IgE sensitization profile in patients with Netherton syndrome

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Author contribution statement

ES, DC, EC and AGC carried out the experiments and data collection. ES and BD recruited the patients. ES and AS performed the statistical analysis. ES, DC and RP conceived the study and assisted in data interpretation. All authors reviewed, edited and approved the final manuscript.

All Authors consent for publication, and confirm that this manuscript is original, has not been published before, is not currently being considered for publication elsewhere, and has not been posted to a preprint server.

Conflict of interest

ES has received consultant arrangements and speakers' bureau participation from Stallergenes, Thermo Fisher Scientific, and non-financial support from Macroarray Diagnostics, Vienna, all outside the submitted work. The other authors have no conflicts of interest to declare that are relevant to the content of this article.

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Data Availability Statement information

The data that support the findings of thisstudy are available on requestfrom the correspondingauthor. The data are not publiclyavailable due to privacy or ethical restrictions.

ETHICAL APPROVAL STATEMENT

The research was conducted ethically following the World Medical Association Declaration of Helsinki. All subjects or caregivers have given their written informed consent and that the study protocol was approved by the institute's committee (106/05).

Dear Editor,

Loss-of-function mutations in the serine protease inhibitor lymphoepithelial Kazal-type related inhibitor (LEKTI) encoding gene, SPINK5, determines a severe autosomal recessive syndromic form of ichthyosis named Netherton syndrome (NS, OMIM 266500)(1). NS is characterized by the association of congenital scaly erythroderma(2) with hair abnormalities(3) and severe atopic manifestations, including atopic dermatitis-like lesions, incoercible itch, environmental and food allergies, hypereosinophilia, and highly increased levels of IgE(4). LEKTI deficiency results in unopposed serine protease activity leading to premature degradation of corneodesmosomal protein components highly accelerated stratum corneum desquamation and a profound skin barrier defect with severe skin inflammation(4),(5).

We sought to extensively characterize the IgE sensitization profile in 10 Italian patients (6 female, mean age 13.8±12.2) whose diagnosis has been confirmed by SPINK5 molecular analysis or anti-LEKTI immunohistochemistry (Table 1). The study received ethical approval from IDI-IRCCS Ethical Committee (106/05) and written informed consent was obtained from all patients or caregivers before participation, following the Declaration of Helsinki. Nine of 10 patients showed increased serum total IgE levels (ranging from 155 to 16,300 kU/L), and blood eosinophilia (ranging from 230 to 850/ μl; Table | 1).

It is widely known that when the total IgE is extremely high, the use of the current singleplex systems can result in non-specific antigen recognition (6). This problem does not occur with multiplexed microarray analytical systems (7). We, therefore, evaluated the IgE sensitization profile in our NS patients, with the ImmunoCAP ISAC® (Thermo Fisher Scientific, Sweden) microarray system, without any background problems resulting in impaired allergenic recognition due to extremely high IgE levels found in these patients (Figure 1 | A). In 2 cases (NS#2 and NS#4) the repetition of the microarray testing at one-year intervals showed comparable values, demonstrating the reproducibility and robustness of the data and also in complex cases with IgE levels >10000 kU/L (data not shown).

Overall, a quite heterogeneous reactivity profile was observed in NS patients: none of the subjects studied was negative, and the molecular recognition ranged from 6.3% to 66.3% of the total allergen components investigated (Figure 1 | B)

Analyzing the genuine molecules of the main indoor and outdoor allergenic sources represented in the microchip, group 1 and 2 molecules from dust mites were by far the most frequently positive in the NS population studied (9/10 patients), followed by the Ole e 1 allergen from the Olive tree (7/10). All other genuine molecules studied were scattered among the patients, being heterogeneously recognized only by some of them (i.e. Cup a 1 from the cypress tree in 3/10 cases, Alt a 1 from Alternaria alternata in 2/10, Art v 1, the major allergen of mugwort pollen in 2/10, Can f 1 from Canis familiaris in 4/10, Fel d 1 from Felis domesticus in 3/11, group 1 and 5 molecules from the grasses in 4/10, and Par j 2 from pellitory in 3/10).

Two patients, curiously the youngest (3-year-old) and the oldest of our series (43-year-old) were strongly reactive to latex, being allergic to Hev b 6 and Hev b 5 respectively, with an anaphylactic reaction after exposure to the allergen, in the second case.

Three patients (NS#1, 2, and 7), had a serious allergy to cow's milk, with recognition of both thermolabile molecules (α-lactalbumin and β-lactoglobulin) and thermostable casein (Bos d 8), in all cases associated with immediate urticarial reactions after ingestion of the food, both raw and cooked. The same 3 patients also had a clinical history suggestive of adverse reactions (in 2 cases anaphylactic) after ingestion of cooked hen's egg (all patients were allergic to both the thermostable protein Gal d 1, and the thermolabile Gal d 2 and Gal d 3). None of the patients had reactivity for Gald5 α-livetine; indeed they regularly ate poultry meat. Interestingly, the remaining 7 patients studied never had any problems with egg or milk. NS patient #2 was also reactive to wheat (Tri at 19; ω-5 gliadin). Four patients also scored reactive for Act d 1 from kiwi. Two patients (NS#4 and 5) had severe fish allergy confirmed by cod parvalbumin reactivity (Gad c 1), with a positive clinical history of anaphylactic events. One of the patients studied had a simultaneous reactivity for 11s globulin from hazelnut (Cor a 9), peanut (Ara h 2), walnut (Jug r 1), and sesame (Ses i 1) 2s Albumins, while another patient was mono reactive to the walnut 2s Albumin-(Jug r 1).

The main plant panallergens available on the array (nsLTP, Profilin, PR-10, and Polcalcin) were also studied. At least one out of the 8 molecules belonging to the nsLTP superfamily scored positive in 8/10 subjects studied, Pru p 3 from peach being the most frequently recognized (5 patients). Eight/10 patients were immunoreactive to profilin panallergen, whilst 3/10 scored positive to PR10 molecules. Since none but one patient was monoreactive to LTPs, but also immunoreactive to PR10 or Profilin, no cases of severe reaction to LTP were recorded in our patients, probably due to the mitigating effect exerted by the multiple vegetable panallergen recognition in LTP allergic patients (8), similar to what already observed in Finnish surveying of 10 patients with NS (9).

None of the NS patients was reactive to polcalcin or cross-reactive carbohydrate determinants (CCDs).

Interestingly, when we considered the IgE reactivity profile overall and applied a hierarchical analysis, we observed the formation of 2 main reactivity clusters. The first, included 3 patients (# 4, # 8 and # 9), who were PR10 reactors, also sensitized to the olive tree, house dust mite molecules and grasses, while the second cluster was characterized mainly by dust mites, profilins and LTPs reactivity.

In conclusion, this study demonstrated the efficacy of a proteomic microarray approach in the study of rare diseases presenting immune defects and characterized by extremely high IgE levels, such as NS, where the assessment of specific reactivity can be hampered due to the limitations of an *in vitro* dosing with singleplex methods. Moreover, constraints related to the availability of small amounts of serum, especially in very young patients, do not represent a limit for multiplexed microarray analytical systems, which allow for obtaining reliable and reproducible results on hundreds of allergenic molecules with a few microliters of the serum. By using this approach, we succeeded in decoding comprehensive reactivity profiles, useful in the clinical management of this rare but severe disease.

Table 1. Demographical and molecular features of Netherton syndrome patients.

Pz#	Age	Sex	IgE (kU/L)	Eos (μl)	*SPINK5* **mutations (NM_-006846.3)**	**Mutation conse-quences**	**LEKTI expres-sion (IHC)**	**Refs**
1	12	F	13,175	800	*c.153delT / c.891C>T*	p.Gln52Lysfs*6 / Altered splicing	Highly reduced	(10,11)

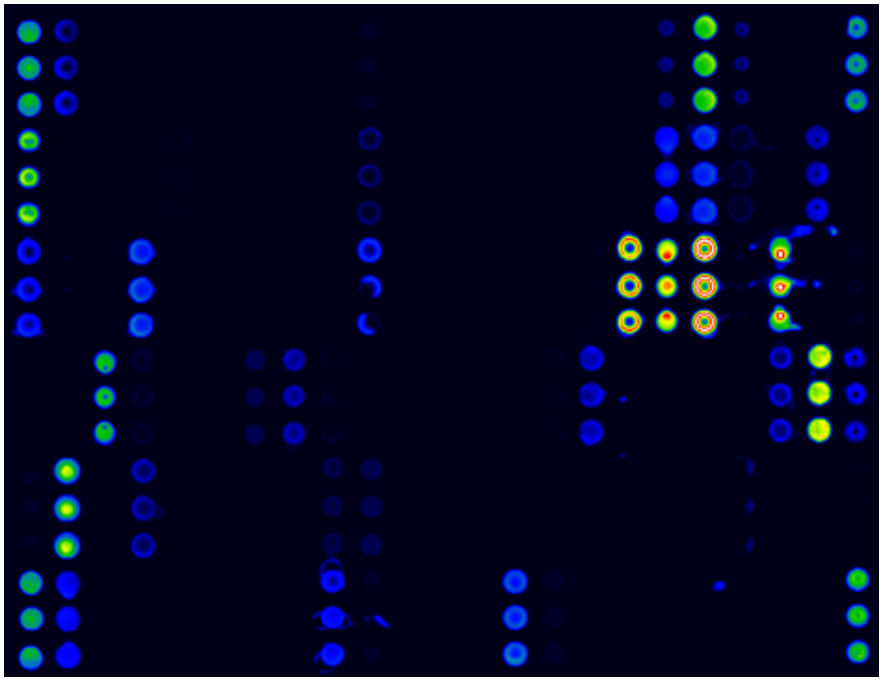
Pz#	Age	Sex	IgE (kU/L)	Eos (µl)	*SPINK5* **mutations (NM_-006846.3)**	**Mutation consequences**	**LEKTI expression (IHC)**	**Refs**
2	3	M	16,300	670	*c.1431-12G>A / c.1431-12G>A*	Altered splicing / Altered splicing	Absent	(12)
3	4	F	21	230	c.1129G>T / c.2667-2A>G	**p.Gly377* / Altered splicing**	Absent	This stu
4	43	M	1,711	750	*c.238dupG / c.238dupG*	p.Ala80Glyfs*19 / p.Ala80Glyfs*19	Absent	(13)
5	4	M	2,145	850	*c.316_-317delGA / c.1302+4A>T*	p.Asp106Trpfs*17 / Altered splicing	Highly reduced	(14,15)
6	19	F	1,718	450	*c.1111C>T / c.2041_-2042delAG*	p.Arg371* / p.Arg681Glufs*28	Absent	(16)
7	5	M	155	300	*c.238dupG / c.891C>T*	p.Ala80Glyfs*19 / Altered splicing	Highly reduced	(11)
8	13	F	1,035	390	*ND / ND*	/	Absent	/
9	13	F	893	650	*c.1302+4A>T / °*	Altered splicing / °	Reduced	(14)
10	22	F	635	560	*ND / ND*	ND / ND	Absent	/

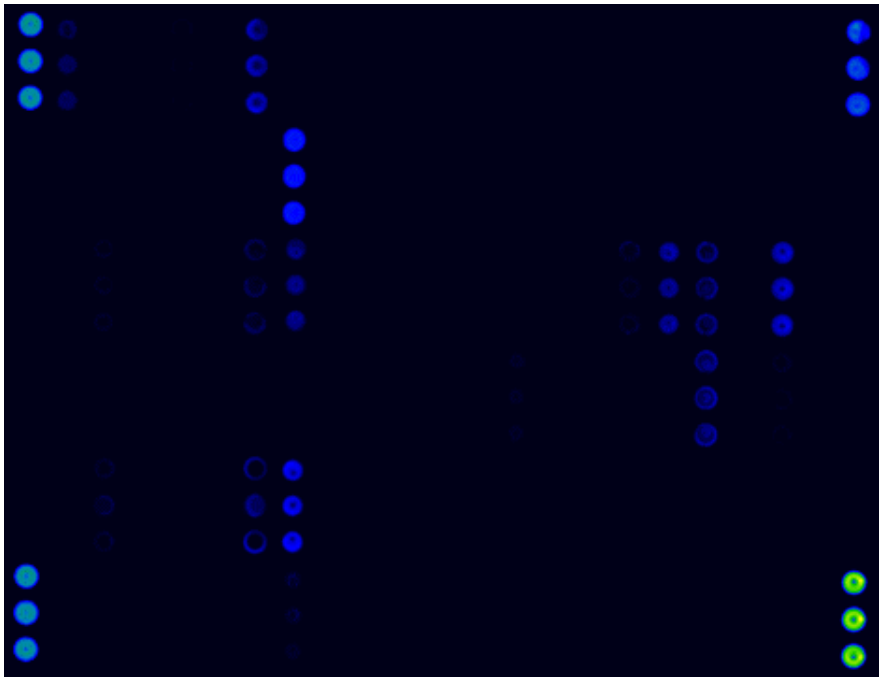
Clinical diagnosis confirmed by SPINK5 genetic testing on genomic DNA from blood and/or immunohistochemistry (IHC) on skin sections from formalin-fixed paraffin-embedded biopsies using anti-LEKTI (*lymphoepithelial Kazal-type-related inhibitor*) polyclonal antibodies directed to the D7D12 or D13D15 C-terminal regions, as described(17).

° The second mutation was not identified; **ND** : not done.

Previously unreported SPINK5 mutations are in bold

A





NS#1NS#4

B

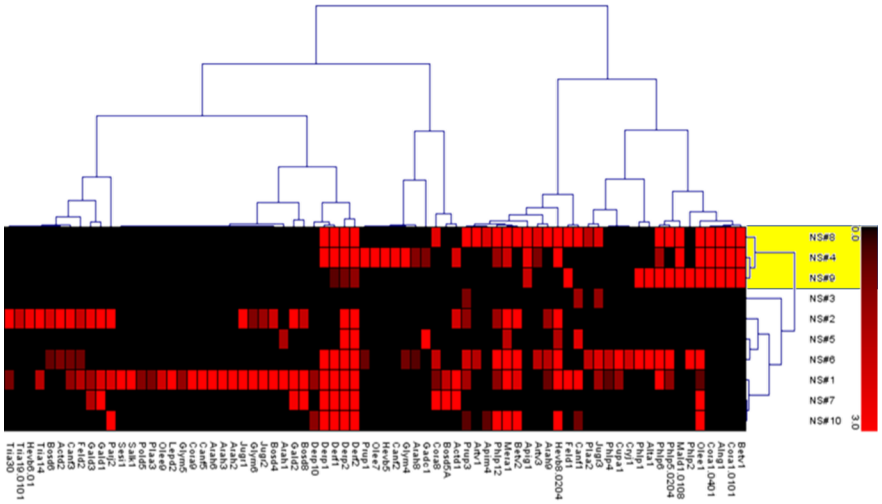


Figure 1

A | Two exemplary cases of multiple IgE reactivity evaluated with proteomic microarray in patients with Netherton syndrome and elevated IgE levels (NS# 1: 13,175 kU / L; NS# 4: 1,711 kU / L);

B | Supervised one-way hierarchical clustering analysis of molecular allergen IgE values. Allergens are reported on the x-axis, subjects on the y-axis. The black to light red scale corresponds to IgE values from negative to strongly positive. Cluster A, including patients # 4, # 8 and # 9, is highlighted in yellow.

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