Functional Regulation of Interleukin-31 Production by its Genetic Polymorphism in Patients with Extrinsic Atopic Dermatitis

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Atopic dermatitis (AD) is a common pruritic skin disease that greatly impairs quality of life (1). The pathophysiology of pruritus in AD is complex, but may involve several proinflammatory cytokines. One of these cytokines, interleukin-31 (IL-31), which is mainly expressed by Th2 cells, signals through a heterodimeric receptor composed of IL-31 receptor A (IL-31RA) and oncostatin M receptor on cutaneous keratinocytes (2). In mice, transgenic overexpression of IL-31 induces severe pruritic dermatitis (2). IL-31 gene transcript is also increased in NC/Nga mice with atopic-like dermatitis (3). In humans, it is increased in pruritic AD skin, but not in non-pruritic psoriatic skin (4). At the protein level, IL-31RA expression is increased on the keratinocytes of AD skin (5). Moreover, Raap et al. (6) reported an association between serum IL-31 level and AD severity.

Schulz et al. (7) first sequencing the IL-31 gene from 690 German families, found 15 main single-nucleotide polymorphisms (SNPs). They reported those having haplotype AA to be at risk of developing intrinsic AD (without allergen-specific immunoglobulin E (IgE)), but not extrinsic AD. Moreover, peripheral blood mononuclear cells (PBMCs) from healthy individuals stimulated with anti-CD3 and anti-CD28 showed 3.8 times greater expression of IL-31 mRNA in individuals with AA than in non-A haplotype carriers. Nevertheless, how IL-31 SNPs are associated with blood IL-31 level or disease severity in AD, particularly in extrinsic AD, is not known.

MATERIALS AND METHODS

We measured blood IL-31, accessed clinical severity, determined IL-31 SNPs, and conducted an *in vitro* transactivation assay in patients with extrinsic AD and controls. From 2008 to 2009, we recruited 105 patients with extrinsic AD defined by serum level IgE higher than 100 IU/ml) (69 males and 36 females, age 52.2 ± 22.4 years, range 1–71 years) and 210 controls without any atopic diseases (115 males and 95 females, age 55.2 ± 18.4 years, range 5–69 years) from the Department of Dermatology based on Hanifin's AD diagnostic criteria (8) and from Department of Health Examination in a medical referral centre. PBMCs were separated for DNA extraction (Gentra Puregene blood kit, Valencia, CA, USA) and sera were stored at –70ºC until measured for IL-31 with a commercial enzyme-linked immunoassay (ELISA) kit according to manufacturer's directions with detection limit set around 50 pg/ml (R&D, Minneapolis, MN, USA). As expected, the extrinsic AD patients had increased serum IgE (mean 497.5 IU/ml) and SCORAD (SCORing Atopic Dermatitis) severity index (45.3). Serum IL-31 was significantly higher in AD patients than in controls (979 pg/ml vs. 212 pg/ml). We also found significant associations between IgE and IL-31, as well as between SCORAD index and IL-31 (Spearman's association, $p = 0.014$ and $p = 0.018$, respectively).

Two IL-31 SNPs were selected for association analysis (Table I). We first selected rs10847385 (IL-31-631 T>G) from the Han Chinese population in the HapMap database. This SNP has a minor allele frequency (MAF) ≥ 0.1, $r^2 \geq 0.8$. We then selected rs7974857 (IL-31 c. 183 C>G G61G). According to Schultz et al., this IL-31 SNP is significant within exon, and has a MAF of ≥ 0.1. Using real-time PCR, we designed FAM- and VIC-labelled probes of IL-31to detect the SNPs: dbSNP:rs7974857 (forward CACGGCAGCGTGTAATTCTG and backward CACTGACCATTTCTCCTCATTCCA) and IL-31 dbSNP:rs10847385 (forward TCAGGGCCCAGAACCAGAGACTGTG and backward TCCAGAGTTATTGCTTGAAATCCTT). All samples were genotyped by TaqMan Allelic Discrimination Assay (Applied Biosystems, Carlsbad, CA, USA) in 96-well microplates by ABI9700 Thermal Cycler and analysed by the System SDS software version 1.2.3.

The promoters of rs10847385 alleles (A/C) were generated and subcloned into pGL3-Basic vector (Invitrogen, Carlsbad, CA, USA) at the Xho and Hind sites. The forward and reverse primers were: 5′-CTCGAGGATATGAACAAGGATTTCAAG-3′ and 5′-AAGCTTTTCTGGAGCCAGATGTGTT-3′, respectively. The constructs with luciferase reporters were transfected by Lipofectamine 2000 (Invitrogen) into Jurkat cells. The luciferase activity in cell lysate was measured (Promega, Madison, WI, USA) in triplicate.

Table I. *There were no associations between genotypic and allelic polymorphisms of IL-31 and the occurrence of extrinsic atopic dermatitis (AD)*

	Genotypic polymorphism			Allelic polymorphism				
SNP	Genotypes	AD ($n=105$)	NC ($n=210$)	Allele	AD	NC	Genotype p	Allele p
RS10847385	TT	79 (75.2)	177 (84.3)	T	184 (87.6)	385 (91.7)	0.062	0.105
	GG	0	2 (0.9)	G	26 (12.4)	35 (8.3)		
	TG	26 (24.8)	31 (14.8)					
RS7974857	CC	86 (81.9)	178 (84.8)	C	191 (91.0)	388 (92.4)	0.516	0.535
	GC	19 (18.1)	32 (15.2)	G	19 (9.0)	32 (7.6)		

SNP: single-nucleotide polymorphisms; NC: normal control.

RESULTS

Both SNPs were tested by χ^2 goodness-of-fit to be in Hardy-Weinberg equilibrium. The two SNPs in those with AD were not found to be significantly different in allele and genotype frequency than they were in normal controls (Table I). IL-31 level was similar in rs10847385 and rs7974857 normal controls with either allele A or C, but significantly lower in rs10847385 AD patients with allele type C than in the patients with allele A (215 ± 173 vs. 1023 ± 342 pg/ml) (Fig. 1A). In contrast, IL-31 level was similar in rs7974857 AD patients regardless of allele. Similarly, the SCORAD index was significantly lower in rs10847385AD patients with allele type C than in those with allele A of (20.2 ± 25.9 vs. 52.6 ± 46.8). These findings suggested that IL-31 genetic polymorphism might regulate IL-31 level and could lead to differences in IgE level and clinical severity.

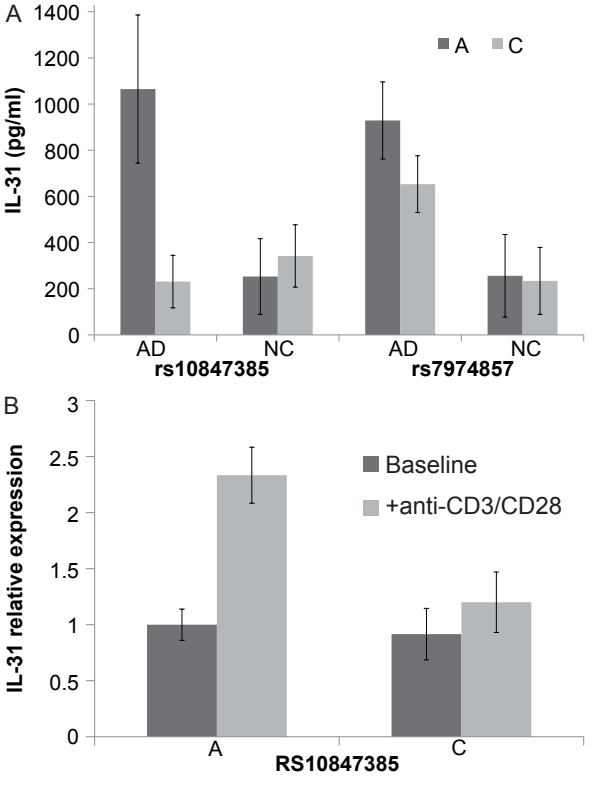

Fig. 1. (A) Blood was collected for IL-31 measurements by enzyme-linked immunoassay (ELISA) and genotyping by PCR from atopic dermatitis (AD) patients and normal controls (NC) ($n = 105$ and 210, respectively). IL-31 was shown in bar graph by diffrent rs10847385 and rs7974857 alleles (A vs. C). While controls showed no difference in their IL-31 levels based on their single-nucleotide polymorphism allele types, AD patients with allele C of rs10847385 had significantly lower levels of serum IL-31 (*$p < 0.05$). (B) Jurkat T cells were transfected with IL-31 promotor constructs with different rs10847385 alleles (A/C) linked with a luciferase reporter. Transfected Jurkat T cells were activated by anti-CD3 and anti-CD28. Luciferase activities measured in allele A/C transfected Jurkat cells were then determined ($n = 3$, *$p < 0.05$).

Next, to investigate whether rs10847385 directly regulated IL-31 production, we made a promoter construct for transactivation and monitored luciferase activity (see Material and Methods). Baseline expression of luciferase activity was similar in transfected cells containing either allele A or C. However, when activated by anti-CD3/CD28 antibodies, allele A-transfected cells had significantly higher luciferase activity than those transfected with allele-C (Fig. 1B), indicating that C allele could cause lower transactivation, which would decrease expression of IL-31. This result was consistent with our *in vivo* data showing AD patients with allele type C had lower serum IL-31 and suggests that under inflammatory conditions, this IL-31 allele can down-regulate IL-31 activities.

DISCUSSION

In this study, IL-31 SNPs were not associated with susceptibility to extrinsic AD, echoing the findings of Schulz's study, which suggested that intrinsic but not extrinsic AD was associated with IL-31 SNP. However, differences in ethnicity, disease onset, and hospital-based design in these two studies may affect results. Although IL-31 SNPs are not associated with AD, IL-31 can affect AD by inducing eosinophils and keratinocytes to produce proinflammatory cytokines (9). In addition, IL-31 increases the TLR-2-dependent secretion of CCL2 after up-regulation of IL-31R in keratinocytes by IFN-γ (10). Our findings, that IL-31 allele can downregulate the expression of IL-31 only under inflammatory conditions, suggest that IL-31 acts as a disease modifier, but not a disease risk factor in AD.

One limitation of this study is that it was a relatively small case-control study. Another limitation is that SNPs other than the two SNPs selected for this study might also have undisclosed associations. Finally, site-directed mutagenic assay (11) in IL-31 might confirm the significance of the locus and clarify the mechanism of increased promoter activity. Nevertheless, by using bioinformatics tool *TFSER* from Japan, we found two putative binding sites near rs10847385, including Cap and Heat shock transcription factor (HSF) within 100 bp (unpublished observation).

In summary, IL-31-631 T>G polymorphism was significantly associated with IL-31 blood level, IgE level, and severity in extrinsic AD patients of Han Chinese origin in Taiwan, but not with their susceptibility to AD. The rs10847385 C allele contributed to inadequate function of the IL-31 promotor.

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