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Letter to the Editor

Homozygous variant p.Ser427Pro in PNPLA1 is a preventive factor from atopic dermatitis

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Main Text

To the Editor:

One of the genetic predisposing factors for atopic dermatitis (AD) is abnormal barrier function in the stratum corneum. Mutations in the filaggrin gene (*FLG*) have become apparent as a cause of this abnormal barrier function [1]. In addition, functional lipids in the epidermis, especially ω-O-esterified ultra-long-chain acylceramide (EOS), are important for skin barrier function [2]. The corneocyte lipid envelope (CLE) is also an essential structure for proper stratum corneum barrier function [2]. CLE is a thin, single lipid layer located between the intercellular lipid layers and the cornified cell envelope (CCE). The CLE is mostly composed of ω-hydroxyceramide with ultra-long-chain (ULC) fatty acids derived from EOS. A number of genes are involved in the formation of the CLE, including *ELOVL* family genes, *CYP4F22*, *SLC27A4*, and *PNPLA1* [3, 4]. Few reports have addressed the association between CLE formation and AD. Thus, to understand the association between AD, and EOS synthesis and CLE formation, we analyzed polymorphisms of genes related to EOS synthesis and CLE formation, and identified a significant association between the *PNPLA1* single-nucleotide polymorphism (SNP) rs4713956 and AD.

In the genes involved in EOS synthesis and CLE formation, we first searched the Human Genetic Variation Database for candidate SNPs that showed minor allele frequencies of greater than 5% and that lead to missense, nonsense, or frameshift mutations (http://www.hgvd.genome.med.kyoto-u.ac.jp/). Nine candidate SNPs in 5 genes (*ALOXE3*, *PNPLA1*, *SLC27A4*, *NIPAL4* and *ELOVL4*) were identified as the subjects of the present analysis (Supplementary Table 1). Then, we genotyped the SNPs in at least 142 healthy controls and 109 AD patients (Supplementary Table 2). In the AD group, we performed a subgroup analysis on the presence/absence of *FLG* mutations, the absence or mild/moderate/severe serum IgE elevation (0-170, 171-10,000 or 10,000- UA/mL), the absence or mild/moderate/severe serum TARC elevation (0-450, 451-10,000 or 10,000- IU/mL), and Investigator Global Assessment (IGA) scores (2-4) for AD severity at the first physical examination.

One of the *PNPLA1* SNPs, rs4713956 (T/C, p.Ser427Pro), showed that the incidence of the CC genotype was significantly low in the AD patient group (AD, CC:CT+TT=26:84; control, CC:CT+TT=54:88; p=0.015; Table 1). Furthermore, the tendency held true for the AD group without *FLG* mutations (AD without *FLG* mutations, CC:CT+TT=20:67; control, CC:CT+TT=54:88; p=0.018). The frequency of CC was also low in other subgroups, i.e. the

AD subgroup showing moderate serum IgE elevation (171-10,000 UA/mL) (CC:CT+TT=5:30; control, CC:CT+TT=54:88; p=0.008), the AD subgroup with moderate serum TARC elevation (451-10,000 UA/mL) (CC:CT+TT=8:35, p=0.018), and the AD subgroup with IGA score 3 (CC:CT+TT=2:15, p=0.032). All the results of SNP analysis including data on other SNPs in other genes are shown in Supplementary Table 2.

Then, we compared the thickness of the stratum corneum and the other layers of the epidermis (the basal, spinous and granular layers) of the skin lesions in the trunk, the arm or the leg of the patients with CC (n=3) and the patients with CT or TT (n=6) in the cohort of the AD group without *FLG* mutations. There was a tendency for the basal, spinous and granular layers to be thicker in the patients with CC than in the patients with CT or TT. Conversely, there was a tendency for the stratum corneum to be thinner in the patients with CC than in the patients with CT or TT (Supplementary Fig. 1).

PNPLA1 is a causative gene of autosomal recessive congenital ichthyosis (ARCI) [5]. From a study of *Pnpla1* knockout mice [6, 7], PNPLA1 was reported to have the function of transferring linoleic acid to the terminal hydroxyl group of ω-hydroxide ceramide and synthesize acylceramide (Fig. 1a). Acylceramide is then transported into the cell membrane

zone or secreted to the intercellular space, where it forms CLE and the intercellular lipid layers. Thus, *PNPLA1* is thought to be extremely important for the skin barrier [6-8].

In the present study, the CC genotype at *PNPLA1* rs4713956 was significantly less prevalent in the AD group than in the control group. We regard homozygosity for *PNPLA1* Pro427 as a strong factor in the prevention of AD, although *PNPLA1* Pro427 deficiency is not sufficient to cause the onset of AD, because a considerable number of individuals in the healthy group were homozygous for Ser427.

In addition, the frequency of the CC genotype was low in some subgroups, including the AD group with moderate serum IgE elevation (171-10,000 UA/mL), the AD group with moderate serum TARC elevation (451-10,000 UA/mL), and the AD group with IGA score 3. Serum IgE and TARC concentrations are affected by previous treatment and environmental factors. Unfortunately, we did not evaluate these factors in the present study. It is necessary to increase the number of AD cases included in the study and to include more detailed information of patients' backgrounds in future study.

PNPLA1 has a conserved catalytic domain called the patatin domain at the N-terminal side and a proline-rich hydrophobic region at the C-terminal side (Fig. 1b). The causative mutations of ARCI are mostly in the patatin domain, but there are a few mutations in other areas, including proline-rich region [9]. Thus, the proline-rich region is presumed to have some function, and rs4713956 (p.Ser427Pro) resides in that region.

In our study, the stratum corneum of the lesional skin was thinner in the patients with the CC genotype than in the patients with the CT or TT genotypes. In cases of ichthyosis due to the loss-of-function of PNPLA1, marked hyperkeratosis was seen, compensating for the skin barrier dysfunction. These results suggest that the skin barrier function might be stronger in the patients with the CC genotype than in the patients with the CT or TT genotypes.

The present study is the first to propose the possibility that a gene associated with EOS synthesis and CLE formation might be involved in the onset of AD. These results presumably suggest a novel concept in which the down-regulated synthesis of EOS and insufficient formation of CLE might be associated with the pathogenesis of AD.

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Conflicts of interest

None of the authors (NW, MK, MS, KT and MA) has any conflict of interest to declare.

Supporting Information

Additional supporting information may be found in the online version of this article:

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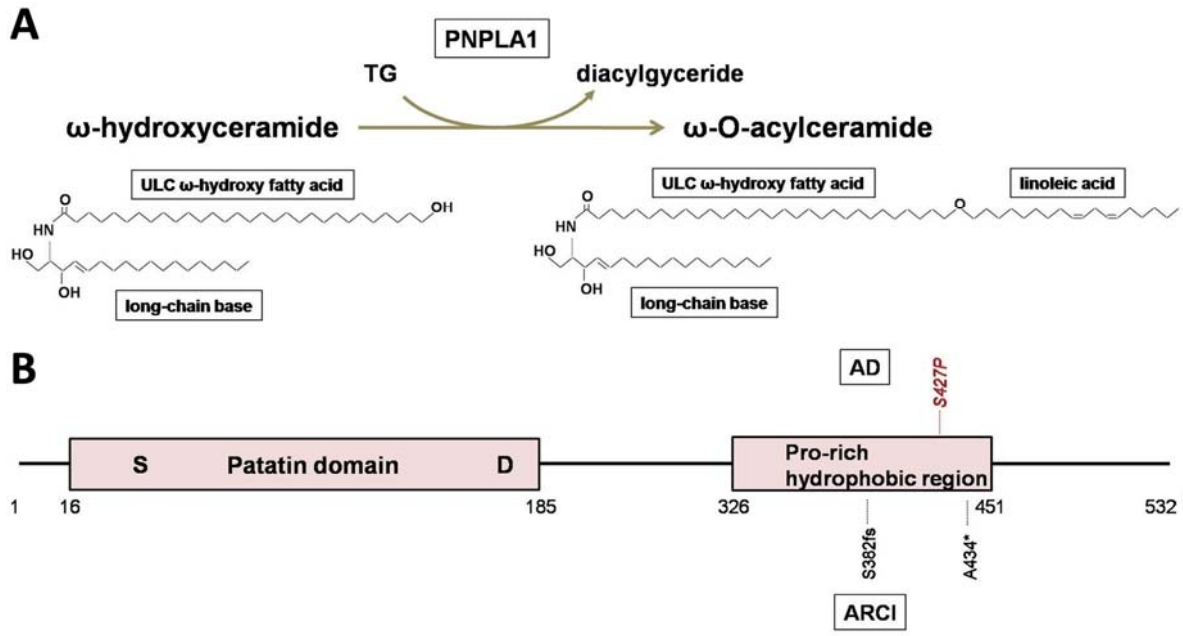

Fig. 1. The function and structure of PNPLA1. **(A)** Enzyme reaction catalyzed by PNPLA1. PNPLA1 catalyzes the transacylation of linoleic acid from the TG molecule on to ULC ω-hydroxyceramide to produce ω-O-acylceramide.[6-9] *TG*, triglyceride. **(B)** Schematic of the primary structure of PNPLA1. Most mutations causative of ARCI are in the patatin domain with the catalytic dyad Ser53 (S) and Asp172 (D). Ser427Pro (S427P) (red) is located in the proline-rich hydrophobic region.

Table 1. *PNPLA1* rs4713956 genotype analysis in the AD patients and subgroups

Patient group				Patient breakdown by genotype			*p* value
				CC	CT+TT	total	
Control				54	88	142	-
AD	Total AD			26	84	110	0.015*
	Subgroups by IgE levels	absent/mild ↑		3	7	10	0.612
		moderate ↑		5	30	35	0.008*
		severe ↑		6	10	16	0.965
	Subgroups by TARC levels	absent/mild ↑		3	7	10	0.612
		moderate ↑		8	35	43	0.018*
		severe ↑		3	4	7	0.799
	Subgroups by IGA scores	2		0	3	3	0.177
		3		2	15	17	0.032*
		4		13	26	39	0.590
	Subgroups with/without *FLG* mutations	*FLG* mutation-positive		6	17	23	0.270
		FLG mutation-negative	Total *FLG* mutation-negative AD	20	67	87	0.018*
			Subgroups by IgE levels: absent/mild ↑	2	6	8	0.457
			moderate ↑	5	24	29	0.032*
			severe ↑	4	9	13	0.607
			Subgroups by TARC levels: absent/mild ↑	3	5	8	0.976
			moderate ↑	6	30	36	0.016*
			severe ↑	2	4	6	0.817
			Subgroups by IGA scores: 2	0	3	3	0.177
			3	2	13	15	0.058
			4	10	20	30	0.630

P values were obtained by statistical analysis between each subgroup and the control. *, statistically significant.
Serum IgE (UA/ml); absent/mild ↑ 1-170, moderate ↑ 171-10,000, severe ↑ 10,000-.
Serum TARC (IU/ml); absent/mild ↑ 1-450, moderate ↑ 451-10,000, severe ↑ 10,000-.