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SUPPORTING INFORMATION

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Identification of a functional *DOCK8* gene polymorphism associated with atopic dermatitis

To the Editor,

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by recurrent eczematous lesions, intense itch, and type 2 immune responses. Interleukin 31 (IL-31) is a cytokine mainly produced from skin-homing CLA^+ T cells.[1,2] Serum IL-31 levels correlate with the disease severity of AD patients,[2] and the administration of an anti-IL-31 receptor antibody alleviates pruritus in AD patients.[3] Thus, IL-31 plays a major role in AD pathogenesis.

Dedicator of cytokinesis 8 (DOCK8) is an evolutionarily conserved guanine nucleotide exchange factor (GEF) for Cdc42.[4] Bi-allelic loss-of-function mutations of *DOCK8* cause a combined immunodeficiency characterized by AD.[5] We have previously shown that knock down of *DOCK8* gene in T cells from healthy controls markedly increases T-cell receptor-mediated *IL31* gene expression.[6] Therefore, DOCK8 acts as a negative regulator for IL-31 induction in human T cells. However, DOCK8 expression is comparable between healthy controls and AD patients,[6] and functional significance of DOCK8 in the disease predisposition remains unknown.

The aim of this study was to evaluate whether *DOCK8* gene polymorphism is associated with AD. We recruited age- and gender-matched 46 AD patients and 46 healthy controls (Table S1). Blood samples were obtained from all participants according to the institutional review board approvals. Gene polymorphisms in all exons of *DOCK8* (Exon 1–48) were analyzed by direct DNA sequencing using exon-specific primers (Table S2). The difference of allele and genotype frequencies between patients and control samples was compared using the chi-square test.

Among the sequences of all the 48 *DOCK8* exons, only the frequency of the *DOCK8* allele encoding T instead of C at position 1790 (*DOCK8* + 1790T allele, rs17673268) showed significantly higher frequency in AD patients than that in the controls (Table 1, 50% vs. 34%; odds ratio [OR] = 1.96; $p = .025$). In addition, the frequency of the TT genotype significantly increased in AD patients (Table 1), suggesting that this genotype contributes to disease susceptibility to AD. Moreover, severe AD (Investigator Global Assessment [IGA] score: 4) was observed only in patients with CT and TT genotypes (Figure S1). When AD patients were subdivided into two populations with IGA scores of 4 or ≤3, the frequency of the TT genotype significantly increased in patients with IGA scores of 4, compared with patients with IGA scores of ≤3 ($p = .029$; Table S3). We made

further comparisons of EASI scores between CT and TT genotypes in moderate-to-severe patients, which indicate that EASI scores were significantly higher in patients with TT genotype than those with CT genotype ($p = .005$; Figure S2).

We previously identified endothelial PAS domain protein 1 (EPAS1) as a master transcriptional factor essential for T-cell receptor-mediated IL-31 induction.[6] Although EPAS1 translocates to the nucleus in response to hypoxia to mediate promoter activation of target genes, this process is negatively regulated by DOCK8, independent of its Cdc42 GEF activity.[6] To better understand whether *DOCK8* +1790T allele affects EPAS1 nuclear localization, the fluorescence intensity of intranuclear EPAS1 was

TABLE 1 Genotype and allele frequencies of *DOCK8* SNP rs17673268 (c.1790C>T; p.Ala597Val) in AD patients and control subjects

	AD patients (*N* = 46)		Healthy controls (*N* = 46)					
Allele	***N***	**Frequency**	***N***	**Frequency**	**Model**	**OR (95% CI)[b]**	**χ^2 value**	***p* Value[§]**
C	46	0.50	61	0.66	C vs. T	0.50 (0.28–0.91)	5.025	.025*
T	46	0.50	31	0.34	T vs. C	1.96 (1.09–3.51)		
Total	92	1.00	92	1.00				
Genotype	**N**	**Frequency**	**N**	**Frequency**	**Model[a]**	**OR (95% CI)[b]**	**χ^2 value**	***p* Value[§]**
CC	13	0.283	20	0.434	Dominant; CC vs. (CT+TT)	0.51 (0.22–1.20)	2.315	.128
CT	20	0.434	21	0.457	Co-dominant 1; CT vs. CC	1.46 (0.60–3.72)	0.652	.419
TT	13	0.283	5	0.109	Co-dominant 2; TT vs. CC	4.00 (1.11–12.87)	5.023	.025*
					Recessive; TT vs. (CC+CT)	3.23 (1.00–8.77)	4.420	.035*
Total	46	1.00	46	1.00	Over-dominant; (CC+TT) vs. CT	1.09 (0.49–2.42)	0.044	.839

[§]Statistical significance, *$p < 0.05$.

Abbreviations: AD, atopic dermatitis; CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.

[a]Multiple logistic regression model: Dominant (major allele homozygotes vs. heterozygotes and minor allele homozygotes), Co-dominant 1 (heterozygotes vs. major allele homozygotes), Co-dominant 2 (minor allele homozygotes vs. major allele homozygotes), Recessive (minor allele homozygotes vs. major allele homozygotes and heterozygotes), Over-dominant (major and minor allele homozygotes vs. heterozygotes).

[b]OR, AD-to-controls with 95% CI.

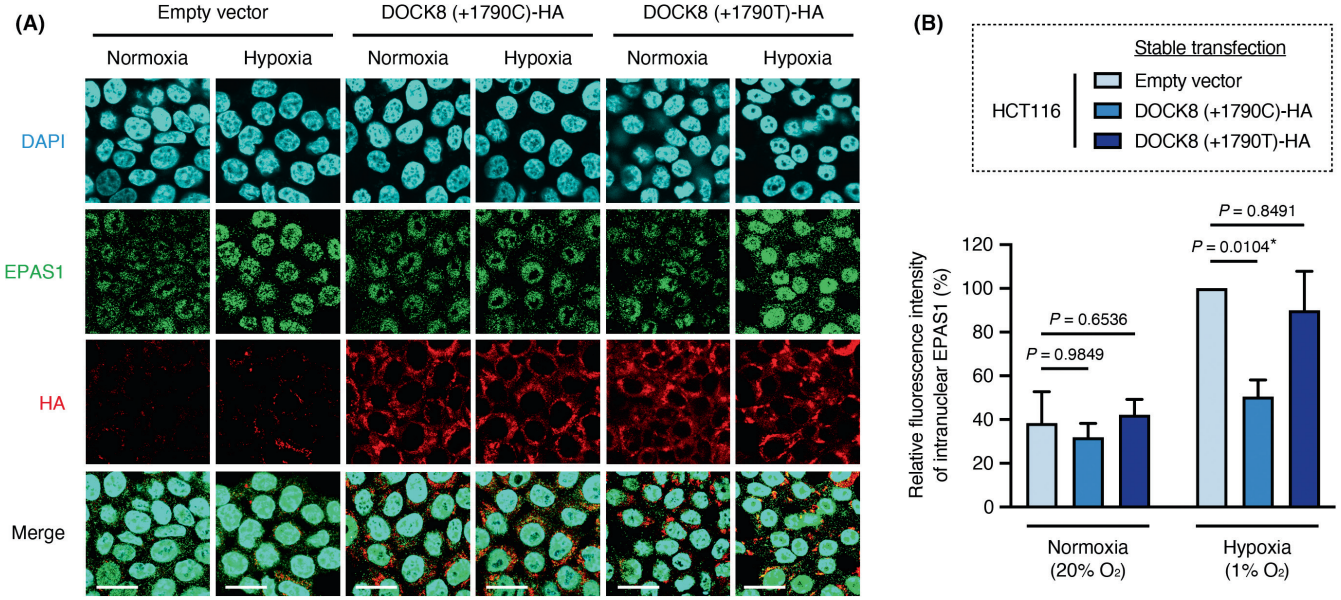

FIGURE 1 Immunofluorescence analyses for subcellular localization of EPAS1 in human HCT116 cells under normoxic or hypoxic conditions. (A) Representative images of EPAS1 (green) and HA (red) expression in HCT116 cells (lacking endogenous DOCK8) stably expressing HA-tagged DOCK8 (+1790C) or DOCK8 (+1790T). DAPI was used to stain nuclei. Scale bars, 20 μm. (B) Comparison of relative fluorescence intensity of intranuclear EPAS1 among each transfectant. Data are expressed as mean ± SD of four independent experiments. In each experiment, 30 cells were counted per group. Data were analyzed by Kruskal–Wallis test with Dunn's multiple comparison test (vs. Empty vector); *$p < .05$

analyzed after stably expressing *DOCK8* +1790C allele or +1790T allele (NM_203447.3:c.1790C > T; p.Ala597Val) in the human cell line HCT116 lacking endogenous DOCK8 protein (Figure S3). When +1790C allele was expressed in HCT116 cells, hypoxia-induced nuclear translocation of EPAS1 was inhibited (Figure 1). However, such inhibitory effect was not seen for +1790T allele (Figure 1). Collectively, these results indicates that the TT genotype of *DOCK8* gene augments IL-31 production by promoting EPAS1 nuclear localization.

In conclusion, we found that *DOCK8* polymorphism at position 1790 (rs17673268) is associated with the susceptibility to AD and revealed its underlying molecular basis. Although this study used a relatively small sample size, our findings provide a novel insight into AD pathogenesis.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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