

Letter to the Editor

IgE allergen component-based profiling and atopic manifestations in patients with Netherton syndrome

To the Editor:

Netherton syndrome (NS; OMIM no. 266500) is an autosomal recessive genodermatosis with atopic dermatitis (AD)-like erythroderma, a bamboo-like hair-shaft defect, and multiple atopic manifestations: asthma, urticaria, angioedema, allergic rhinitis, increased IgE levels, and blood hypereosinophilia.¹ NS is caused by serine protease inhibitor, Kazal type 5 (*SPINK5*) mutations leading to defective lymphoepithelial Kazal-type related inhibitor (LEKTI), a serine protease inhibitor expressed in the upper epidermal skin layers.¹ Loss of LEKTI inhibition results in a severe skin barrier defect.¹ *SPINK5* polymorphisms, especially the 420Lys LEKTI variant, have been associated with atopy and AD.²

We studied in detail the atopic symptoms in 10 Finnish patients with NS with *SPINK5* mutations. Our patients with NS had multiple allergies initiating neonatally and increasing rapidly in early childhood (Table I and see Tables E1 and E2 in this article's Online Repository at www.jacionline.org). Increased serum total IgE levels (up to 17,433 kU/L) were seen in 9 of 10 patients, and 8 of 10 had blood hypereosinophilia (Table I and see Table E2). Urticaria, asthma, and angioedema were common (Table I). We evaluated the IgE antibody profile specifically with the ImmunoCAP ISAC microarray (Thermo Fisher Scientific, Waltham, Mass) in 6 patients with NS 0.4 to 49.5 years of age, enabling the most precise IgE profiling in patients with NS to date (see the Methods section in this article's Online Repository at www.jacionline.org). Repeated ISAC microarray testing within the first 3 years for 2 patients (patients T4 and T6) showed that the initial IgE antibodies increased in intensity and new IgE sensitizations occurred rapidly for both, reaching 17% to 21% at 2 years and 35% by 3.2 years of age (Fig 1 and see Table E1). A similar trend in increasing specific IgE (sIgE) levels to multiple allergens with increasing age was noted for other patients (see Table E2).

Overall, the sIgE antibodies to allergens triggering clinical symptoms represented common food allergens, such as egg, cow's milk, wheat, and nuts, as well as pollens and animal proteins (see Table E1). All patients reacted against 14% to 37% of the allergen components. In comparison, considering that our study was not a case-control study, a cohort of 140 German pediatric patients with AD studied on an ISAC microarray with 95 allergen components showed mean sensitization to 7.6 ± 7.7 of allergens, ranging from 0% (in 14% of patients) to 34%, and higher numbers of sIgE responses associated with higher total IgE levels (3–26,567 kU/L).³ All our patients with NS had IgE antibodies to multiple potentially anaphylactic stable allergens, such as lipid transfer proteins (LTPs; 83%), storage proteins (17% to 33%), and tropomyosin (33%; see Table E1).⁴ Interestingly, patients with NS were sensitized to 1 to 8 different LTPs, especially hazelnut, peach, and mugwort, each in 4 of 6 patients, although LTP allergy is rare in Northern Europe.⁴ LTPs are important allergens in the Mediterranean region, but in Finland only 20% of 100 children (2–13 years old) and 12% of 100 adults (40–60 years old) studied for various allergies were sensitized to at least 1 of the 9 LTPs on the microarray (own unpublished data). None of 49 Swedish allergic children aged 3 months to

18 years had LTP IgEs.⁵ LTPs were not associated with anaphylaxis in our patients with NS. IgE microarrays are beneficial in patients with NS because they simply reveal the full extent of sensitization and sources of clinically relevant allergic reactions that should be avoided. Numerous targeted sIgEs would be needed to obtain equivalent data. However, sIgEs might be more sensitive than microarrays, and sIgEs allow analysis of specific allergen components not included in microarrays.

Three patients had anaphylactic reactions induced by egg (patient H1 at age 6 years), wheat (patient T4 at age 0.9 years), and cow's milk (patient T6 at age 6 years), and all had increased IgE levels for the allergens' stable components (see Tables E1 and E2). Otherwise stable allergens have elicited only mild oral allergy symptoms or skin flaring, which can be attributable to sensitization to the labile components of the allergens (Table I and see Table E1). Patients H2, H3, and T5 were sensitized to multiple storage proteins, but no severe allergic reactions have occurred, possibly because of strict elimination from their diets.

Oral allergen desensitization was beneficial for 2 patients. Patient H1 had wheat desensitization from 6 years of age, and she currently tolerates small amounts. Patient H2 started milk desensitization at 5.4 years of age for a period of 1.5 years. He currently tolerates small amounts of milk. A decrease in sIgE levels was seen after desensitization for both patients (see Table E2). Successful induction of oral tolerance to wheat and milk has previously been reported in 1 patient with NS.⁶ Severe allergic reactions are common in patients with NS, and thus oral desensitization should be considered.

All patients with NS were sensitized to at least 1 panallergen (profilins, pathogenesis-related 10 [PR-10] proteins, and tropomyosins), which are cross-reactive allergens inducing sensitization to multiple allergens.⁴ Eighty-three percent had IgE antibodies for PR-10 proteins, 67% for profilins, and 33% for tropomyosins. Patients T5 and T6 had antibodies for all 3 panallergens, and patient H1 had antibodies for PR-10 proteins and profilin. Patient H2 was sensitized to profilins only, and patients H3 and T4 were sensitized to PR-10 proteins (see Table E1). In 3113 allergic Italian patients only 1.2% recognized all 3 panallergens, 13.6% recognized 2 panallergens, and 86.4% recognized only 1 panallergen.⁷

We diagnosed eosinophilic esophagitis (EoE) in patient H2, which is the first reported NS case to our knowledge (see Table E3 in this article's Online Repository at www.jacionline.org). T helper cells and a mixed IgE- and non-IgE-mediated allergic response to allergens play a role in the pathogenesis of EoE. Most pediatric patients with EoE have allergies, asthma, eczema, chronic rhinitis, peripheral eosinophilia, increased IgE levels, nausea, vomiting, diarrhea, and failure to thrive, all features seen in patients with NS.⁸ Gastroesophageal reflux, irritability, and poor feeding were noted neonatally in patient H2. All symptoms responded rapidly to cisapride started at age 6 months. Concomitant cisapride and omeprazole, occasionally combined with ranitidine, have been used beneficially. Short breaks in the medication have resulted in relapsing symptoms. Gastroesophageal reflux and diarrhea are common in patients with NS and were observed in 7 of 10 patients but concurrently only in patient H2 (See Table E3). Increased fecal calprotectin and antitrypsin levels, reflecting intestinal inflammation and protein loss, respectively, were found

TABLE I. Atopic features in patients with NS

Patient ID	Age (y)	Atopy heritage	AD/U/AO/AS/R	IgE (kU/mL)	Eos (E9/L)	Current symptomatic allergies/eliminated foods	Subsided allergies
H1	8.5	+	AD/-/AO/-/-	972	0.83 (13%)	Egg white/A Cow's milk/OAS Wheat/SF, AO, OAS Rye/AO, OAS Barley/AO, OAS, SF Buckwheat/SF Fish/SF Birch cross-reactive vegetables and fruits/OAS Nuts/El Kiwi/El Celery/El	
H2	7.3	+	AD/U/AO/AS/R	17,433	1.47 (21%)	Egg white/AO, OAS, GI Cow's milk/AO, OAS, GI, SF Wheat/OAS Birch cross-reactive vegetables and fruits/OAS Barley/El Rye/El Fish/El Birch/R, AS Timothy grass/R Cat/SF Dog/SF Horse/SF	Beef/SF Pork/SF
H3	14.2	-	AD/-/-/-/R	2,025	0.64 (8%)	Kiwi/OAS Nuts/OAS Birch/R	Cow's milk/SF Egg white/SF Barley/SF Citrus fruits/SF Soy/SF
T1	10.5	+	AD/-/-/-/-	4,740	1.03 (8%)	Banana/OAS, SF Egg white/OAS, SF Cat/SF, AO Horse/SF, AO	Cow's milk/SF, GI Wheat/SF, GI
T2	7.5	+	AD/-/-/-/-	4,780	ND	Carrot/OAS, SF Watermelon/OAS, SF Egg white/OAS, SF Cat/P	
T3	4.7	+	AD/-/-/-/-	2,049	0.59 (4%)	Peanuts/OAS, SF Watermelon/OAS, SF Cat/P	
T4	2.9	+	AD/-/-/AS/-	240	1.80 (20%)	Wheat/A Barley/SF, GI Egg white/AO, GI	
T5	51.4	+	AD/U/AO/-/-	11,425	0.36 (4%)	Almond/SF, OAS, GI Coconut/SF, OAS, GI Nuts/SF, OAS, GI Raw carrot/SF, OAS, GI Kiwi/SF, OAS, GI Chickpea/SF, OAS, GI Fish/SF, OAS, GI Crustacean/SF, OAS, GI Birch/R, SF Timothy grass/R, SF	
T6	3.9	+	AD/U/AO/AS/-	7,229	2.76 (20%)	Cow's milk/A Egg white/GER Wheat/GER Rye/GER Barley/GER Chicken/GER Beef/GER Cat/SF	
T7	1.3	+	AD/-/-/-/-	19	0.35 (3%)	Cow's milk/GER Rye/GER Corn/GER Plum/GER	

Age indicates age at last evaluation. *Atopy heritage* indicates AD, asthma, or allergies in 1 or both parents or siblings.

A, Anaphylaxis; AD, erythrodermic atopic dermatitis; AO, angioedema; AS, asthma; El, eliminated for caution; Eos, highest blood eosinophilia; GER, gastroesophageal reflux; GI, gastrointestinal symptoms (vomiting and diarrhea); IgE, highest total serum IgE level; ND, no data; OAS, oral allergy symptoms; P, pruritus; R, rhinoconjunctivitis; SF, skin flaring; U, urticaria.

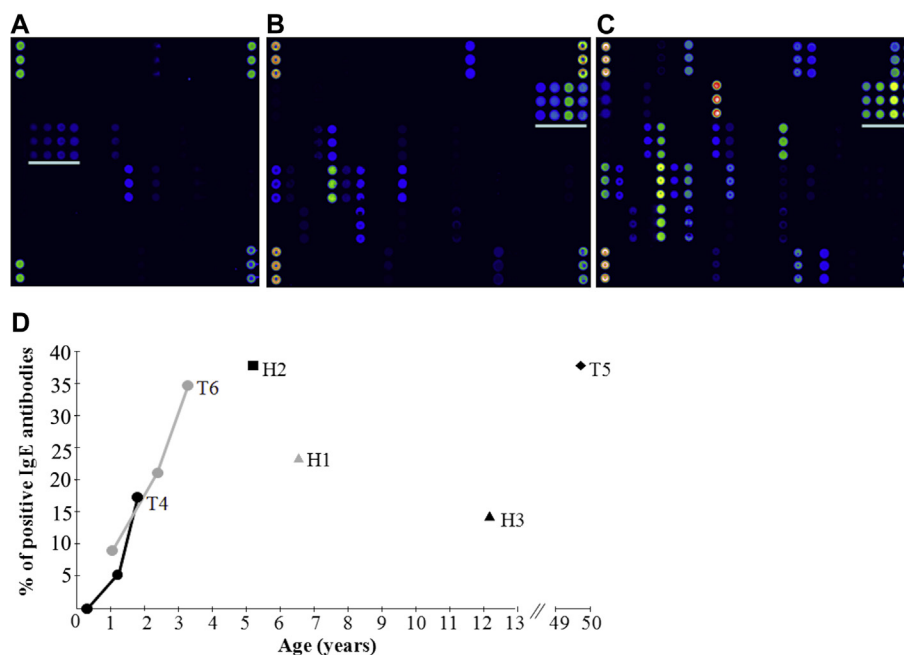


FIG 1. Rapid and numerous IgE sensitizations reflected by using the ISAC microarray in patients with NS. **A–C,** A rapid increase in the number and intensity of IgE antibodies in 3 consecutive ISAC microarrays for patient T6 to 9 (9%) of 103 components at age 1.0 year (Fig 1, **A**), 23 (21%) of 112 at 2.3 years (Fig 1, **B**), and 39 (35%) of 112 at 3.2 years age (Fig 1, **C**) are shown. An increase in intensity was seen from blue (>0.3 ISAC standardized units [ISU]) to green (>30 ISU) to yellow (>80 ISU) to red (>100 ISU). The white bar underlines the cow's milk allergens Bos d 4, Bos d 5, Bos d 6, and Bos d 8 on both microarrays. Individual allergens are listed in Table E1. **D,** Percentage of positive ISAC microarray IgE antibody levels in relation with age for patients T4 (black circles) and T6 (gray circles) connected by solid lines (3 consecutive ISAC microarrays were done for both patients). Patient H1, Gray triangle; patient H2, black square; patient H3, black triangle; and patient T5, black diamond.

in 4 of 7 patients within the first months. The gastrointestinal tract is a major site for food allergen sensitization and an important site of early normal microbial contact. Duodenal biopsy specimens from patients H2 and T1 were normal, and immunostaining excluded deficiency in enteroendocrine cells or major antimicrobial peptides (see Table E3 and the Methods section in this article's Online Repository).

Many allergens contain serine or cysteine protease activity, which disrupts the epithelial barrier, and some induce inflammatory responses, causing greater sensitization. Whether LEKTI inhibits the activity of environmental proteases is not known. Loss of LEKTI inhibition of allergen proteases combined with increased allergen penetration caused by severe barrier defect could contribute to the rapid and vast allergic sensitization observed in patients with NS. Reduced LEKTI expression in sinonasal epithelium is associated with chronic rhinosinusitis and allergies, but LEKTI is not expressed in the gut epithelium.⁹ Disruption of the airway epithelial barrier might also predispose patients with NS to increased sensitization by inhaled allergens. Further studies will be needed to elucidate whether the early and promiscuous sensitization to especially LTP allergens, which are more ingestion than inhalation induced, could be explained by a functional barrier defect in the mucous membranes of the intestine in patients with NS and whether the intestinal microbiome might contribute to this effect.

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MATERIALS AND METHODS

Patients

NS patients were recruited from the University central hospitals in Helsinki (H1-H3) and Tampere (T1-T7). Written informed consent was obtained and this study was approved by the Coordinating Ethical Review Board of the Helsinki and Uusimaa hospital district, Finland.

Serum IgE levels and Immucap ISAC microarray analyses

Serum immunoglobulin levels for total IgE (S-IgE), specific IgE (sIgE) to allergens (ImmunoCAP) and allergen components (ImmunoCAP ISAC microarray) were determined according to the manufacturer's instructions (Thermo Fisher Scientific, Uppsala, Sweden). Data was analyzed with the Microarray Image Analysis Software. The ISAC microarray is a semi-quantitative assay containing 112 (103 in the previous version) individual allergen components. Allergen-specific IgE-antibodies are expressed in arbitrary ISAC Standardized Units (ISU): <0.3 = negative to very low, $0.3-0.9$ = Low, $1.0-14.9$ = Moderate to High, ≥ 15 = Very high.

Gastrointestinal biopsy immunostaining

Formalin-fixed, paraffin-embedded duodenal biopsies from H2 and T1 were analyzed with standard immunohistology protocols with monoclonal antibodies against LEKTI (clone 6A108), human beta defensin 5 (hBD5) (clone 8C8), chromogranin A (CgA) (clone DAK-A3), and 5-hydroxytryptamine (5-HT) (clone NB120-16007). The bound antibodies were detected with ImmPRESS Universal Antibody Polymer Detection Kit, and AEC SK 4200. Duodenum slides obtained from elective surgery patients were used as controls.

Table E1. Positive allergen component-specific IgE antibodies on the ISAC microarray in NS patients.

			H1	H2	H3	T4			T5	T6				
		Age (years)	6.6	5.2	12.2	0.4	1.1	1.8	49.5	1.0	2.3	3.2		
		Positive (n/total)	26/112	38/103	16/112	0/103	6/112	19/112	41/112	9/103	23/112	39/112		
		Positive (%)	23	37	14	0	5	17	37	9	21	35		
	Allergengroup/allergen	S (%)	8(31%)	28(74%)	8(50%)	0	6(100%)	11(58%)	21(51%)	8(89%)	11(48%)	16(41%)	N	N/6
Foods	Egg white	Component	*A	*	*			*				*		
	Ovomucoid	S Gal d 1	0.4				2.4	2.9					2	33 %
	Ovalbumin	S Gal d 2	0.9	10			0.4	1.4		0.3	6.5	20	4	67 %
	Ovotransferrin	S Gal d 3		1.9			0.6	7.7				1	3	50 %
	Milk		*	*	*							*A		
	α-lactalbumin	S Bos d 4	0.3	16						1.6	9.5	31	3	50 %
	β-lactoglobulin	S Bos d 5		26					0.6	1.8	13	40	4	67 %
	Casein	S Bos d 8		16						2.5	14	59	2	33 %
	Lactoferrin	S Bos d LF		3.6								0.3	2	33 %
	Wheat		*	*				*A				*		
	Wheat LTP	S Tri a 14"	12				6.3	11				0.4	3	50 %
	α-amylase/trypsininhibitor	S Tri a aA_Ti	16	2.8			0.4	5.7					3	50 %
	Tri a gliadin	S Tri a gliad""		0.8									1	17 %
	Fish		*						*			*		
	Carp	S Cyp c 1""		0.6									2	33 %
	Cod	S Gad c 1						0.3	22				1	17 %
	Kiwi				*				*					
	Cysteineprotease	S Act d 1	18		0.7								2	33 %
	Thaumatococcus protein	C Act d 2							1.6				1	17 %
	Nut/seedstorageproteins													
	Walnut	S Jug r 1"			7.4 *				7.3 *				2	33 %
	Peanut	S Ara h 2			0.4 *				1.6 *				2	33 %
	Peanut	S Ara h 6"			0.6 *				1 *				2	33 %
	Cashew	S Ana o 2		0.4	*				*	0.8			2	33 %
	Hazelnut	S Cor a 9		1.8	*				*				1	17 %
	Brazil nut	S Ber e 1			*				0.9 *				1	17 %
	Buckwheat	S Fag e 2"	*						1				1	17 %

	Soy	S	Gly m 5		0.5	*								1	17 %
	Soy	S	Gly m 6		3.5									1	17 %
Animals	Animalproteins														
	Cat	S	Fel d 1		65 *			6.7	37	7.2	34	79*	4	67 %	
	Cat	S	Fel d 4		21 *			2.4	3.9	1.1	7	27*	4	67 %	
	Dog	S	Can f 1		64 *		4.5	14					2	33 %	
	Dog	S	Can f 2		46 *					1.6	7.1	40	2	33 %	
	Horse	S	Equ c 1"		*			6.6	4.6		5.5	31	3	50 %	
	Mouse	S	Mus m 1		27			4	18		4.9	15	4	67 %	
	Serum albumin														
	Bovineserum albumin	C	Bos d 6		54 *					2.4	27	79 *	2	33 %	
	Cat	C	Fel d 2		22 *						1.8	5.8	2	33 %	
	Dog	C	Can f 3		49 *						1.3	5.8	2	33 %	
	Horse	C	Equ c 3		98 *						0.8	5.8	2	33 %	
Pollens	Grass				*				*						
	Timothygrass	S	Phl p 1		71				27				2	33 %	
	Timothygrass	S	Phl p 2						104				1	17 %	
	Timothygrass	S	Phl p 4		0.7								1	17 %	
	Timothygrass	S	Phl p 5						113				1	17 %	
	Timothygrass	S	Phl p 6						111				1	17 %	
	Timothygrass	C	Phl p 7						4.2				1	17 %	
	Timothygrass	S	Phl p 11		1.1								1	17 %	
	Bermuda grass	S	Cyn d 1		26				6.2				2	33 %	
	Otherpollens														
	Olive	S	Ole e 1		0.8								1	17 %	
	Olive	S	Ole e 9”								0.8	1.7	1	17 %	
	Ragweed	S	Amb a 1		0.8								1	17 %	
	Saltwort	S	Sal k 1		0.4								1	17 %	
	Cypress	S	Cup a 1							1			0.7	2	33 %
	Mugwort	S	Art v 1							18				1	17 %
	Japanescedar	S	Cry j 1			0.4								1	17 %
Pan-allergens	PR-10 proteins														
	Birch	C	Bet v 1	39	*	20 *		7.7	29 *			124	5	83 %	
	Alder	C	Aln g 1	0.5		3.4		0.8	5.4			24	5	83 %	
	Hazel pollen	C	Cor a 1.01	2.3		1.2		0.7	5			6.4	5	83 %	

[illegible]

	NPC2 family	S	Der p 2		6.3	60							2	33 %
	NPC2 family	S	Eur m 2""		2.1								1	17 %

Age, the age in years when the ISAC microarray with 103 (an earlier version) or 112 components was performed. "component not on ISAC 103, ""component only on ISAC 103. The positive number and percent of components on the ISAC microarray. Stable food allergens are highlighted in bold. <0.3 ISU, negative to very low (blank); 0.3-0.9 ISU low (white); 1–14.9 ISU moderate to high (light grey), ≥15 ISU very high (dark grey). S, mainly species-specific allergen; C, cross-reactive allergen.⁴ N, patients positive for allergen component; N/6, percentage of patients positive for allergen component. Allergic symptoms ever received from the allergen source are marked by an asterisk*; A, anaphylaxis.

[illegible]

[illegible]

Table E3. Gastrointestinal findings in NS patients.

Case	GER	Diarrhea	Calprotectin (<100 ug/g) (Age)	Alpha1-antitrypsin (<268 ug/g) (Age)	Duodenal histology (Age)	Duodenal immunohistology CgA, 5-HT, hBD5	Esophageal biopsy
H1	+	-	143-1232^{a)} (5.2)	420- >800 (6.1)	ND	ND	ND
H2	+	+	187-476^{b)} (5.3)	478-953^{c)} (5.3)	Normal (2.3)	Normal (2.3)	Moderate esophagitis with marked eosinophilia 60 (0.4)-80 (1.4) eosinophils per HPF and eosinophilic spongiosis.
H3	-	-	ND	ND	ND	ND	ND
T1	-	+	ND	1000-2400 (1.5)	Normal (1.5)	Normal (1.5)	ND
T2	-	-	ND	ND	ND	ND	ND
T3	-	-	ND	ND	ND	ND	ND
T4	-	+	15 (2.3)	169 (2.3)	ND	ND	ND
T5	-	+	38 (50)	175 (5.0)	ND	ND	ND
T6	+	-	82 (3.5)	95 (3.5)	ND	ND	ND
T7	+	-	235 (0.75)	152 (0.75)	ND	ND	ND

Abnormal fecal calprotectin and alpha1-antitrypsin levels are indicated in bold. Age, in brackets, indicates last measurement in years. GER, gastroesophageal reflux. ND, no data. Levels normalized by the age of a) 3 years, b) 5.3 years, c) 4.3 years. CgA, chromogranin A. 5-HT, 5-hydroxytryptamine. hBD5, human beta defensin 5. HPF, high power field.