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-31RA 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 IL-31 mRNA expression 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 transactivation assay in vitro than in AD patients (8). We also found significant associations between IgE and IL-31, as well as between SCOrad index and IL-31 (9). As expected, the extrinsic AD patients had increased serum IL-31 (mean 497.5 IU/ml) and SCORAD (SCORing atopic Dermitis) severity index (10). Serum IL-31 was significantly higher in AD patients than in controls (979 pg/ml vs. 21 pg/ml). We also found significant associations between IgE and IL-31, as well as between SCORAD index and IL-31 (11). The present study aimed to investigate the genetic basis of IL-31.

MATERIALS AND METHODS

We measured blood IL-31, assessed 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 (12) and from Department of Health Examination in a medical referral centre. PBMCs were separated for DNA extraction (Genta Puregene blood kit, Valencia, CA, USA) and sera were stored at −70°C until measured for IL-31 with a commercial enzyme-linked immunosorbent assay (ELISA) kit according to manufacturer’s directions with the 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 (13). 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 (14). The present study aimed to investigate the genetic basis of IL-31.

SNP Genotypes AD (n=105) NC (n=210)

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>AD (%)</th>
<th>NC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS10847385</td>
<td>TT</td>
<td>79 (75.2)</td>
<td>177 (84.3)</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>0</td>
<td>2 (0.9)</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>26 (24.8)</td>
<td>31 (14.8)</td>
</tr>
<tr>
<td>RS7974857</td>
<td>CC</td>
<td>86 (81.9)</td>
<td>178 (84.8)</td>
</tr>
<tr>
<td></td>
<td>GC</td>
<td>19 (18.1)</td>
<td>32 (15.2)</td>
</tr>
</tbody>
</table>

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

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RESULTS

Both SNPs were tested by \( \chi^2 \) 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 rs10847385 AD 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.

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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REFERENCES