Helsinki guidelines. Mutational searches for *COL7A1* were undertaken using genomic DNA isolated from peripheral blood, as previously described [5]. We found that seven probands (20%) from non-consanguineous parents in 35 Japanese probands possessed the c.6216 + 5G > T (IVS74 + 5G > T) mutation in intron 74 (Fig. 1A), which was first reported in Japanese recessive DEB patient by Kon et al. [6]. The frequency of the mutation was comparable to those of previously reported Japanese recurrent mutations [4] in our cohort (data not shown).

To confirm whether the recurrence of c.6216 + 5G > T mutation was due to a founder effect, we performed haplotype analysis of *COL7A1* using six intragenic polymorphisms as markers. As a result, all mutant alleles were found to harbor the same six intragenic markers (Fig. 1A). Among these markers, c.4613G > A substitution (rs2229824) was rare in Japanese, detected in only two alleles among the 1552 alleles (0.13%) in the Kyoto Human Gene Variation Database (HGVD, http://www.genome.med.kyoto-u.ac.jp/SnpDB/). The frequency of c.7984-13delT (rs397989501, identical to c.7984-7delT [rs66737445]) was 7.14% in the Kyoto HGVD. In this database, the c.6216 + 5G > T mutation was not identified. These results suggested that the c.6216 + 5G > T mutant alleles were derived from a common ancestor and the mutation probably occurred on the rare c.4613G > A allele and spread in the Japanese population.

To disclose the consequences of the c.6216 + 5G > T mutation on splices, reverse transcription-PCR (RT-PCR) was performed, as previously reported [7], using total RNA extracted from the skin of a proband (Family 4) with c.6216 + 5G > T homozygote. The reverse-transcribed cDNA of exon 72–78 (502 bp) was amplified with 38 cycles of PCR, 63 °C of annealing temperature, and the following primers: 5′-ctctggtagcttcctgcctg-3′ and 5′-cacaccctgtctcctttgg-3′.

Three major transcripts (404, 502, and 582 bp) were identified (Fig. 2A). Direct sequencing of the respective bands revealed the following consequences (Fig. 2E). First, the upper band contained a 582 bp-transcript generated by retention of the entirety of intron 74 (80 bp), resulting in a frameshift and pre-termination within exon 82 (Fig. 2B). Second, the middle band contained the wild-type 502 bp-transcript. The sequence of this band included small contamination peaks following exon 73, identical to the sequence of exon 75 (Fig. 2C). This band may thus contain a small amount of a 466 bp-transcript generated by skipping the entire exon 74 (36 bp), resulting in an inframe deletion. Third, the lower band contained a 404 bp-transcript generated by deletion of the last 62 bp of exon 73 and the entire exon 74, inducing a frameshift and pre-termination within exon 76 (Fig. 2D).

The c.6216 + 5G > T nucleotide substitution was not located in the splicing consensus AG-GT sequence, but at the +5 position, where G is reportedly present in 75–86% [8]. Hamada et al. previously revealed that c.6216 + 5G > T mutation generated normal transcript and mutant transcript including intron 74 [9]. Our RT-PCR experiments using c.6216 + 5G > T homozygous proband, in whom all *COL7A1* mRNA was transcribed from c.6216 + 5G > T mutant allele, clearly showed that this mutation induced plural aberrant splicing transcripts in addition to the normal transcript.

Not only three homozygous probands for the c.6216 + 5G > T mutation but also four compound heterozygous probands with PTC generating mutation showed a mild clinical phenotype. Careful examination showed no skin symptoms in their parents. Immunofluorescence studies for type VII collagen were performed in four cases, showing normal or slightly reduced expression in the epidermal basement membrane zone (Fig. 1B), similar to Hamada's case [9]. As mentioned above, the phenotype of patients with the c.6216 + 5G > T leaky splice site mutation was relatively mild. Our result suggested that the c.6216 + 5G > T mutation exhibits mild pathogenic effects in DEB although the phenotype also depends on the nature of the second mutation alleles. The first case of c.6216 + 5G > T mutation was a severe recessive DEB of compound heterozygote with c.427 − 3C > G (IVS3 − 3C > G) mutation [6].