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KEYWORDS

egg white, food allergy, IgD, IgG4, ovomucoid

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTION

NI, MY, AK, HM, EN, YH, and KO collected the samples. NI wrote the manuscript. YO organized the study. All authors read and approved the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Severe cold urticaria can point to an underlying clonal mast cell disorder

To the Editor,

Gain-of-function mutations of KIT, most frequently D816V, cause clonal mast cell disorders (CMCD) and drive severe anaphylaxis in patients with systemic mastocytosis (SM). The connection between CMCD and chronic inducible urticaria (CIndU) has not been investigated before.

Cold urticaria (ColdU) is characterized by the appearance of wheals and/or angioedema in response to body cooling and rewarming. In cold-induced anaphylaxis (ColdA), there is a sudden cold-induced onset of at least two of the following: (a) involvement of the skin and/or mucosal tissue, (b) respiratory involvement, (c) reduced blood pressure or associated symptoms, or (d) gastrointestinal

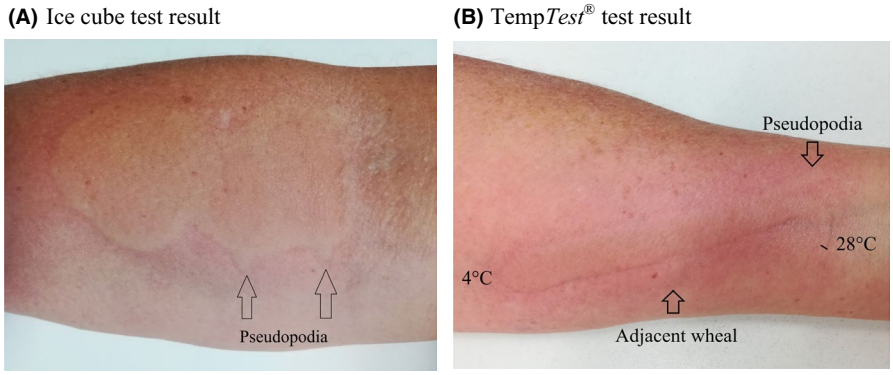

FIGURE 1 Cold stimulation test (CST) results on the volar forearms. Panel A shows wheal and flare reactions with pseudopodia after stimulation with a melting ice cube placed in a non-latex glove. Panel B shows an unusually strong wheal and flare reaction after stimulation with Temp*Test*®: 10 millimeter wide wheal on the scale from 4°C to 28°C, pseudopodia and an additional wheal in proximity to stimulated area

symptoms. Clinical presentations of ColdU depend on the potency and duration of exposure to different cold triggers (air, fluids, surfaces), individual cold sensitivity thresholds and other yet to be defined factors. The diagnosis of ColdU relies on the patient's history and positive cold stimulation testing (CST) with an ice cube and/or Temp*Test*® or other less commonly used methods. Critical temperature threshold (CTT) is the highest Temp*Test*® temperature, and the critical stimulation time threshold (CSTT) is the shortest time required to induce a wheal in CST.[1]

Here, we describe detailed characteristics of a 65-year-old female patient who presented with a 19-year history of ColdU with increasing severity two years before evaluation and compare them to characteristics of 35 other subsequent ColdU patients comprehensively evaluated at the Urticaria Center of Reference and Excellence (UCARE) at the University Clinic Golnik.[2] The following data indicated a severe ColdU (Table 1): (a) wheals induced by air temperatures of 24°C or lower, (b) an unusual 5-minute episode of dyspnea after drinking cold water, and (c) high disease activity with a 30-second CSTT and a 28°C CTT, with ice cube- and Temp*Test*®-induced pseudopodial whealing, and whealing beyond the Temp*Test*®-stimulated skin area (Figure 1A,B). Because these findings pointed to unusually severe ColdU as compared to 35 control ColdU patients (Table 1), we determined the patient's basal serum tryptase levels, which were elevated twice in a four-month period (17.3, 23.0 ng/mL). An underlying CMCD was suspected and confirmed by a positive KIT D816 V mutation test in circulating blood leukocytes (1.0% mutational burden) and in the bone marrow. No cytomorphological features of SM were found in the bone marrow.

The diagnosis of SM is established if: (a) at least a major and one minor or (b) three minor criteria defined by the World Health Organization are fulfilled.[3] Our patient did not meet these criteria. Since she presented with symptoms of mast cell activation, the KIT D816V mutation and lacked signs of cutaneous mastocytosis, she was diagnosed with a monoclonal mast cell activation syndrome (MMAS).[4] MMAS has not been previously described in patients with ColdU, probably because they are hardly ever assessed for a CMCD.

Our findings suggest that severe reactivity to cold triggers, in patients with ColdU, can point to comorbid MMAS and the possibility that MMAS is a risk factor for severe reactions. Our patient's reaction to drinking cold water, a single, short-lived episode of dyspnea with spontaneous resolution, may have been ColdA or, more likely, a severe local response. Screening CIndU patients with severe disease for an elevated tryptase and the presence of the KIT D816V mutation could help to clarify the rates and role of activating KIT mutations as a potential driver for severe reactions. It could also improve our approach to the management and treatment of patients with severe KIT D816V-positive ColdU, for example, by the use of selective tyrosine-kinase inhibitors, such as avapritinib or other KIT-targeting therapies, and of omalizumab, which has been shown to benefit patients with CIndU and effectively prevent anaphylaxis in patients with MMAS and SM.[5,6]

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KEYWORDS

basal serum tryptase, clonal mast cell disorder, cold urticaria, systemic mastocytosis

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CONFLICT OF INTEREST

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TABLE 1 Characteristics of study participants

Characteristics	Described patient	Comparative group (N = 35)
A. Demographics and baseline characteristics		
Age (years)	65	Median (range): 40 (18–73)
Female gender	Yes	66% (23/35)
BMI (kg/m^2)	24.0	Median (range): 24.9 (18.0–36.0)
B. Patient history data		
Age at onset of ColdU (years)	46	Median (range): 33 (9–60)
Early onset of ColdU (≤18 years old)	No	9% (3/35)
Duration of ColdU before evaluation (months)	228[f]	Median (range): 60 (2–384)
Wheals induced by cold	Yes	100% (35/35)
Induced by air temperatures of ≤20°C	Yes	66% (23/35)
Induced by air temperatures of >20°C	Yes[f]	11% (4/35)
Induced by contact with solid surfaces	Yes	37% (13/35)
Induced by localized contact with cold liquids	Yes	43% (15/35)
Maximal duration of wheals (min)	45	Median (range): 60 (5–720)
Itch induced by immersion in the sea	Yes	63% (22/35)
The shortest time to onset of itch (minute/s)	1[f]	Median (range): 5 (1–20), N=18
Angioedema induced by cold	Yes	34% (12/35)
Maximal duration of angioedema (minutes)	45	Median (range): 60 (20–720), N=11
Oropharyngeal or laryngeal symptoms induced by cold	Yes[f]	31% (11/35)
Gastrointestinal symptoms induced by cold	No	14% (5/35)
Respiratory symptoms induced by cold	Yes	37% (13/35)
Induced by ingestion of cold drinks	Yes[f]	0% (0/35)
Induced by air temperatures of ≤20°C	No	9% (3/35)
Induced by air temperatures of ≤20°C, no asthma	No	3% (1/35)
Induced by immersion in the sea	No	11% (4/35)
Symptoms of reduced blood pressure induced by cold	No	29% (10/35)
Wind is an aggravating factor	Yes	60% (21/35)
Increased humidity is an aggravating factor	Yes	49% (17/35)
Asthma	No	34% (12/35)
At least one: asthma, allergic rhinitis, allergic conjunctivitis, or atopic dermatitis	No	40% (14/35)
Thyroid disease	No	17% (6/35)
Systemic reaction to a Hymenoptera sting	No	3% (1/35)
Positive family history of ColdU	No	6% (2/35)
C. Cold stimulation testing (CST) results[a]		
Positive ice cube test	Yes	66% (23/35)
The shortest CSTT ever determined (seconds)	30[f]	Median (range): 300 (30–300), N=22
Ice cube-induced pseudopodial whealing	Yes[f]	9% (2/23)
Positive Temp*Test*®	Yes	43% (15/35)
The highest CTT ever determined (°C)	**28**[f]	Median (range): 17 (6–25), N=15
Temp*Test*®-induced wheal diameter	10[f]	Median (range): 9 (5–15), N=11
Temp*Test*®-induced pseudopodial whealing	Yes[f]	0% (0/11)
Whealing beyond stimulated skin area	Yes[f]	0% (0/11)
D. Patient-reported outcome		
ACUSI score (5–18)	13[f]	Median (range): 10 (6–17)

(Continues)

TABLE 1 (continued)

Characteristics	Described patient	Comparative group (N = 35)
E. Diagnosis		
Cold contact urticaria[b]	Yes	60% (21/35)
Ice cube and Temp*Test*® positive	Yes	43% (15/35)
Ice cube positive at least once, Temp*Test*® never positive	No	17% (6/35)
Ice cube never positive, Temp*Test*® positive at least once	No	0% (0/35)
Localized ColdU	No	3% (1/35)
Localized cold reflex urticaria	No	6% (2/35)
Systemic atypical ColdU suspected (negative local CST)	No	31% (11/35)
ColdA	No	34% (12/35)
Comorbid other form of CIndU	No	17% (6/35)
Comorbid CSU	No	17% (6/35)
F. Laboratory findings		
Total IgE level (IU/mL)	113	Median (range): 97 (9–2050), N=30
Basal serum tryptase (ng/mL)[c]	**23.0**	Median (range): 5.5 (2.9–15.9), N=33
Elevated basal serum tryptase (>11.4 ng/mL)	Yes	15% (5/33)
Basal serum tryptase >8.0 ng/mL[d]	Yes	18% (6/33)
KIT D816V mutation in circulating blood leukocytes	Yes	0% (0/3)
KIT D816V mutation in the bone marrow	Yes	0% (0/2)
Positive cryoglobulin test	No	28% (9/32)
Positive cold agglutinin test at 4°C	Yes	44% (15/34)
Cold agglutinin titer at 4°C[e]	2	Mean ±SD: 1.4 ± 2.9, N=34

Note: Comparative group: A total of 35 cold urticaria patients represent a comparative group. Results are shown as percentage (%) of total for categorical variables and median (range) or mean ±SD for numerical variables.

Abbreviations: ACUSI, Acquired Cold Urticaria Severity Index; BMI, body mass index; CIndU, chronic inducible urticaria; ColdA, cold-induced anaphylaxis; ColdU, cold urticaria; CST, cold stimulation testing; CSTT, critical stimulation time threshold; CSU, chronic spontaneous urticaria; CTT, critical temperature threshold; IgE, immunoglobulin E; N, number of patients; SD, standard deviation.

All patients:

Values exceeding the range of a comparative group are in bold.

[a]CST results from past evaluations were also obtained.

[b]Whealing on cold-stimulated area on the forearm is obligatory for diagnosis.

[c]The highest level of basal serum tryptase ever determined.

[d]Cutoff value for likelihood of hyper-alpha-tryptasemia.

[e]The numbers are a reciprocal expression of cold agglutinin titers (ie, 2 = agglutination at 1:2 dilution).

[f]Described patient: Values indicating a more severe disease.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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GPR109A deficiency promotes IL-33 overproduction and type 2 immune response in food allergy in mice

To the Editor,

Food allergy (FA) is an uncontrolled hyperreactive immune response resulting in loss of tolerance to food allergens.[1] Defects in epithelial barrier frequently precede the development of allergic responses to food allergens.[2] The G-protein-coupled receptor 109A (GPR109A) is expressed by epithelium and innate immune cells in the intestine.[3] Earlier, GPR109A has been reported to mediate anti-lipolytic or anti-inflammatory effects as a receptor for butyrate and niacin.[3,4] Here, we sought to determine the unique role of GPR109A in FA by employing the oral ovalbumin (OVA) sensitization-induced FA model in wild-type (WT) and *Gpr109a*$^{-/-}$ mice.

Compared with OVA-sensitized WT mice, OVA-sensitized *Gpr109a*$^{-/-}$ mice exhibited more severe clinical features of FA, as evidenced by reduced body weight and temperature, and increased diarrhea and intestinal permeability (Figure 1A–D). Pathological markers of FA, serum OVA-IgE and mucosal mast cell protease-1 (mMCP-1)levels were also the greatest in OVA-sensitized *Gpr109a*$^{-/-}$ mice (Figure 1E,F). Intestinal barrier damage is generally manifested as decreased tight junction protein expression.[5] Compared with the WT mice, OVA-sensitized *Gpr109a*$^{-/-}$ mice expressed reduced occludin, claudin-1, and zonula occludens (ZO)-1, but not ZO-2 (Figure 1G). Jejunum histological examination confirmed an exacerbated FA phenotype in OVA-sensitized *Gpr109a*$^{-/-}$ mice, evidencing by damaged intestinal villi (Figure 1H). No difference in the above FA markers was observed between untreated *Gpr109a*$^{-/-}$ and WT mice (Figure S1). These data indicate a role for GPR109A in the modulation of FA development and epithelial integrity.

GPR109A is expressed in both immune cells and epithelial cells in the intestine.[3] We further investigated whether GPR109A deficiency in the hematopoietic or nonhematopoietic cell compartment plays a more important role in FA by bone marrow chimera (BMC) transfer experiments. Four groups of BMC mice, WT → WT/OVA, *Gpr109a*$^{-/-}$ → *Gpr109a*$^{-/-}$/OVA, WT → Gpr109a$^{-/-}$/OVA, *Gpr109a*$^{-/-}$ → WT/OVA, were monitored for FA development. Symptoms of FA were most severely observed in the *Gpr109a*$^{-/-}$ → *Gpr109a*$^{-/-}$+OVA group (Figure S2A-E), followed by WT → Gpr109a$^{-/-}$/OVA group, which lacked GPR109A in nonimmune cells and *Gpr109a*$^{-/-}$ → WT/OVA group, which lacked GPR109A in immune cells, suggesting that in addition to its role in nonimmune cells, GPR109A expression in immune cells also plays an important role in FA development.

To decipher the mechanism linking GPR109A deficiency-induced epithelial dysfunction and allergic immune cell responses, we analyzed the epithelial-derived alarmin cytokine IL-33 that promotes a type 2 immune response and mediates FA propagation.[6,7] The RT-qPCR analysis revealed increased IL-33 expression in jejunum of OVA-sensitized *Gpr109a*$^{-/-}$ mice compared with the WT mice (Figure 2A). Western blot analyses further showed that OVA-sensitized *Gpr109a*$^{-/-}$ mice had increased production of full-length IL-33 and the cleaved form with enhanced activity,[8] compared with OVA-sensitized WT group (Figure 2B). A similar increase of IL-33 was observed in *ex vivo* isolated jejunal epithelia (Figure S3).

BMC experiments suggest that GPR109A deficiency on immune cells plays a role in promoting disease. Thus, we analyzed