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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.

Acknowledgements

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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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Figure Legend

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-.

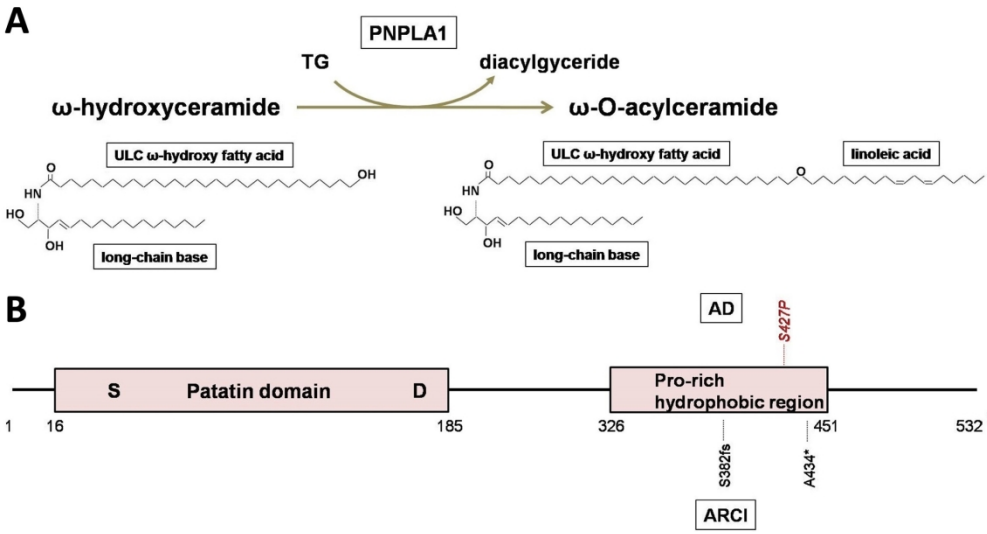

Fig. 1. The function and structure of PNPLA1. (A) Enzyme reaction catalyzed by PNPLA1. PNPLA1 catalyzes the transacylation of linoleic acid from the TG molecule on to ULC ω-hydroxyceramide to produce ω-O-acylceramide.[6-9] TG, triglyceride. (B) Schematic of the primary structure of PNPLA1. Most mutations 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.

199x108mm (300 x 300 DPI)

Supplementary Information

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

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SUPPLEMENTARY METHODS

Patients and control groups

This study was approved by the Ethics Committee of Nagoya University and was performed in accordance with the Declaration of Helsinki. The diagnosis of atopic dermatitis (AD) was based on the diagnostic criteria of Hanifin and Rajka [1], and was made by consultant dermatologists. Blood samples for genetic analyses were collected with written informed consent from AD patients and normal healthy controls at Nagoya University Hospital and affiliated hospitals or clinics. Blood sampling other than for genetic analysis, including for serum IgE, specific IgE antibodies, TARC and LDH, and a questionnaire about medical history, family history, allergies, age of onset and so on were performed on a voluntary basis.

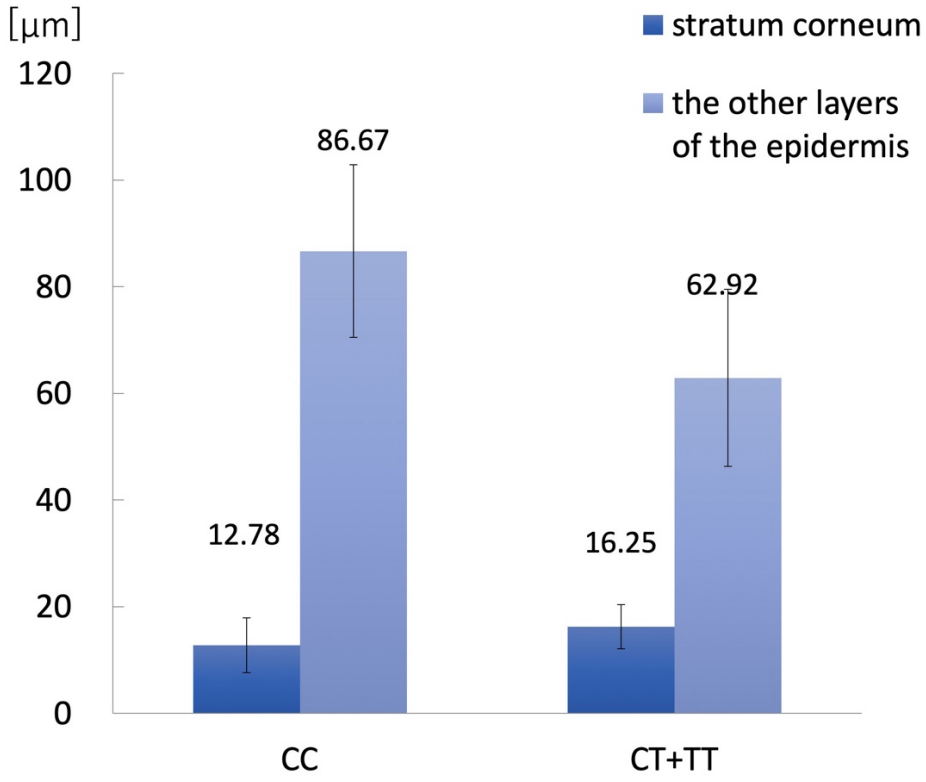
Analysis of the genes associated with corneocyte lipid envelope (CLE) formation

Based on the literature, we selected 20 genes that are involved in CLE formation. The SNPs present in these genes were listed with reference to the Human Genetic Variation Database provided by Kyoto University <http://www.hgvd.genome.med.kyoto-u.ac.jp/> (accessed April 2018). This study regards AD as a disease with a high incidence; therefore, SNPs with a minor allele frequency of greater than 5% were targeted for analysis. SNP analysis was performed with two-color fluorescence probe methods (TaqMan® SNP Genotyping Assay (Applied Biosystems Life Technologies, Foster City, California) and KASP™ genotyping assay (LGC Genomics, Hoddeson, United Kingdom)) using LightCycler® 480 II (Roche Diagnostics, Mannheim, Germany).

Statistical analyses

Statistical analyses were performed using SPSS Statistics 20 (IBM, Armonk, NY). The correlation between genotypes and AD was examined by Chi-squared test. Values of $p<0.05$ were considered to be significant.

SUPPLEMENTARY FIGURE



Supplementary Fig. 1. Histopathological samples (tissue sections of skin biopsy specimens stained with hematoxylin-eosin) from lesional skin in the trunk, the arm or the leg of 3 CC-genotyped and 6 CT- or TT-genotyped AD patients were collected, and the thicknesses of the stratum corneum and the other epidermal layers (the basal, spinous and granular layers) were measured. To evaluate the thickness of the stratum corneum, the distance between the top of the stratum corneum and the top of the uppermost granular layer was measured in each tissue section. In addition, to evaluate the thickness of the other layers of the epidermis (the basal, spinous and granular layers), the distance between the top of the uppermost granular layer and the top of the dermal papilla was measured in each tissue section. The average of values measured at 3 points was defined as the thickness value of each specimen. The average thickness of the stratum corneum in the patients with the genotype CC (n=3) and with the genotypes CT or TT (n=6) was 12.78 μm and 16.25 μm, respectively. The average thicknesses of the other layers of the epidermis (the basal, spinous and granular layers) in the patients with the CC genotype and with the CT or TT genotypes was 86.67 μm and 62.92 μm, respectively. The thicknesses of the stratum corneum and the other epidermal layers showed no significant difference between CC and CT/TT.

SUPPLEMENTARY TABLE

Supplementary Table 1. SNPs involved in CLE-related genes

Gene	loci	rs ID	Ref/Alt	Function	
ALOXE3	17p13.1	rs3027232	G/A	missense	p.Pro11Leu
PNPLA1	6p21.31	rs74946910	G/A	missense	p.Ala136Ser
		rs34598813	A/G	missense	p.Glu193Gly
		rs12199580	C/A	missense	p.Pro328His
		rs12197079	C/T	missense	p.Thr395Met
		rs4713956	T/C	missense	p.Ser427Pro
SLC27A4	9q34.11	rs2240953	G/A	missense	p.Gly209Ser
NIPAL4	5q33.3	rs6860507	A/G	missense	p.Arg194Gly
ELOVL4	6q14.1	rs3812153	T/C	missense	p.Met299Val

SNPs more than 5% allele frequency.
SNPs that cause missense, nonsense, or frameshift mutations are extracted.

Supplementary Table 2. CLE-related gene SNP genotyping

ALOXE3 rs3027232					Patient group	Patient breakdown by genotype				*p* value
						AA	AG	GG	total	
Control						43	76	23	142	
AD	total AD					29	64	16	109	0.711
	subgroups by IgE levels	absent/mild ↑				1	8	1	10	0.255
		moderate ↑				8	24	3	35	0.25
		severe ↑				4	8	4	16	0.662
	subgroups by TARC levels	absent/mild ↑				2	8	0	10	0.209
		moderate ↑				10	26	7	43	0.651
		severe ↑				2	5	0	7	0.466
	subgroups by IGA score	2				0	3	0	3	0.276
		3				1	14	2	17	0.058
		4				13	21	5	39	0.854
	subgroups with/without *FLG* mutation	*FLG* mutation-positive				7	13	2	22	0.687
		FLG mutation-negative	Total FLG mutation-negative AD			22	51	14	87	0.696
			subgroups by IgE levels	absent/mild ↑		1	6	1	8	0.466
				moderate ↑		5	21	3	29	0.172
				severe ↑		2	7	4	13	0.306
			subgroups by TARC levels	absent/mild ↑		2	6	0	8	0.366
				moderate ↑		5	24	7	36	0.14
				severe ↑		2	4	0	6	0.556
			subgroups by IGA scores	2		0	3	0	3	0.276
				3		1	12	2	15	0.108
				4		8	17	5	30	0.923

PNPLA1 rs74946910					Patient group	Patient breakdown by genotype				*p* value
						AA	AG	GG	total	
Control						4	28	111	143	
AD	total AD					2	23	84	109	0.853
	subgroups by IgE levels	absent/mild ↑				0	0	10	10	0.243
		moderate ↑				1	11	23	35	0.312
		severe ↑				0	1	14	15	0.355
	subgroups by TARC levels	absent/mild ↑				0	1	9	10	0.632
		moderate ↑				1	11	30	42	0.65
		severe ↑				0	1	6	7	0.839
	subgroups by IGA score	2				0	1	2	3	0.819
		3				1	1	15	17	0.324
		4				0	9	29	38	0.518
	subgroups with/without *FLG* mutation	*FLG* mutation-positive				0	3	20	23	0.519
		FLG mutation-negative	Total FLG mutation-negative AD			2	20	64	86	0.794
			subgroups by IgE levels	absent/mild ↑		0	0	8	8	0.322
				moderate ↑		1	10	18	29	0.199
				severe ↑		0	1	11	12	0.505
			subgroups by TARC levels	absent/mild ↑		0	1	7	8	0.772
				moderate ↑		1	10	24	35	0.504
				severe ↑		0	1	5	6	0.897
			subgroups by IGA scores	2		0	1	2	3	0.819
				3		1	1	13	15	0.362
				4		0	8	21	29	0.443

PNPLA1 rs12199580	Patient group			Patient breakdown by genotype				*p* value
				AA	AC	CC	total	
Control				6	51	86	143	
AD	total AD			9	48	67	124	0.427
	subgroups by IgE levels	absent/mild ↑		1	3	6	10	0.683
		moderate ↑		2	15	18	35	0.637
		severe ↑		3	6	7	16	0.049*
	subgroups by TARC levels	absent/mild ↑		1	3	6	10	0.637
		moderate ↑		5	16	22	43	0.167
		severe ↑		0	5	2	7	0.155
	subgroups by IGA score	2		1	1	1	3	0.056
		3		1	6	10	17	0.948
		4		4	15	20	39	0.282
	subgroups with/without *FLG* mutation	*FLG* mutation-positive		3	9	13	25	0.265
		FLG mutation-negative	Total FLG mutation-negative AD	5	34	51	90	0.82
			subgroups by IgE levels: absent/mild ↑	1	1	6	8	0.275
			subgroups by IgE levels: moderate ↑	1	13	15	29	0.648
			subgroups by IgE levels: severe ↑	2	5	6	13	0.192
			subgroups by TARC levels: absent/mild ↑	1	1	6	8	0.275
			subgroups by TARC levels: moderate ↑	3	15	18	36	0.412
			subgroups by TARC levels: severe ↑	0	4	2	6	0.293
			subgroups by IGA scores: 2	1	1	1	3	0.056
			subgroups by IGA scores: 3	1	5	9	15	0.898
			subgroups by IGA scores: 4	2	12	16	30	0.723

PNPLA1 rs4713956	Patient group			Patient breakdown by genotype				*p* value
				CC	CT	TT	total	
Control				54	67	21	142	
AD	total AD			26	65	19	110	0.051
	subgroups by IgE levels	absent/mild ↑		3	6	1	10	0.729
		moderate ↑		5	22	8	35	0.027*
		severe ↑		6	8	2	16	0.962
	subgroups by TARC levels	absent/mild ↑		3	6	1	10	0.729
		moderate ↑		8	26	9	43	0.06
		severe ↑		3	3	1	7	0.967
	subgroups by IGA score	2		0	2	1	3	0.364
		3		2	12	3	17	0.094
		4		13	17	9	39	0.463
	subgroups with/without *FLG* mutation	*FLG* mutation-positive		6	12	5	23	0.475
		FLG mutation-negative	Total FLG mutation-negative AD	20	53	14	87	0.056
			subgroups by IgE levels: absent/mild ↑	2	5	1	8	0.69
			subgroups by IgE levels: moderate ↑	5	18	6	29	0.099
			subgroups by IgE levels: severe ↑	4	8	1	13	0.577
			subgroups by TARC levels: absent/mild ↑	3	4	1	8	0.981
			subgroups by TARC levels: moderate ↑	6	23	7	36	0.053
			subgroups by TARC levels: severe ↑	2	3	1	6	0.972
			subgroups by IGA scores: 2	0	2	1	3	0.364
			subgroups by IGA scores: 3	2	10	3	15	0.165
			subgroups by IGA scores: 4	10	15	5	30	0.884

SLC27A4 rs2240953		Patient group		Patient breakdown by genotype				*p* value
				AA	AG	GG	total	
Control				7	47	88	142	
AD	total AD			7	40	63	110	0.723
	subgroups by IgE levels	absent/mild ↑		0	3	7	10	0.735
		moderate ↑		2	14	19	35	0.705
		severe ↑		1	8	7	16	0.364
	subgroups by TARC levels	absent/mild ↑		1	3	6	10	0.787
		moderate ↑		2	20	21	43	0.27
		severe ↑		0	2	5	7	0.786
	subgroups by IGA score	2		0	1	2	3	0.926
		3		0	5	12	17	0.582
		4		5	17	17	39	0.061
	subgroups with/without *FLG* mutation	*FLG* mutation-positive		2	9	12	23	0.592
		FLG mutation-negative	Total FLG mutation-negative AD	5	31	51	87	0.873
			subgroups by IgE levels: absent/mild ↑	0	1	7	8	0.338
			subgroups by IgE levels: moderate ↑	2	14	13	29	0.232
			subgroups by IgE levels: severe ↑	0	6	7	13	0.506
			subgroups by TARC levels: absent/mild ↑	1	2	5	8	0.622
			subgroups by TARC levels: moderate ↑	1	18	17	36	0.165
			subgroups by TARC levels: severe ↑	0	1	5	6	0.551
			subgroups by IGA scores: 2	0	1	2	3	0.926
			subgroups by IGA scores: 3	0	4	11	15	0.552
			subgroups by IGA scores: 4	4	13	13	30	0.085

NIPAL4 rs6860507		Patient group		Patient breakdown by genotype				*p* value
				AA	AG	GG	total	
Control				10	68	65	143	
AD	total AD			14	47	48	109	0.284
	subgroups by IgE levels	absent/mild ↑		1	2	7	10	0.239
		moderate ↑		5	13	17	35	0.286
		severe ↑		1	8	7	16	0.98
	subgroups by TARC levels	absent/mild ↑		1	3	6	10	0.559
		moderate ↑		7	16	20	43	0.142
		severe ↑		1	3	3	7	0.766
	subgroups by IGA score	2		0	3	0	3	0.198
		3		3	5	9	17	0.182
		4		4	14	21	39	0.406
	subgroups with/without *FLG* mutation	*FLG* mutation-positive		4	9	10	23	0.239
		FLG mutation-negative	Total FLG mutation-negative AD	10	38	38	86	0.479
			subgroups by IgE levels: absent/mild ↑	1	2	5	8	0.443
			subgroups by IgE levels: moderate ↑	4	11	14	29	0.387
			subgroups by IgE levels: severe ↑	0	7	6	13	0.605
			subgroups by TARC levels: absent/mild ↑	1	3	4	8	0.772
			subgroups by TARC levels: moderate ↑	5	13	18	36	0.274
			subgroups by TARC levels: severe ↑	1	3	2	6	0.625
			subgroups by IGA scores: 2	0	3	0	3	0.198
			subgroups by IGA scores: 3	2	5	8	15	0.474
			subgroups by IGA scores: 4	3	11	16	30	0.529

ELOLV4 rs3812153		Patient group			Patient breakdown by genotype				*p* value
					TT	TC	CC	total	
Control					90	47	6	143	
AD	total AD				72	37	6	115	0.926
	subgroups by IgE levels	absent/mild ↑			3	6	1	10	0.114
		moderate ↑			25	9	1	35	0.637
		severe ↑			10	6	0	16	0.684
	subgroups by TARC levels	absent/mild ↑			4	4	2	10	0.066
		moderate ↑			30	13	0	43	0.298
		severe ↑			4	3	0	7	0.766
	subgroups by IGA score	2			3	0	0	3	0.417
		3			9	7	1	17	0.72
		4			24	14	1	39	0.858
	subgroups with/without *FLG* mutation	*FLG* mutation-positive			15	10	0	25	0.498
		FLG mutation-negative	Total FLG mutation-negative AD		57	27	6	90	0.672
			subgroups by IgE levels	absent/mild ↑	3	4	1	8	0.274
				moderate ↑	20	8	1	29	0.828
				severe ↑	8	5	0	13	0.718
			subgroups by TARC levels	absent/mild ↑	3	3	2	8	0.028*
				moderate ↑	25	11	0	36	0.417
				severe ↑	4	2	0	6	0.877
			subgroups by IGA scores	2	3	0	0	3	0.417
				3	8	6	1	15	0.741
				4	19	10	1	30	0.977

ALOXE3 rs3027232		Patient group			Patient breakdown by genotype			*p* value
					AA	AG+GG	total	
Control					43	99	142	
AD	total AD				29	80	109	0.523
	subgroups by IgE levels	absent/mild ↑			1	9	10	0.173
		moderate ↑			8	27	35	0.386
		severe ↑			4	12	16	0.661
	subgroups by TARC levels	absent/mild ↑			2	8	10	0.491
		moderate ↑			10	33	43	0.372
		severe ↑			2	5	7	0.926
	subgroups by IGA score	2			0	3	3	0.256
		3			1	16	17	0.034*
		4			13	26	39	0.716
	subgroups with/without *FLG* mutation	*FLG* mutation-positive			7	15	22	0.885
		FLG mutation-negative	Total FLG mutation-negative AD		22	65	87	0.417
			subgroups by IgE levels	absent/mild ↑	1	7	8	0.281
				moderate ↑	5	24	29	0.154
				severe ↑	2	11	13	0.258
			subgroups by TARC levels	absent/mild ↑	2	6	8	0.751
				moderate ↑	5	31	36	0.048*
				severe ↑	2	4	6	0.87
			subgroups by IGA scores	2	0	3	3	0.256
				3	1	14	15	0.053
				4	8	22	30	0.692

PNPLA1 rs74946910		Patient group			Patient breakdown by genotype			*p* value
					AA	AG+GG	total	
Control					4	139	143	
AD	total AD				2	107	109	0.617
	subgroups by IgE levels	absent/mild ↑			0	10	10	0.593
		moderate ↑			1	34	35	0.982
		severe ↑			0	15	15	0.512
	subgroups by TARC levels	absent/mild ↑			0	10	10	0.593
		moderate ↑			1	41	42	0.88
		severe ↑			0	7	7	0.651
	subgroups by IGA score	2			0	3	3	0.772
		3			1	16	17	0.488
		4			0	38	38	0.297
	subgroups with/without *FLG* mutation	*FLG* mutation-positive			0	23	23	0.419
		FLG mutation-negative	Total FLG mutation-negative AD		2	84	86	0.831
			subgroups by IgE levels	absent/mild ↑	0	8	8	0.633
				moderate ↑	1	28	29	0.846
				severe ↑	0	12	12	0.557
			subgroups by TARC levels	absent/mild ↑	0	8	8	0.633
				moderate ↑	1	34	35	0.982
				severe ↑	0	6	6	0.679
			subgroups by IGA scores	2	0	3	3	0.772
				3	1	14	15	0.409
				4	0	29	29	0.364

PNPLA1 rs12199580		Patient group			Patient breakdown by genotype			*p* value
					AA	AC+CC	total	
Control					6	137	143	
AD	total AD				9	115	124	0.279
	subgroups by IgE levels	absent/mild ↑			1	9	10	0.399
		moderate ↑			2	33	35	0.695
		severe ↑			3	13	16	0.017*
	subgroups by TARC levels	absent/mild ↑			1	9	10	0.399
		moderate ↑			5	38	43	0.07
		severe ↑			0	7	7	0.58
	subgroups by IGA score	2			1	2	3	0.017*
		3			1	16	17	0.744
		4			4	35	39	0.14
	subgroups with/without *FLG* mutation	*FLG* mutation-positive			3	22	25	0.11
		FLG mutation-negative	Total FLG mutation-negative AD		5	85	90	0.634
			subgroups by IgE levels	absent/mild ↑	1	7	8	0.276
				moderate ↑	1	28	29	0.853
				severe ↑	2	11	13	0.081
			subgroups by TARC levels	absent/mild ↑	1	7	8	0.276
				moderate ↑	3	33	36	0.31
				severe ↑	0	6	6	0.61
			subgroups by IGA scores	2	1	2	3	0.017*
				3	1	14	15	0.653
				4	2	28	30	0.56

SLC27A4 rs2240953			Patient group		Patient breakdown by genotype			*p* value
					AA	AG+GG	total	
Control					7	135	142	
AD	total AD				7	103	110	0.622
	subgroups by IgE levels	absent/mild ↑			0	10	10	0.473
		moderate ↑			2	33	35	0.85
		severe ↑			1	15	16	0.819
	subgroups by TARC levels	absent/mild ↑			1	9	10	0.492
		moderate ↑			2	41	43	0.942
		severe ↑			0	7	7	0.547
	subgroups by IGA score	2			0	3	3	0.699
		3			0	17	17	0.349
		4			5	34	39	0.08
	subgroups with/without *FLG* mutation	*FLG* mutation-positive			2	21	0	0.457
		FLG mutation-negative	Total FLG mutation-negative AD		5	82	87	0.788
			subgroups by IgE levels	absent/mild ↑	0	8	8	0.522
				moderate ↑	2	27	29	0.668
				severe ↑	0	13	13	0.411
			subgroups by TARC levels	absent/mild ↑	1	7	8	0.358
				moderate ↑	1	35	36	0.577
				severe ↑	0	6	6	0.58
			subgroups by IGA scores	2	0	3	3	0.699
				3	0	15	15	0.379
				4	4	26	30	0.088

NIPAL4 rs6860507			Patient group		Patient breakdown by genotype			*p* value
					AA	AG+GG	total	
Control					10	133	143	
AD	total AD				14	95	109	0.117
	subgroups by IgE levels	absent/mild ↑			1	9	10	0.723
		moderate ↑			5	30	35	0.164
		severe ↑			1	15	16	0.909
	subgroups by TARC levels	absent/mild ↑			1	9	10	0.723
		moderate ↑			7	36	43	0.064
		severe ↑			1	6	7	0.466
	subgroups by IGA score	2			0	3	3	0.631
		3			3	14	17	0.128
		4			4	35	39	0.498
	subgroups with/without *FLG* mutation	*FLG* mutation-positive			4	19	23	0.096
		FLG mutation-negative	Total FLG mutation-negative AD		10	76	86	0.229
			subgroups by IgE levels	absent/mild ↑	1	7	8	0.556
				moderate ↑	4	25	29	0.222
				severe ↑	0	13	13	0.325
			subgroups by TARC levels	absent/mild ↑	1	7	8	0.556
				moderate ↑	5	31	36	0.183
				severe ↑	1	5	6	0.371
			subgroups by IGA scores	2	0	3	3	0.631
				3	2	13	15	0.378
				4	3	27	30	0.568

ELOLV4 rs3812153		Patient group			Patient breakdown by genotype			*p* value
					TT	TC+CC	total	
Control					90	53	143	
AD	total AD				72	43	115	0.957
	subgroups by IgE levels	absent/mild ↑			3	7	10	0.039*
		moderate ↑			25	10	35	0.346
		severe ↑			10	6	16	0.974
	subgroups by TARC levels	absent/mild ↑			4	6	10	0.15
		moderate ↑			30	13	43	0.411
		severe ↑			4	3	7	0.755
	subgroups by IGA score	2			3	0	3	0.186
		3			9	8	17	0.422
		4			24	15	39	0.872
	subgroups with/without *FLG* mutation	*FLG* mutation-positive			15	10	25	0.78
		FLG mutation-negative	Total FLG mutation-negative AD		57	33	0	0.951
			subgroups by IgE levels	absent/mild ↑	3	5	8	0.149
				moderate ↑	20	9	29	0.539
				severe ↑	8	5	13	0.919
			subgroups by TARC levels	absent/mild ↑	3	5	8	0.149
				moderate ↑	25	11	36	0.467
				severe ↑	4	2	6	0.856
			subgroups by IGA scores	2	3	0	3	0.186
				3	8	7	15	0.467
				4	19	11	30	0.967

ALOXE3 rs3027232		Patient group		Patient breakdown by genotype			*p* value
				AA+AG	GG	total	
Control				119	23	142	
AD	total AD			93	16	109	0.741
	subgroups by IgE levels	absent/mild ↑		9	1	10	0.603
		moderate ↑		32	3	35	0.254
		severe ↑		12	4	16	0.373
	subgroups by TARC levels	absent/mild ↑		10	0	10	0.168
		moderate ↑		36	7	43	0.989
		severe ↑		7	0	7	0.247
	subgroups by IGA score	2		3	0	3	0.445
		3		15	2	17	0.637
		4		34	5	39	0.607
	subgroups with/without *FLG* mutation	*FLG* mutation-positive		20	2	22	0.389
		FLG mutation-negative	Total FLG mutation-negative AD	73	14	87	0.982
			subgroups by IgE levels: absent/mild ↑	7	1	8	0.781
			subgroups by IgE levels: moderate ↑	26	3	29	0.424
			subgroups by IgE levels: severe ↑	9	4	13	0.183
			subgroups by TARC levels: absent/mild ↑	8	0	8	0.215
			subgroups by TARC levels: moderate ↑	29	7	36	0.643
			subgroups by TARC levels: severe ↑	6	0	6	0.284
			subgroups by IGA scores: 2	3	0	3	0.445
			subgroups by IGA scores: 3	13	2	15	0.772
			subgroups by IGA scores: 4	25	5	30	0.948

PNPLA1 rs7494691(		Patient group			Patient breakdown by genotype			*p* value
					AA+AG	GG	total	
Control					32	111	143	
AD	total AD				25	84	109	0.915
	subgroups by IgE levels	absent/mild ↑			0	10	10	0.093
		moderate ↑			12	23	35	0.143
		severe ↑			1	14	15	0.155
	subgroups by TARC levels	absent/mild ↑			1	9	10	0.356
		moderate ↑			12	30	42	0.407
		severe ↑			1	6	7	0.614
	subgroups by IGA score	2			1	2	3	0.656
		3			2	15	17	0.313
		4			9	29	38	0.865
	subgroups with/without *FLG* mutation	*FLG* mutation-positive			3	20	23	0.308
		FLG mutation-negative	Total FLG mutation-negative AD		22	64	86	0.58
			subgroups by IgE levels	absent/mild ↑	0	8	8	0.131
				moderate ↑	11	18	29	0.078
				severe ↑	1	11	12	0.255
			subgroups by TARC levels	absent/mild ↑	1	7	8	0.51
				moderate ↑	11	24	35	0.263
				severe ↑	1	5	6	0.741
			subgroups by IGA scores	2	1	2	3	0.656
				3	2	13	15	0.417
				4	8	21	29	0.544

PNPLA1 rs12199580		Patient group		Patient breakdown by genotype			*p* value
				AA+AC	CC	total	
Control				57	86	143	
AD	total AD			57	67	124	0.314
	subgroups by IgE levels	absent/mild ↑		4	6	10	0.995
		moderate ↑		17	18	35	0.349
		severe ↑		9	7	16	0.207
	subgroups by TARC levels	absent/mild ↑		4	6	10	0.995
		moderate ↑		21	22	43	0.295
		severe ↑		5	2	7	0.097
	subgroups by IGA score	2		2	1	3	0.347
		3		7	10	17	0.917
		4		19	20	39	0.321
	subgroups with/without *FLG* mutation	*FLG* mutation-positive		12	13	25	0.446
		FLG mutation-negative	Total FLG mutation-negative AD	39	51	90	0.6
			subgroups by IgE levels: absent/mild ↑	2	6	8	0.4
			subgroups by IgE levels: moderate ↑	14	15	29	0.401
			subgroups by IgE levels: severe ↑	7	6	13	0.325
			subgroups by TARC levels: absent/mild ↑	2	6	8	0.4
			subgroups by TARC levels: moderate ↑	18	18	36	0.27
			subgroups by TARC levels: severe ↑	4	2	6	0.192
			subgroups by IGA scores: 2	2	1	3	0.347
			subgroups by IGA scores: 3	6	9	15	0.991
			subgroups by IGA scores: 4	14	16	30	0.49

PNPLA1 rs4713956		Patient group		Patient breakdown by genotype			*p* value
				CC+CT	TT	total	
Control				121	21	142	
AD	total AD			91	19	110	0.592
	subgroups by IgE levels	absent/mild ↑		9	1	10	0.676
		moderate ↑		27	8	35	0.247
		severe ↑		14	2	16	0.805
	subgroups by TARC levels	absent/mild ↑		9	1	10	0.676
		moderate ↑		34	9	43	0.338
		severe ↑		6	1	7	0.974
	subgroups by IGA score	2		2	1	3	0.382
		3		14	3	17	0.758
		4		30	9	39	0.217
	subgroups with/without *FLG* mutation	*FLG* mutation-positive		18	5	0	0.394
		FLG mutation-negative	Total FLG mutation-negative AD	73	14	87	0.791
			subgroups by IgE levels: absent/mild ↑	7	1	8	0.861
			subgroups by IgE levels: moderate ↑	23	6	29	0.427
			subgroups by IgE levels: severe ↑	12	1	13	0.481
			subgroups by TARC levels: absent/mild ↑	7	1	8	0.861
			subgroups by TARC levels: moderate ↑	29	7	36	0.492
			subgroups by TARC levels: severe ↑	5	1	6	0.897
			subgroups by IGA scores: 2	2	1	3	0.382
			subgroups by IGA scores: 3	12	3	15	0.592
			subgroups by IGA scores: 4	25	5	30	0.792

SLC27A4 rs2240953		Patient group		Patient breakdown by genotype			*p* value
				AA+AG	GG	total	
Control				54	88	142	
AD	total AD			47	63	110	0.451
	subgroups by IgE levels	absent/mild ↑		3	7	10	0.612
		moderate ↑		16	19	35	0.404
		severe ↑		9	7	16	0.158
	subgroups by TARC levels	absent/mild ↑		4	6	10	0.904
		moderate ↑		22	21	43	0.125
		severe ↑		2	5	7	0.615
	subgroups by IGA score	2		1	2	3	0.866
		3		5	12	17	0.486
		4		22	17	39	0.04*
	subgroups with/without *FLG* mutation	*FLG* mutation-positive		11	12	0	0.372
		FLG mutation-negative	Total FLG mutation-negative AD	36	51	87	0.614
			subgroups by IgE levels: absent/mild ↑	1	7	8	0.145
			subgroups by IgE levels: moderate ↑	16	13	29	0.087
			subgroups by IgE levels: severe ↑	6	7	13	0.564
			subgroups by TARC levels: absent/mild ↑	3	5	8	0.976
			subgroups by TARC levels: moderate ↑	19	17	36	0.108
			subgroups by TARC levels: severe ↑	1	5	6	0.289
			subgroups by IGA scores: 2	1	2	3	0.866
			subgroups by IGA scores: 3	4	11	15	0.386
			subgroups by IGA scores: 4	17	13	30	0.059

NIPAL4 rs6860507		Patient group		Patient breakdown by genotype			*p* value
				AA+AG	GG	total	
Control				78	65	143	
AD	total AD			61	48	109	0.822
	subgroups by IgE levels	absent/mild ↑		3	7	10	0.133
		moderate ↑		18	17	35	0.739
		severe ↑		9	7	16	0.895
	subgroups by TARC levels	absent/mild ↑		4	6	10	0.372
		moderate ↑		23	20	43	0.903
		severe ↑		4	3	7	0.895
	subgroups by IGA score	2		3	0	3	0.116
		3		8	9	17	0.557
		4		18	21	39	0.352
	subgroups with/without *FLG* mutation	*FLG* mutation-positive		13	10	23	0.86
		FLG mutation-negative	Total FLG mutation-negative AD	48	38	0	0.852
			subgroups by IgE levels: absent/mild ↑	3	5	8	0.347
			subgroups by IgE levels: moderate ↑	15	14	29	0.781
			subgroups by IgE levels: severe ↑	7	6	13	0.963
			subgroups by TARC levels: absent/mild ↑	4	4	8	0.804
			subgroups by TARC levels: moderate ↑	18	18	36	0.624
			subgroups by TARC levels: severe ↑	4	2	6	0.558
			subgroups by IGA scores: 2	3	0	3	0.116
			subgroups by IGA scores: 3	7	8	15	0.56
			subgroups by IGA scores: 4	14	16	30	0.433

ELOLV4 rs3812153		Patient group			Patient breakdown by genotype			*p* value
					TT+TC	CC	total	
Control					137	6	143	
AD	total AD				109	6	115	0.699
	subgroups by IgE levels	absent/mild ↑			9	1	10	0.399
		moderate ↑			34	1	35	0.713
		severe ↑			16	0	16	0.405
	subgroups by TARC levels	absent/mild ↑			8	2	10	0.029*
		moderate ↑			43	0	43	0.172
		severe ↑			7	0	7	0.58
	subgroups by IGA score	2			3	0	3	0.721
		3			16	1	17	0.744
		4			38	1	39	0.639
	subgroups with/without *FLG* mutation	*FLG* mutation-positive			25	0	25	0.298
		FLG mutation-negative	Total FLG mutation-negative AD		84	6	90	0.408
			subgroups by IgE levels	absent/mild ↑	7	1	8	0.276
				moderate ↑	28	1	29	0.853
				severe ↑	13	0	13	0.451
			subgroups by TARC levels	absent/mild ↑	6	2	8	0.01*
				moderate ↑	36	0	36	0.21
				severe ↑	6	0	6	0.61
			subgroups by IGA scores	2	3	0	3	0.721
				3	14	1	15	0.653
				4	29	1	30	0.83

SUPPLEMENTARY REFERENCE

1. Hanifin JM, Rajka G. Diagnostic features of atopic eczema. Acta. Dermatol. Venereol. 92 (1980) 44-47.