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Eosinophilic esophagitis and colonic mucosal eosinophilia in Netherton syndrome

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Key words:

Netherton, eosinophilic esophagitis, colonic mucosal eosinophilia, LEKTI, IgE

List of abbreviations:

NS: Netherton syndrome

EoE: eosinophilic esophagitis

CME: colonic mucosal eosinophilia

IgE: immunoglobulin E; sIgE: specific immunoglobulin E

MIM: Mendelian Inheritance in Man

HPF: high-power field

SD: standard deviation

SFED: six-food elimination diet

Capsule summary

Nutritional management in Netherton syndrome (NS) is complicated by frequent digestive symptoms. We report the presence of eosinophilic esophagitis and colonic mucosal eosinophilia in NS. Digestive endoscopies should be considered in NS patients with growth retardation and digestive symptoms.

To the editor:

Netherton syndrome (NS, MIM#256500) is a severe autosomal recessive disorder characterized by chronic skin inflammation and a high prevalence of atopic manifestations and allergies. NS is caused by loss-of-function mutations in *SPINK5* encoding an anti-serine protease (LEKTI) expressed in stratified epithelia¹. Although skin investigations analyzing the association between the dermatitis and the disruption of skin barrier are now documented¹, little is known about the digestive consequences of NS. Nutritional difficulties are major complications with frequent severe failure to thrive. Food allergies rapidly become a dominant trait, persist throughout life, and are related to a high level of total and specific IgE (sIgE) to foods². Clinical manifestations may involve digestive symptoms, and recently eosinophilic esophagitis (EoE) in one NS child has been reported². We shared this experience of EoE in one NS child in our French reference center (MAGEC Center, Necker-Enfants-Malades Hospital, Paris) and thus included digestive endoscopy in the routine work-up of NS patients when presenting with digestive symptoms. Blood and mucosal samples were collected in a registered collection (French Ministry of Health N° DC-2009-955).

Twelve patients (mean age: 6.5 years [1-15]) with NS who had a severe cutaneous involvement and digestive symptoms were referred to the pediatric gastro-intestinal department for further investigation and dietary adaptation. *SPINK5* genetic testing was not available but NS diagnosis was confirmed by the total lack of LEKTI expression in cutaneous immunostaining in all patients as previously described³. All had growth retardation with mean height -1.6 SD [-3 - -0.5], and mean weight -1.5 SD [-3 - -0.5]. All had experienced chronic abdominal pain from early infancy and 4 had persistent vomiting and diarrhea. Other allergic manifestations had been anaphylaxis in 4 patients, angioedema in 2, oral allergy syndrome in 2, urticaria in 3 and asthma in 4 patients. Diet was normal in only 1 patient whereas 11 patients were carrying out an elimination diet of staple foods (milk and dairy products, egg

and/or wheat), in association with more specific eliminations in 9 patients (e.g. peanuts, fish, soy), based on parents observation. Skin testing was not possible regarding to major skin alteration. Difficulties in feeding had led to enteral feeding in 6 patients (mean age: 6.5 years). Total IgE were constantly above normal value for age (mean IgE 4627 kU/L [42-27211]). Almost every patient had positive sIgE for staple foods (cow milk, egg white, wheat) (table). Endoscopy showed EoE (with >15 eosinophils/HPF⁴) in 4/12 patients among which 3 were in contact with at least 4 of the 6 foods considered as major allergens in the so-called “6 food elimination diet” (SFED)⁵. Among these 8 patients with reduced number (2/8) or no eosinophils (6/8), all but 1 had a food elimination diet involving at least 3 of the SFED. One patient, nonetheless, did not have EoE despite normal oral feeding. Medians of total IgE were significantly higher in NS patients with EoE (8195 kUI/L L [2586-27211]) than in NS patients without EoE (659.5 kUI/L [42-4744]) with $p=0.02$. Among the 10 patients who had a colonoscopy, 8 (including the 4 patients with EoE) had > 20 eosinophils/HPF (upper level of tissue eosinophilia in a pediatric review of eosinophilic colitis⁶), one had >10 eosinophils/HPF (moderate tissue eosinophilia) and one had a maximum number of 10 eosinophils/HPF only in the sigmoid colon. Noticeably, in one child with severe esophageal eosinophilia and who was not following any diet, adherence to the modified SFED (SFED plus elimination of foods oriented by allergy testing) reduced the mucosal eosinophils count from 100/HPF to 15/HPF in the esophagus and from 34/HPF to 9/HPF in the colon. The expression of LEKTI was tested in the esophageal mucosa of all NS patients, and compared to 15 routine esophageal samples (without EoE), and to 10 esophageal samples of patients with EoE without NS (with comparative analysis of a positive normal skin control and the demonstration of internal negative control areas for every sample). In all control samples, an extracellular expression of LEKTI was observed in the esophageal mucosa. In non NS EoE patients, LEKTI immunostaining was always positive, either normal (8/10) or reduced (2/10).

In contrast, none of the NS patients showed any esophageal immunoreactivity for LEKTI (image).

This study thus shows a high frequency of established EoE or increased esophageal eosinophilia in NS patients, despite elimination of the foods considered as major EoE triggers.

This suggests that EoE might be a major trait of NS. The esophageal epithelium is a squamous epithelium and is affected by SPINK5 deficiency, similarly to skin, and thus may exhibit a noticeable defect in epithelial barrier such as seen in the skin. It remains unclear whether LEKTI deficiency could favor the IgE response to allergens and then lead to high levels of total and sIgE as observed in NS. This is in good agreement with a recent study in atopic dermatitis confirming that *SPINK5* polymorphism was associated with high IgE serum levels⁷. EoE during NS might thus be related to both LEKTI deficiency in the esophagus and the high level of IgE-mediated food reactivity. Colonic mucosal eosinophilia (CME) happens to be also a trait of NS. The finding was not surprising, owing to the frequency of digestive symptoms during NS. An association between different digestive locations of eosinophilic disorders has already been described and might be present in patients with NS⁸.

Further studies should confirm that EoE and CME are probably common features of NS. Digestive endoscopies should be considered in NS patients with very high total IgE levels (above 2000 kU/L), growth retardation and digestive symptoms. In addition to current handling of NS patients, an appropriate care of the digestive tract involvement might largely help patients and caretakers and could be a crucial step.

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Legends:

Figure: LEKTI immunostaining (Santa Cruz Biotechnology™) in esophageal samples:

A, B: control patients: granular immunostaining.

C, D: non NS EoE patients: slightly reduced (C) or significantly reduced reactivity (D, arrows).

E, F: NS EoE patients: total lack of immunoreactivity.

G: positive immunohistochemical control on normal skin

H: negative immunohistochemical control on normal intestinal epithelium

A, C, E, F: original magnification x 20

B, D, G, H: original magnification x 40.

Table:

Biological and endoscopic findings in NS patients.

RC: right colon; TC: transverse colon; LC: left colon; S: sigmoid colon; R: rectum.

Statistics:

Quantitative data expressed as mean/median [min-max] or number of patients and percentage as appropriate. Qualitative data represented in the form of frequency and percentage. Prism 6.01 (GraphPad Software Inc., California USA) was used to analyze significant difference (Mann-Whitney U test) between patients with EoE and NS patients without EoE. P value was taken as significant when < 0.05 .

Patient (gender, age)	1 (Girl, 2years)	2 (Girl, 6years)	3 (Girl, 1year)	4 (Boy, 11years)	5 (Girl, 5years)	6 (Boy, 15years)	7 (Boy, 7years)	8 (Boy, 1year)	9 (Boy, 3years)	10 (Girl, 15years)	11 (Girl, 8years)	12 (Boy, 4years)
Blood eosinophils (/mm ³)	4000	NA	500	1000	500	1900	700	900	600	500	300	200
Total IgE (kU/L)	839	2586	153	4744	3277	27211	706	13113	613	42	220	2023
Specific IgE kU/L	Milk: 0,8 Wheat: 1,5 Egg white: 7,6 Peanut: NA Soy: <0,1	Milk: 385 Wheat: 70,2 Egg white: 73,8 Peanut: 59,8 Soy: 31,6 Fish: 76,3	Milk: 64,4 Wheat: 7,3 Egg white: 14,6 Peanut: <0,1 Soy: <0,1 Fish: <0,1	Milk: 0,2 Wheat: 1,1 Egg white: 0,4 Peanut: NA Soy: 0,2 Fish: NA	Milk: 68 Wheat: NA Egg white: >100 Peanut: 0,5 Soy: NA Fish: >100 Tree nuts: 0,9	Milk: 1,4 Wheat: 16,1 Egg white: 5,3 Peanut: 37,4 Soy: 7,3 Fish: NA	Milk: 16,9 Wheat: 3,6 Egg white: 2,2 Peanut: NA Soy: 0,9 Fish: NA	Milk: 2,5 Wheat: 16,3 Egg white: 5,5 Peanut: 18,8 Soy: 8,5 Fish: 0,6	Milk: 12,5 Wheat: 12,5 Egg white: 42,6 Peanut: 0,6 Soy: NA Fish: NA Treenuts : 12,5	Egg White <0,1 Egg Yolk <0,1 other: not done	Milk: <0,1 Wheat: 0,7 Egg white: 0,2 Peanut: <0,1 Soy: <0,1 Fish: <0,1	Milk: 15,2 Wheat: 10 Egg white: 3,9 Peanut: 17,7 Soy: 5,5 Fish: 0,2 Beef: 5,6
Eosinophilic Esophagitis	No (0 Eo/HPF)	Yes (60 Eo/HPF)	No (0 Eo/HPF)	No (0 Eo/HPF)	Yes (22 Eo/HPF)	Yes (100 Eo/HPF)	No (0 Eo/HPF)	Yes (20 Eo/HPF)	No (10 Eo/HPF)	No (6 Eo/HPF)	No (0 Eo/HPF)	No (1 Eo/HPF)
CME (Eo/HPF)	RC=31 TC=29 LC=29 S=13 R=7	RC=13 TC=18 LC=7 S=7 R=11	RC=22 TC=27 LC=NA S=17 R=25	RC=NA TC=NA LC=12 S=22 R=8	RC=11 TC=9 LC=28 S=1 R=5	RC=30 TC=34 LC=18 S=5 R=13	RC=20 TC=14 LC=12 S=5 R=5	RC=20 TC=9 LC=21 S=11 R=4	RC=34 TC=28 LC=26 S=24 R=36	Not done	RC=9 TC=9 LC=5 S=10 R=6	Not done

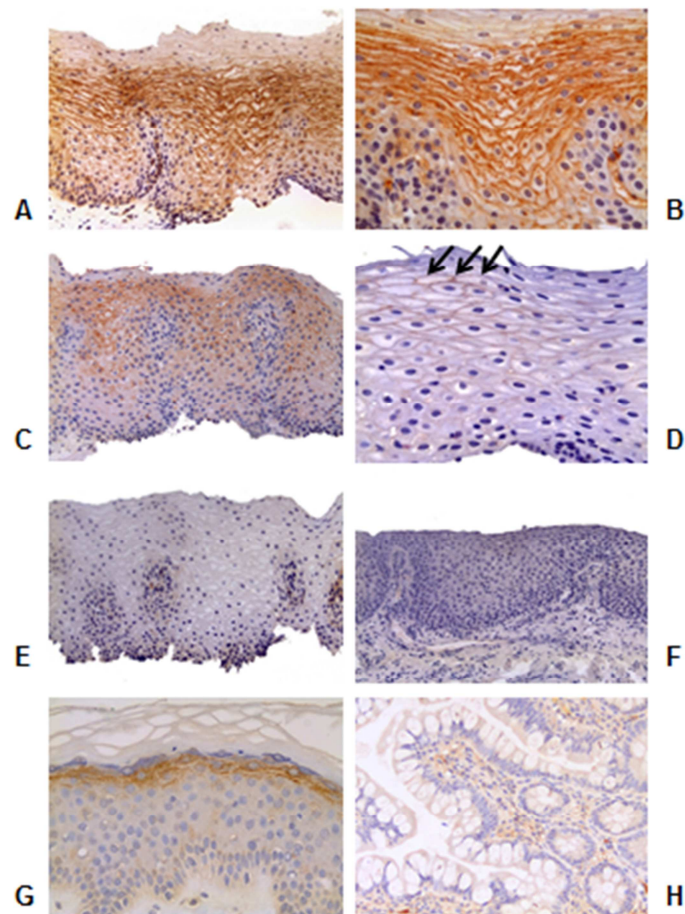


Figure: LEKTI immunostaining in esophageal samples.