Purified IgA from all sera detected the 110-kDa band (Fig. 2d). In contrast, $F(ab)_2$ fragments show only very weak reactivity with PIGR-I (Fig. 2d). The weak reactivity was probably caused by residual uncut IgA in $F(ab)_2$ fragments. Thus, this result indicated that patient IgA bound to PIGR through physiological, but not immunological binding.

The results of these studies clearly indicated that PIGR is not autoantigen in IEN-type IgA pemphigus, although the nature of the 80-kDa protein is still unknown. We also performed other additional experiments, which are described in Supplemental Results and Discussion.

Conclusion

Although autoantigen of IEN-type IgA pemphigus could not be identified, this study provided at least two important insights.

Firstly, we developed a novel IgA-IP using peptide M. Peptide M is a synthetic peptide of 50 amino acids of streptococcal M protein with an additional C-terminal cysteine residue (9). Peptide M binds to IgA with high specificity and affinity and was used to detect tissue bound IgA (5), prompted us to use Protein M in our IgA-IP system. This method should facilitate future studies of identification of autoantigens in various IgA-related diseases.

Secondly, this study confirmed the expression of PIGR by keratinocyte, for the first time by biochemical and molecular biological methods. PIGR is synthesized, delivered to basolateral plasma membranes and bound by polymeric IgA or IgM in secretory epithelia (7,10). PIGR is a type I transmembranous glycoprotein with extracellular, transmembranous and intracellular domains. The extracellular region of PIGR produced by proteolytic cleavage is known as secretory component (SC) (11). After PIGR binds to IgA or IgM, the complexes are internalized and transcytosed to apical surfaces of epithelia. Then, secretory IgA is generated by proteolysis at the cleavage site of PIGR and released into lumen (9).

Previous IF of normal human skin with 2 different anti-SC antibodies showed the presence of SC in epidermis (6). Staining patterns were different between the two antibodies; that is, one stained basement membrane zone and another stained epidermal cell surfaces. These different patterns were speculated to be caused by variable epitope expression of SC in epidermis.

In addition, two previous studies using mainly morphological and biological techniques showed expression of SC/PIGR in keratinocytes (12,13). These studies indicated anti-inflammatory role of SC/PIGR in inflammatory skin diseases via inhibition of IFN-gamma-induced expression of ICAM-1 and HLA-DR in keratinocytes (12,13). From the results in the previous studies and the finding in our study, PIGR should play an important immunological role in epidermis, which is always exposed to external pathogens. Functions of PIGR in secretory immune system in epidermis should be studied in the future.

Acknowledgements

We gratefully appreciate the secretarial work of Ms. Tomoko Tashima and Ms. Mami Nishida. We thank the patients for their participation. We thank the patients for their participation. This study was supported by Grants-in-Aid for Scientific Research (No. 20390308, 20591331, 21659271, 23591634, 23791298, 23791299, 23791300, 23791301, 24659534, 24591672, 24591640, 24791185), and Supported Program for the Strategic Research Foundation at Private Universities from the Ministry of Education, Culture, Sports, Science and Technology; and by 'Research on Measures for Intractable Diseases' Project: matching fund subsidy (H23-028 to K. Iwatsuki, and H24-038 to T. Hashimoto) from the Ministry of Health, Labour and Welfare. The study was also supported by grants from the Kaibara Morikazu Medical Science Promotion Foundation, Ishibashi Foundation, Kanae Foundation for the Promotion of Medical Science, Takeda Science Foundation, Chuo Mitsui Trust and Banking Company, Limited, and Nakatomi Foundation.

Author contribution

Atsunari Tsuchisaka, Norito Ishii, Kwesi Teye, Ryosuke Sogame and Hiroshi Koga performed the experiments. Atsunari Tsuchisaka and Takashi Hashimoto wrote the manuscript. Takahiro Hamada, Daisuke Tsuruta, Chika Ohata and Minao Furumura collected essential reagents and samples. Atsunari Tsuchisaka and Takashi Hashimoto revised and re-revised the manuscript.

Conflict of interests

The authors have declared no conflicting interests.

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Supporting Information

Additional supporting data may be found in the supplementary information of this article.

Data S1. Materials and methods.

Data S2. Results and discussions.

Figure S1. Immunoblotting of KU-8 cell extract.

Figure S2. (a) Genomic structure of PIGR with exon-intron organization.

Figure S3. IF of cultured KU-8 cells, HaCaT cells and NHKs with IEN-type IgA pemphigus patient sera and normal control sera.

Figure S4. Immunoblotting of IP products of keratinocyte cell extracts with anti-PIGR (C-term) pAb.

Figure S5. IF of PIGR-I expressing COS7 cells for anti-c-Myc mAb, anti-PIGR (C-term) pAb, 5 IEN-type IgA pemphigus sera and 13 normal control sera.

Figure S6. SDS-PAGE of purified IgA (lane 1), digested IgA by pepsin for 48 hrs (lane 2), and purified $F(ab)_2$ fragments by peptide M and protein L (lane 3) by stained with Coomassie Brilliant Blue.

Figure S7. (a) IF of PIGR-II expressing COS7 cells for anti-c-Myc mAb, anti-PIGR (C-term) pAb, 2 IEN-type IgA pemphigus sera and 2 normal control sera.

Figure S8. (a) Adsorption experiments of lamina lucida-type LABD patient serum with total lysate from transfected COS7 cells for PIGR-I (lane 2 and 3) and transfected COS7 cells for empty vector (lane 4 and 5) by immunoblotting.

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DOI: 10.1111/exd.12410
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Mutation-dependent effects on mRNA and protein expressions in cultured keratinocytes of Hailey–Hailey disease

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Abstract: Hailey–Hailey disease (HHD) is a dominantly inherited skin disease caused by mutations in *ATP2C1* gene, which encodes secretory pathway Ca^{2+}/Mn^{2+}-ATPase protein 1. The precise mechanism remains unclear. In this study, to understand molecular basis of HHD, we examined expression of mRNA and protein in cultured keratinocytes derived from three HHD patients with different mutations. We showed that reduced expression of mRNA and protein in patient with p.Gln504X, but not in patients with p.Pro307His and c.1308+1G>A. RT-PCR analysis for patient with c.1308+1G>A revealed in-frame exon skipping. Reduction of mRNA and protein in p.Gln504X was considered to be caused by nonsense-mediated mRNA decay. p.Pro307His located adjacent to Ca^{2+}-binding residue may induced conformational change, which leads to defective Ca^{2+} transport. In-frame shorter transcript caused by c.1308+1G>A may have slightly reduced activity, which accounted for mild phenotype of the patient. These results clarified the pathogenic effects of different causative mutations in development of skin lesions.

Key words: Ca^{2+} – Hailey–Hailey disease – keratinocyte – mutation – P-type ATPase

Accepted for publication 1 April 2014

Background

Hailey–Hailey disease (HHD; MIM 169600) is an autosomal dominant hereditary skin disease with abnormal keratinocyte adhesion and differentiation. The prevalence of HHD is estimated to be 1:50 000 (1). HHD shows vesicular or erosive lesions on the intertriginous areas from the third or fourth decade.

Responsible gene for HHD is *ATP2C1* gene on chromosome 3q22.1, which encodes human secretory pathway Ca^{2+}/Mn^{2+}-ATPase protein 1 (SPCA1), a Ca^{2+} pump at Golgi apparatus (2,3). SPCA1 belongs to P-type ATPase superfamily (1) and is consisted of actuator domain (A), nucleotide-binding domain (N), phosphorylation domains (P) and five stalk helices (S1–S5) in the cystoplasm, as well as 10 transmembrane helices (M1-M10). SPCA1 has high-affinity Ca^{2+}-binding site formed by p.Glu308 in M4 and p.Asn738 and p.Asp742 in M6 (Fig. 1a) (4).

To date, more than 140 pathological mutations scattered throughout *ATP2C1* gene have been described with no indication of mutational hotspots or clustering of mutations (5–7). Previous studies detected all types of mutations, including nonsense mutations (20%), frameshift mutations leading to premature termination codons (PTCs) (30%), splice-site mutations (19%), missense mutations (28%) and in-frame deletions or insertions (3%) (8,9).

However, precise mechanism of development of skin lesions in HHD remains unclear, mainly because of difficulty to obtain skin samples due to rarity of HHD and lack of animal model (10).

Questions addressed

In this study, we provide a wider understanding of the molecular basis of the disease, by determining the role of three novel *ATP2C1* mutations in impaired expression of *ATP2C1* mRNA and SPCA1 protein.

Experiment design

Genetic mutation analysis with heteroduplex scanning and following sequencing of PCR products was performed using genomic DNAs from three HHD patients with different disease severity. Using cultured keratinocytes from the patients, mRNA and protein levels were examined by quantitative real-time PCR (qPCR) and immunoblotting, respectively. Furthermore, RT-PCR was performed for possible splice-site mutation. See supporting information for details (Data S1).

Result

Three patients with different clinical severity were examined in this study. Patient 1, a 72-year-old Japanese male, showed the mildest clinical manifestations with dusky erythemas restricted to the groins for 32 years, without any skin lesions on other intertriginous areas (Fig. 1b,c). Patient 2, a 49-year-old Japanese male, showed intermediate skin lesions with dusky erythemas and papules with tiny erosions on the neck, axillae and groins for 9 years (Fig. 1d,e). Patient 3, an 82-year-old Japanese male, showed the most severe skin lesions with scaly erythematous plaques, vesiculopustules and painful erosions on all the intertriginous areas for 12 years (Fig. 1f,g).

Direct nucleotide sequencing of genomic DNA disclosed a heterozygous G>A transition at invariant splice donor site consensus sequence GT within intron 15 (c.1308+1G>A) in patient 1 (Fig. 1h,i). This mutation was predicted to alter mRNA splicing. Patient 2 had a heterozygous C>A transition at nucleotide 920

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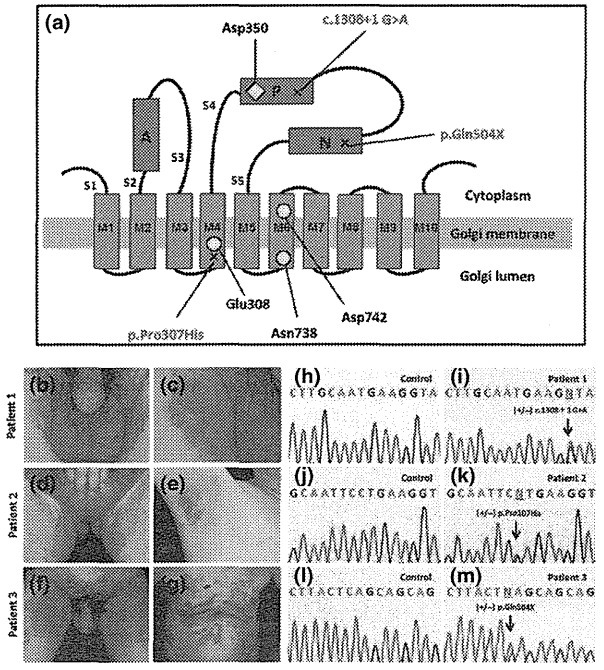

Figure 1. (a) Molecular structure of SPCA1. Actuator domain (A), phosphorylation domain (P), nucleotide-binding domain (N) and 5 stalk helices (S) in the cytoplasm, and 10 transmembrane helices (M). Putative Ca^{2+}-binding residues (p.Glu308, p.Asn738 and p.Asp742) in M domain are indicated by yellow circles. Phosphorylation site (p.Asp350 in P domain) is indicated by a yellow rhombus. The sites of *ATP2C1* mutations found in the 3 patients are also indicated. (b–g) Clinical features. (b, c) Patient 1. (d, e) Patient 2. (f, g) Patient 3. (h–m) Mutation studies of *ATP2C1* gene. (h, i) Patient 1 showed heterozygous G>A transition at +1 position of exon 15 donor splice site (c.1308+1G>). (j, k) Patient 2 showed heterozygous C>A transition at nucleotide 920 which converts proline residue (CCT) to histidine residue (CAT) (p.Pro307His G>A). (l, m) Patient 3 showed heterozygous C>T transition at nucleotide 1510 which converts glutamine residue (CAG) to stop codon (TAG) (p.Gln504X).

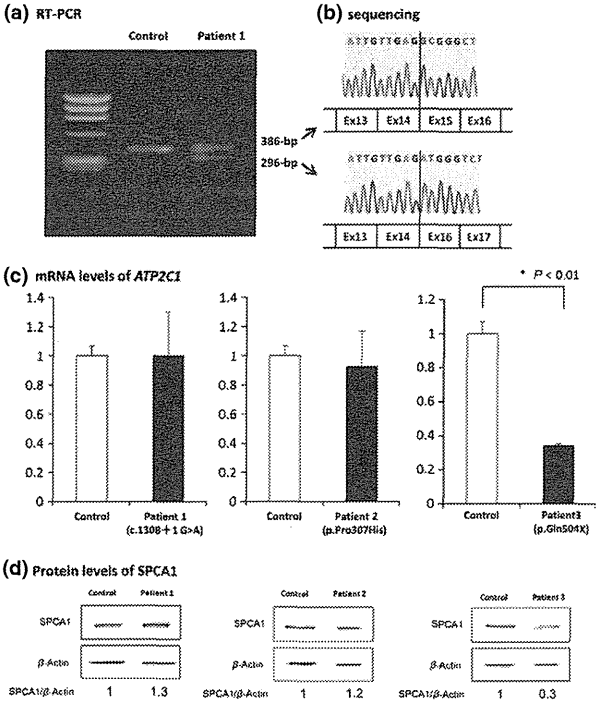

Figure 2. (a, b) Studies of RT-PCR and direct sequencing in patient 1 with c.1308+1G>A. (a) RT-PCR analysis revealed 296-bp mutant transcript and 386-bp normal transcript. (b) Sequencing for mutated transcript showed in-frame exon 15 skipping. (c, d) Studies of expression levels of *ATP2C1* mRNA and SPCA1 protein. (c) qPCR analysis revealed reduced expression of mRNA only in patient 3 with p.Gln504X (*$P < 0.01$). (d) Immunoblotting with densitometry analysis showed reduced expression of protein only in patient 3.

(c.920C>A), which converts proline residue (CCT) to histidine residue (CAT) (p.Pro307His) (Fig. 1j,k). Patient 3 had a heterozygous C>T transition at nucleotide 1510 (c.1510C>T), which converts glutamine residue (CAG) to stop codon (TAG) (p.Gln504X) (Fig. 1l,m). c.1308+1G>A is present in P domain, p.Pro307His in M4 domain, and p.Gln504X in N domain (Fig. 1a). These mutations have not been reported previously. None of these mutations were found in 100 ethnically matched control DNA samples.

RT-PCR analysis across the mutation site for cDNA from keratinocytes of patient 1 with c.1308+1G>A identified aberrant 296-bp band, in addition to 386-bp normal band (Fig. 2a). Direct sequencing for subcloned 296-bp band showed in-frame exon 15 skipping (Fig. 2b).

To determine pathogenicity of *ATP2C1* mutations, we first assessed *ATP2C1* mRNA levels by qPCR using keratinocyte cultures derived from the three HHD patients and normal control (Fig. 2c). mRNA levels in patients with c.1308+1G>A and p.Pro307His did not differ from that in normal control. In contrast, patient 3 with p.Gln504X showed statistically significant reduction in *ATP2C1* mRNA expression ($P < 0.01$, Welch's *t*-test).

We also examined protein levels of SPCA1 by immunoblotting and densitometry (Fig. 2d). SPCA1 protein levels in patients with c.1308+1G>A and p.Pro307His were same as that in normal control, while patient with p.Gln504X showed reduced SPCA1 protein expression. Expression levels of SPCA1 standardized by beta-actin expression were 1 for control and 0.3 for patient 3.

Conclusions

In this study, low expression levels of both mRNA and protein were observed in patient 3 with p.Gln504X, suggesting that the nonsense mutation leads to marked reduction of mutated *ATP2C1* mRNA via nonsense-mediated mRNA decay, accounting for the severe phenotype in patient 3. Thus, haploinsufficiency caused by nonsense or insertion/deletion mutations, which lead to PTCs, should be the major pathogenic mechanism in HHD with dominant inheritance.

In contrast, both mRNA and protein levels in patient 2 with p.Pro307His and patient 1 with c.1308+1G>A did not reduce. However, p.Pro307His is located adjacent to Ca^{2+}-binding residue, p.Glu308, and may affect Ca^{2+}-passage, which accounts for the intermediate phonotype in patient 2. RT-PCR analysis in patient 1 revealed mutant transcript with in-frame skipping of exon 15, which may account for the mild phenotype in patient 1. Although exon 15 is included in P domain, c.1308+1G>A should retain phosphorylated aspartic acid residue (p.Asp350) in exon 13 (Fig. 1a).

Therefore, in HHD patients with non-PTC mutations showing normal level of SPCA1 protein, production of SPCA1 with impaired function should also cause haploinsufficiency. To confirm above speculations, we need more information to be

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provided by future studies. For example, abnormal cytoplasmic Ca^{2+} may be examined by mutagenesis and functional studies.

Abnormal cytoplasmic Ca^{2+} caused by haploinsufficiency of SPCA1 may alter post-translational modifications, including glycosylation, folding, trafficking and sorting, in cell adhesion molecules, leading to acantholytic changes seen in HHD (11–13).

This study showed that different mutations in HHD result in distinct expression of mRNA and protein, which may relate to clinical phenotypes.

Acknowledgement

See supporting information for details (Data S2).

Author contribution

MM, SN and KT performed the researches. HO and SI contributed samples. T. Hamada, CH and MF designed this study. MM and T. Hashimoto wrote the paper.

Conflict of interest

The authors state no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Materials and methods.
Data S2. Acknowledgments.

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LETTER TO THE EDITOR

A case of bullous pemphigoid associated with psoriasis vulgaris showing Hailey–Hailey disease-like histopathological changes in regenerated epidermis without genomic mutation in *ATP2C1* or *ATP2A2* gene

Editor

Bullous pemphigoid (BP) histopathologically shows subepidermal blisters, but not acantholysis. However, a few BP patients were reported in association with pemphigus,[1] Hailey–Hailey disease (HHD)[2] and transient acantholytic dermatosis (TAD or Grover disease).[3–5] We report a unique BP case associated with psoriasis vulgaris, which histopathologically showed acantholysis in regenerated epidermis below subepidermal bullae.

A 72-year-old Japanese female, who had psoriasis vulgaris for 5 years, showed many blisters and erosions with itchy erythemas on the whole body for 1 year (Fig. 1a). Oral mucosa was not involved. In addition, many hyperkeratotic erythematous plaques were seen on the entire body (Fig. 1b).

Histopathology for bullous lesion revealed a large subepidermal bulla with extensive regenerated epidermis below the bulla (Fig. 1c). Infiltrates of neutrophils and eosinophils were found in the blister and upper dermis. In the regenerated epidermis, prominent acantholysis with many dyskeratotic keratinocytes was seen (Fig. 2c, d). However, such acantholysis was not seen either in overlying epidermis or in peripheral non-bullous areas. No atypical keratinocytes suggesting actinic keratosis or squamous cell carcinoma (SCC) were seen. Histopathology for hyperkeratotic lesion confirmed the diagnosis of psoriasis vulgaris.

Direct immunofluorescence (IF) revealed IgG and C3 deposits to basement membrane zone (BMZ) (Fig. 2a). Indirect IF of normal human skin detected IgG anti-BMZ antibodies, which reacted with epidermal side of 1 M NaCl-split skin (Fig. 2b). Indirect IF of monkey oesophagus showed IgG reactivity with cell surfaces, but not BMZ (Fig. 2c). In addition, indirect IF of rat bladder showed IgG reactivity with transitional epithelia.

Enzyme-linked immunosorbent assays (ELISAs) detected IgG antibodies to BP180, but not BP230, desmoglein 1

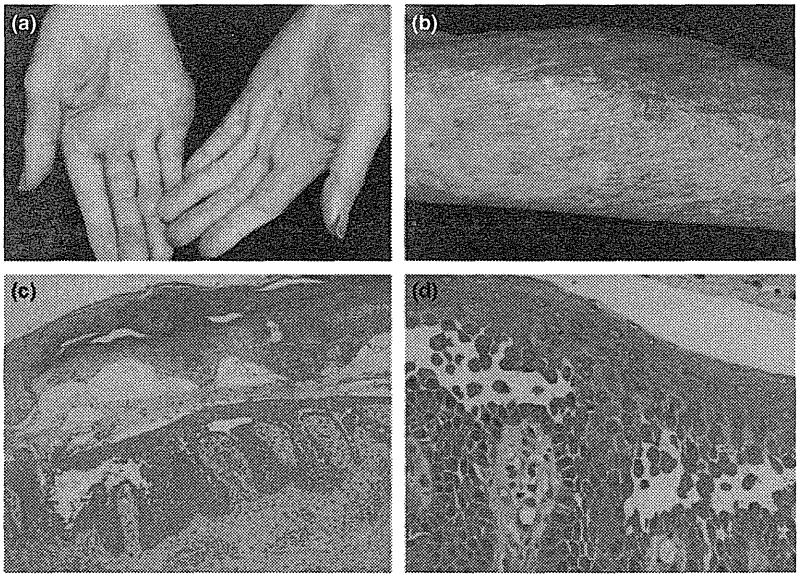

Figure 1 (a) Blistering skin lesions on the palms. (b) Psoriatic lesions on the lower leg. (c, d) Histopathological features (HE staining) (c: 200x, d: 400x).

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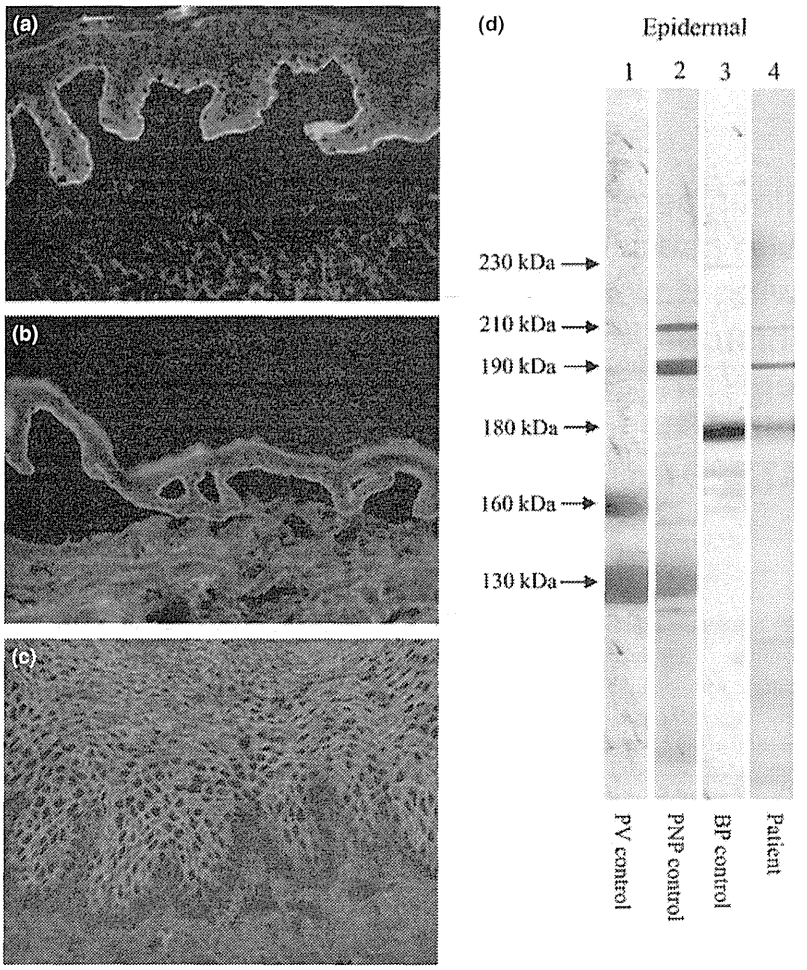

Figure 2 (a) Direct IF for deposit of C3. (b) IgG indirect IF of 1M NaCl-split skin. (c) IgG indirect IF of monkey oesophagus. (d) IgG immunoblotting of normal human epidermal extracts. Control pemphigus vulgaris (PV) serum reacted with the 160 kDa Dsg1 and the 130 kDa Dsg3 (lane 1), control PNP serum reacted with the 210 kDa envoplakin and the 190 kDa periplakin (lane 2) and control BP serum reacted with the 230 kDa BP230 and the 180 kDa BP180 (lane 3). The patient serum reacted with envoplakin, periplakin and BP180 (lane 4).

(Dsg1) or Dsg3. Novel ELISAs detected IgG antibodies to desmocollin 1 (Dsc1), but not to Dsc2 or Dsc3 (Ishii *et al.*, in preparation).

Immunoblotting of normal human epidermal extracts detected IgG antibodies to BP180, envoplakin and periplakin (Fig. 2d). Immunoblotting also showed strong IgG reactivity with recombinant protein of BP180 NC16a domain. However, immunoblot analyses of recombinant protein of BP180 C-terminal domain, concentrated culture supernatant of HaCaT cells, normal human dermal extracts or purified human laminin-332 showed negative results.

Thorough mutation analyses using genomic DNAs showed no mutations in both *ATP2C1* and *ATP2A2* genes, responsible genes for HHD and Darier disease, respectively.[6,7] Extensive studies showed no malignancy.

From these results, we made a diagnosis of BP associated with psoriasis vulgaris, which developed acantholysis restricted to BP lesions. Oral prednisolone 30 mg per day cleared the bullous lesions.

We excluded the possibility of concurrence of PF or PV by negative results in Dsg ELISAs. The possibility that associated actinic keratosis or SCC caused acantholysis was also excluded, because of involvement of non-sun-exposed sites, no tumour formation and no malignant cells histopathologically.

Because no mutations were detected in *ATP2C1* and *ATP2A2* genes, and there were no typical clinical manifestations for both

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diseases, this case was unlikely to be complicated by these diseases.

Two cases with histopathological acantholytic changes associated with BP were diagnosed as TAD.[4,5] Therefore, the possibility that TAD-like changes occurred specifically in the regenerative epidermis in our case cannot be excluded.

Positive IgG reactivity with monkey oesophagus and rat bladder epithelia, as well as envoplakin, periplakin and Dsc, suggested the diagnosis of paraneoplastic pemphigus (PNP).[8,9] However, because our patient showed neither PNP-like clinical findings nor malignancy, the diagnosis of PNP was excluded. Nevertheless, it is still possible that these autoantibodies to epidermis caused the acantholytic changes.

Psoriasis vulgaris patients occasionally developed BP.[10] However, because there was no case of concurrent BP and psoriasis showing acantholysis, relationship between psoriasis and acantholytic changes remains unknown.

In conclusion, acantholysis seen in our case might be developed by autoantibodies to plakins or Dsc1 or by undetermined structural or genetic abnormality in epidermal keratinocytes.

Acknowledgements

We gratefully appreciate the secretarial work of Ms. Tomoko Tashima, Ms. Mami Nishida, and Ms. Shoko Nakamura. We thank the patient for her participation.

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DOI: 10.1111/jdv.12521

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Dermatologic Therapy, Vol. 27, 2014, 233–235
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DERMATOLOGIC THERAPY
ISSN 1396-0296

THERAPEUTIC HOTLINE

Successful treatment with narrow-band UVB therapy for a case of generalized Hailey–Hailey disease with a novel splice-site mutation in *ATP2C1* gene

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ABSTRACT: Hailey–Hailey disease (HHD) is a rare autosomal dominant disorder characterized by development of recurrent blisters, erosions, and crustations in the intertriginous areas. The treatment of HHD is often challenging, and various methods have been tried. We report here a case of a 45-year-old woman with a generalized form of HHD that was dramatically improved and well controlled by narrow-band ultraviolet B phototherapy.

KEYWORDS: Hailey–Hailey disease, narrow-band UVB phototherapy

Introduction

Hailey–Hailey disease (HHD) is an autosomal-dominant disorder with recurrent blisters and erosion with crusts on the intertriginous areas. HHD cases involving large skin areas have been rarely reported as generalized HHD (1,2). There are various therapy options for HHD, including topical corticosteroids, antibiotics, both topical and oral antimycotics, and retinoids, but their effectiveness has been variable. In untreated skin lesions of HHD, a significant infiltration of T cells has been demonstrated. Narrow-band (NB) ultraviolet B (UVB) is known to have immunological effects and is used for treating various immune-modulated skin disorders, such as psoriasis, atopic dermatitis, and vitiligo. Thus, we applied NB-UVB therapy for HHD. We report here a case of generalized HHD successfully treated with NB-UVB phototherapy.

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Case report

A 45-year-old Japanese woman visited us complaining of generalized eruptions. The patient

noticed erythematous plaques on the axillae and groins several years ago, which extended to the trunk, arms, and thighs. Treatments with topical corticosteroid were used for 2 years without any improvement of the skin lesions.

Physical examination revealed small erythematous papules with crusts and scales spread on the whole body (FIG. 1A). In addition, macerated erythemas and milium-sized pustules were seen on the groins (FIG. 1B). Bacterial culture of exudate from a skin lesion on the groin was negative.

Histopathology for skin biopsy from erythematous lesion on the arm showed hyperkeratosis, parakeratosis, suprabasal acantholysis, with a few dyskeratotic cells, in the epidermis, as well as dense lymphoid cell infiltration in the superficial dermis (FIG. 2A). Acantholytic cells showed well-defined nucleus and clear cytoplasm. Direct immunofluorescence of the biopsy specimen showed no immune deposits.

ATP2C1 gene analysis of genomic DNA from this patient found a heterozygous splice-site mutation, c.1891-1G>T in intron 20 (FIG. 2B). This mutation has not been reported. However, because mRNA from this patient was not available, confirmation study for mutated transcripts could not be performed. From these clinical, histopathological, and genetic findings, the diagnosis of generalized HHD was made.

One month treatment with topical corticosteroid twice daily showed no efficacy. Oral etretinate 50 mg/day, topical and oral antibiotics, and dapsone were not effective either. NB-UVB therapy was then commenced with an initial dose of 300 mJ/cm^2 per week. The dose increased by 50 mJ/cm^2 at each subsequent visit until 500 mJ/cm^2 (total: eight treatments, cumulative dosage: 2000 mJ/cm^2). Dramatic improvement was observed 2 weeks later. Almost complete remission was achieved 2 months later (FIG. 1C,D), and NB-UVB therapy discontinued. Because there was slight recurrence of the skin lesions 6 months after discontinuation of NB-UVB therapy, this therapy was resumed and proved to be effective again.

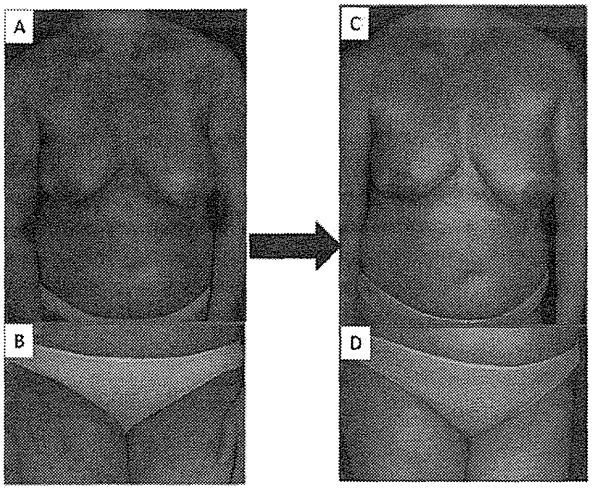

FIG. 1. Clinical features. Clinical pictures before narrow-band ultraviolet B therapy for the skin lesions on the trunk (A) and groins (B). (C,D) Clinical pictures for the skin lesions on the same sites after narrow-band ultraviolet B therapy.

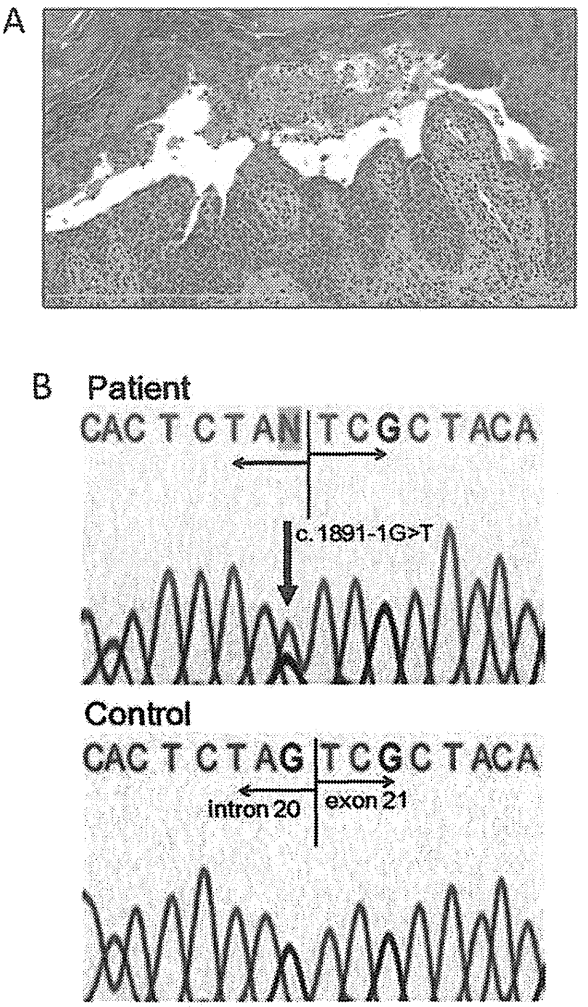

FIG. 2. (A) Histopathological findings of biopsy taken from a skin lesion. (B) *ATP2C1* gene analysis of genomic DNA showing a heterozygous G > T transition at last residue in intron 20 (indicated by an arrow in the upper panel for the patient). The lower panel shows the results of normal control genomic DNA.

Comments

Although HHD lesions are caused by mutations in *ATP2C1* gene (3), there are still mysterious issues in HHD, including late onset at late teens or later, predilection for the neck, axillae, and intertriginous areas, and tendency to improve at late adulthood. Definite pathogenetic mechanism in generalized HHD is also unknown, but bacterial and herpes simplex virus infections, arthropod infestation, and drug allergy were suggested as possible triggers (2). In our case, no bacteria grew on culture, topical and oral antibiotics were ineffective, and no specific previous medications were reported. Although our case showed a novel splice-site mutation, the pathogenic role of this mutation could not be further studies due to lack of cultured keratinocytes or mRNA from the skin or keratinocytes. Thus, cause for the generalized HHD lesion in our case was unknown. Future studies of transfection of mutated cDNA into cultured normal human keratinocytes may unravel the abnormal function of this mutation.

In our case, the extensive skin lesions could not be controlled with topical and oral corticosteroids or retinoids. Histopathology of skin biopsy from this case showed dense lymphoid cell infiltration in the upper dermis, suggesting that, in addition to genetic defect, cellular immunity may contribute to the pathogenesis of generalized HHD. This assumption was further supported by previous study, which reported beneficial effect of tacrolimus treatment in HHD (4) Immune-suppressive effects of NB-UVB is successfully used in treatments for various immune-modulated skin disorders, including atopic dermatitis and vitiligo vulgaris. Local NB-UVB therapy decreased the number of epidermal T lymphocytes and dendritic cells (5). As for the effects of UV radiation on HHD, in a case of concurrent psoriasis and HHD, suberythema UVB therapy rapidly improved psoriatic plaques with no adverse reaction to HHD lesions (6). Therefore, we treated our case with NB-UVB therapy, resulting in dramatic improvement. This result further suggested involvement of immunological mechanism in our case.

Various influences of UV radiation on skin lesions of HHD have been documented. Suppression of *ATP2C1* mRNA expression on keratinocytes by UVB irradiation was reported (7), and latent acantholytic lesions was manifestated by UV radiation (8). These reports suggested that UV radiation may exacerbate HHD lesions. On the other hand, UVB radiation alone was not enough to provoke pathogenic changes in HHD keratinocytes in vitro (9).

In our case, skin lesions with extensive infiltration of lymphocytes were improved by NB-UVB radiation, suggesting an important role of T-lymphocyte-mediated immunity in the pathogenesis in HHD. The results in this study suggested NB-UVB therapy as an effective and safe method for the treatment of not only generalized HHD but also common localized-type HHD.

Funding

None.

Conflict of interests

The authors have no conflict of interest to disclose.

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Wien Med Wochenschr (2014) 164:274–277
DOI 10.1007/s10354-014-0290-0

wmw
Wiener Medizinische Wochenschrift

Autoimmunity versus Autoinflammation - Friend or Foe?

Nobuo Kanazawa · Georgi Tchernev · Uwe Wollina

Received: 16 April 2014 / Accepted: 17 June 2014 / Published online: 9 July 2014
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Abstract "Autoimmunity" is a designation dependent on the conventional immunological issue of self/non-self discrimination. Identification of novel target autoantigens is still an important issue ongoing in classical tissue-specific autoimmune bullous diseases and autoimmune connective tissue diseases. In contrast, synchronized with the paradigm shift of the fundamental aspect of immunity to danger sensing/signaling, distinct collagen-like diseases have been defined by the genetic mutations causing dysregulated innate immunity/inflammation and have been designated as "autoinflammatory" diseases. Due to the clinical and etiological similarities, the concept of autoinflammatory diseases has expanded to include non-hereditary collagen-like diseases, tissue-specific chronic idiopathic inflammatory diseases and metabolic diseases. On the other hand, various genetic causes of autoimmune diseases have been identified and the border of these two pathophysiologies is becoming obscure. Instead, a variable mixture of both autoimmunity and autoinflammation can cause each inflammatory phenotype with a variable level of antigen specificity.

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Keywords Autoimmunity · Autoinflammation

Autoimmunität versus Autoinflammation - Freund oder Feind?

Zusammenfassung Unter „Autoimmunität" versteht man konventionell das immunologische Problem der Diskriminierung von Selbst und Nicht-Selbst. Die Identifizierung weiterer Target-Autoantigene bei den klassischen gewebe-spezifischen autoimmunen bullösen Dermatosen und den autoimmunen Bindegewebserkrankungen bleibt auch weiterhin ein wichtiges Anliegen. Dabei hat sich das Verständnis der fundamentalen Aspekte der Immunologie hin entwickelt zum Themenkomplex der Gefahrenerkennung (danger sensing) und Signalübertragung. Bei den Kollagenosen wurden teils genetische Mutationen entdeckt, die verantwortlich zeichnen für Störungen der Immunität und Entzündungskaskade. Die autoinflammatorischen Erkrankungen wurden definiert. Aufgrund klinischer und ätiologischer Ähnlichkeiten wurde das Konzept der autoinflammatorischen Erkrankungen auf nicht hereditäre Bindegewebserkrankungen, gewebe-spezifische chronisch idiopathisch-entzündliche Erkrankungen und metabolische Erkrankung ausgedehnt. Andererseits wurden verschiedene genetische Ursachen der Autoimmunerkrankungen entdeckt, so dass das die Grenzen der klassischen Pathologien verschwimmen. In der Tat kann eine Mischung von Autoimmunität und Autoinflammation nahezu jeden Entzündungs-Phänotyp mit variablem Level der Antigenspezifität auslösen.

Schlüsselwörter Autoimmunität · Autoinflammation

The immune system is working for protection of the living things from harmful things, such as invasive patho-

gen and internal malignancy. At first, discriminating self and nonself had been considered the fundamental aspect of immunity, and "autoimmunity" was designated for immune reaction to the self ("auto") antigens. Identification of major histocompatibility complex and concept of central and peripheral tolerance (clonal deletion and anergy as the mechanism, respectively) explained the basis of self-recognition. However, recent research on regulatory T cells (T_{reg}) and tolerogenic dendritic cells has added further implications in keeping unresponsiveness to the self [1, 2].

Clinically, several kinds of chronic inflammatory disorders have been applied for autoimmune diseases, which target self-antigens. They are divided into two categories: tissue-specific autoimmune diseases caused by type II allergy and immune complex-mediated systemic autoimmune diseases caused by type III allergy. In the skin-specific autoimmune bullous diseases, such as pemphigus and pemphigoid, molecular identification of pathogenic antibodies and the corresponding antigens, desmoglein, and type XVII collagen, respectively, provided insights into the molecular basis of skin structure [3]. Interestingly, another severe bullous disease, staphylococcal scalded skin syndrome is caused by a proteolytic exotoxin, which injures homophilic desmoglein junction [4]. Type VII collagen is a target antigen in autoimmune epidermolysis bullosa aquisita, and is genetically deficient in congenital dystrophic epidermolysis bullosa, suggesting that an autoantibody causes functional defect of the corresponding antigen. By analysis of the cases showing lesional immunoglobulin deposit without detection of known autoantibodies, novel target antigens are still continuously discovered [5]. However, it is mostly unclear how the autoantibodies are generated [6].

Systemic autoimmune diseases include the classical connective tissue diseases (rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, and polymyositis/dermatomyositis) except for rheumatoid fever, in which self-antigen mimicking a part of *Group A Streptococcus* becomes the target after streptococcal infection. Serum antinuclear antibody level is elevated in most of these diseases, and the disease-specific tissue-nonspecific autoantibodies against DNA, DNA-binding proteins, and other nuclear/cytoplasmic proteins, are used as markers for diagnosis and for appreciation of the disease activity. However, their pathogenic role is mostly unclear as compared with the case of skin-specific autoimmune bullous diseases. Nevertheless, recent identification of autoantibodies against novel antigens has defined some specific subtypes of dermatomyositis [7]. Interestingly, antibody against MDA5, which acts as an intracellular receptor for viral RNA and belongs to RIG-I-like receptors, is specific for clinically amyopathic dermatomyositis, which is frequently accompanied with rapidly progressing interstitial pneumonitis [8, 9].

Besides the classical autoimmune connective tissue diseases, some related disorders are still accompanied with specific autoantibodies, such as Sjögren's syndrome, antiphospolipid antibody syndrome, and antineutrophil cytoplasmic antibody-related vasculitis. In contrast, the remaining systemic disorders with chronic inflammation, such as adult Still's disease, Behcet's disease, Sweet syndrome, Weber-Christian disease, and sarcoidosis, are negative for autoantibodies and are driven by activated neutrophils and/or macrophages. Therefore, these diseases are considered to be autoimmune-like, but rather related with nonspecific hyper-reactivity or latent infection. These characteristic features are shared with other tissue-specific chronic idiopathic inflammatory diseases, such as urticaria, psoriasis, and inflammatory bowel diseases including Crohn's disease and ulcerative colitis.

Recently, several distinct diseases, whose phenotypes are similar to these chronic idiopathic inflammatory diseases, have been defined by the causative genetic mutations. As these mutations cause dysregulation of innate immunity/inflammation, the defined diseases have been designated as "autoinflammatory" diseases [10]. These processes have been synchronized with the paradigm shift of the fundamental aspect of immunity, from self/nonself discrimination to danger sensing/signaling [11]. Familial Mediterranean fever (FMF) and related hereditary periodic fever syndromes are the prototypic autoinflammatory disorders and most of them are caused by dysregulated activation of NOD-like receptors (NLR) P3 inflammasome, which senses various dangerous stimuli to induce interleukin (IL)-1β secretion, such as bacterial RNA, imidazoquinolin, and contact allergen [12]. As referred to the membranous toll-like receptors (TLR), NLR have been shown to act as intracellular sensors for various pathogen- or danger-associated molecular patterns [13]. Therefore, it is conceivable that these hereditary diseases resemble infectious or allergic diseases. Although heterozygous *NLRP3* mutations-oriented cryopyrin-induced periodic fever syndromes (CAPS) include formerly called familial cold urticaria, febrile attacks seem to occur periodically or even "automatically", without any apparent triggers. The category of hereditary autoinflammatory diseases has rapidly expanded to include more numbers of diseases, such as pyogenic pustular diseases and systemic granulomatosis [14]. Differentiation of pyogenic arthritis, pyoderma gangrenosum and acne syndrome with *PSTPIP1* mutations among pyoderma gangrenosum and/or cystic acne patients, identification of deficiency for IL-36 receptor antagonist with *IL36RN* mutations among generalized pustular psoriasis patients, and distinction of early onset sarcoidosis with *NOD2* mutations from sarcoidosis have indicated the fact that such monogenic diseases constitute at least a part of sporadic common diseases [15–17].

Then, the previously described autoantibody negative chronic idiopathic inflammatory disorders are considered as acquired autoinflammatory diseases, which share clinical features and the putatively dysregulated inflammatory pathways with hereditary autoinflammatory diseases. Adult Still's disease, Behcet's disease, and Weber-Christian disease show periodic febrile

attacks with skin rash and are similar to CAPS, FMF, and Nakajo-Nishimura syndrome with *PSMB8* mutations, respectively. Sweet syndrome is associated with Majeed syndrome with *LPIN2* mutations [18, 19]. Moreover, Schnitzler syndrome with various clinical similarities to CAPS shows dramatic improvement by anti-IL-1β therapy, suggesting the underlying activation of NLRP3 inflammasome [20]. Thus, autoimmune and autoinflammatory diseases seem apparently distinguished by their clinical and/or genetic features.

Furthermore, some metabolic diseases such as gout, pseudogout, and type 2 diabetes mellitus, which are caused by chronic inflammation due to monosodium urate crystals, calcium pyrophosphate crystals, and hyperglycemia, respectively, are categorized as another class of autoinflammatory diseases [21]. In these diseases, self molecule-induced activation of NLRP3 inflammasome is considered responsible, and anti-IL-1β therapy is effective. Similarly, it has been shown that obesity-induced metabolic syndrome is mediated by TLR4 activation in adipocytes through saturated fatty acid, which can be called as homeostatic inflammation rather than autoinflammation [22].

On the other hand, it is well known that genetic background is important for development of systemic autoimmune diseases. Various mouse strains have been reported to be autoimmune-prone, such as *lpr* (deficient for *fas*), *gld* (deficient for *fas ligand*), *Dnase1-/-*, *Ctla4-/-*, and *Pd1-/-* mice [23, 24]. These mutations are related with dysregulation of apoptosis, clearance of apoptosis, and negative costimulatory (coinhibitory) signaling, which lead to breakage of tolerance. In humans, Aicardi-Goutieres syndrome, which shows the characteristic features of overlapping with cerebellar viral infection and systemic lupus erythematosus, is caused by deficiency for various exonucleases, such as TREX1/DNaseIII [25]. Identification of such various genetic causes for autoimmune diseases is making the border obscure between these diseases and hereditary autoinflammatory diseases.

Interestingly, *Il1rn-/-* mice show rheumatoid arthritis-like autoimmune and psoriasis-like T cell-independent autoinflammatory phenotypes, while *IL1RN* deficiency causes an autoinflammatory syndrome termed deficiency for IL-1 receptor antagonist in humans [26-28]. It may sound strange that both systems can be dysregulated on a monogenic background. However, considering that innate and adaptive immunity not only regulate but also activate each other, it is not surprising that both phenotypes of autoimmunity and autoinflammation are observed in the same body. Actually, elevation of nonspecific autoantibodies can be observed during development of Nakajo-Nishimura syndrome [17]. To develop the autoimmune phenotype (T/B cell-mediated autoantigen-specific hyper-reactivity), autoinflammatory responses (dysregulated signaling from the autoantigen through some pattern recognition receptor such as TLRs) should be involved [29]. Very recently, it has been reported that the repetitive percutaneous stimulation with imidazoquinolin generated a new model of systemic lupus erythematosus with high serum level of anti-double-stranded DNA antibodies [30]. Collectively, a variable mixture of both autoinflammation and autoimmunity can cause the inflammatory phenotype with a variable level of antigen specificity, depending on the lesional tissue, triggering factors, and other genetic backgrounds.

Conflict of interest
None declared.

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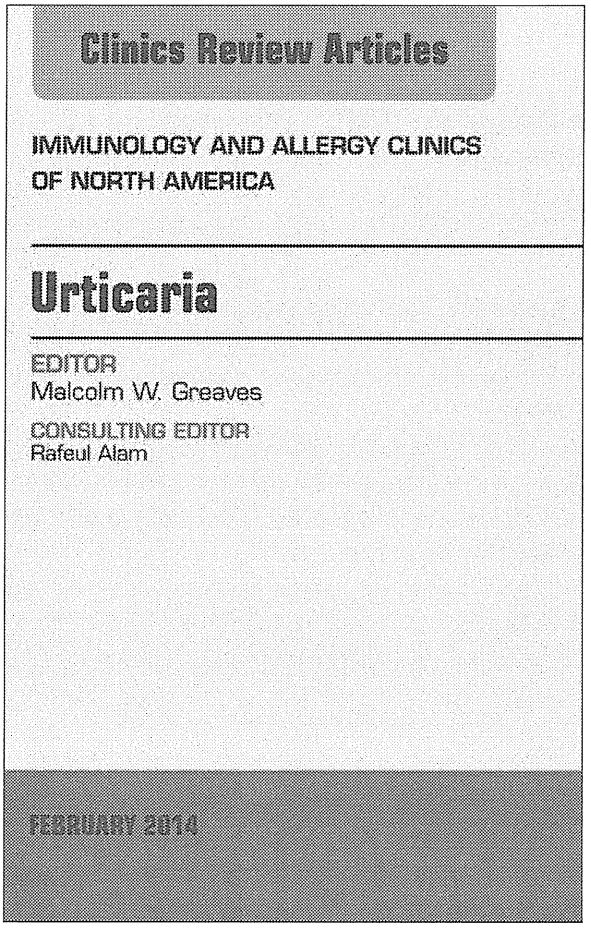

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Hereditary Disorders Presenting with Urticaria

Nobuo Kanazawa, MD, PhD

KEYWORDS

• KIT • C1-inhibitor • Bradykinin • NLRP3 inflammasome • IL-1β • NLRP12 • PLCγ2 • Autoinflammatory syndrome

KEY POINTS

- Hereditary disorders presenting with urticaria are not common and may not be encountered by most physicians.
- Hereditary disorders presenting with urticaria can be easily missed or misdiagnosed without correct knowledge.
- With proper diagnosis and understanding of the genetic cause and consequent pathogenesis, disease-specific essential therapeutic regimens can be offered.
- Recent discovery of the genetic origins for rare cases with distinct hereditary cold urticaria encourages examination of more cases.
- With rapid progress in genetic analysis, further insights into undefined hereditary urticaria will emerge in the near future.
- The knowledge obtained is promising for the development of novel therapeutics.

INTRODUCTION

Hereditary diseases listed in the latest clinical guideline for urticaria include *KIT* mutations–induced urticaria pigmentosa (mastocytosis), *C1NH* mutations–induced hereditary angioedema (HAE), and *NLRP3* mutations–induced cryopyrin-associated periodic syndromes (CAPS).[1] Although acquired somatic mutations in the *KIT* gene have a central role in the pathogenesis of mastocytosis, some germline *KIT* mutations have been reported in rare familial cases of pediatric mastocytosis.[2] HAE is a potentially life-threatening disease, and a precise diagnosis is required for replacement therapy of complement component 1 inhibitor (C1-INH).[3] CAPS are the most studied hereditary autoinflammatory disorders with dysregulated inflammasome signaling,

Funding Sources: Japan Society for the Promotion of Science, Ministry of Health, Labor and Welfare.
Conflict of Interest: None.
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Immunol Allergy Clin N Am 34 (2014) 169–179
http://dx.doi.org/10.1016/j.iac.2013.08.001 **immunology.theclinics.com**
0889-8561/14/$ – see front matter © 2014 Elsevier Inc. All rights reserved.

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for which a precise diagnosis is also critical for early intervention with anti-interleukin (IL)-1β therapy.[4]

In recent years, distinct syndromes with urticarial skin lesions, termed familial cold-induced autoinflammatory syndrome 2 (FCAS2) and FCAS3, have been designated as NLRP12-associated periodic syndromes (NAPS12) and PLCG2-associated antibody deficiency and immune dysregulation (PLAID), respectively, by identification of their genetic origins.[5–7] Moreover, there still remain more than a few cases with genetically undefined hereditary urticaria. The diseases discussed in this article are summarized in Table 1.

MASTOCYTOSIS

Mastocytosis (also known as mast cell disease, OMIM #154800) is divided into cutaneous mastocytosis (CM) and systemic mastocytosis (SM).[2] CM includes urticaria pigmentosa (UP), mastocytoma of the skin, and diffuse CM. In contrast to CM confined to the skin, SM is defined by mast cell infiltration in at least one extracutaneous lesion with or without cutaneous involvement (Table 2). CM is more commonly observed in children, especially before 6 months of age, but also affects adults mainly in the third to fourth decade.[8] Whereas pediatric CM spontaneously regresses before puberty in most cases, UP in adults has a significant risk of progression to SM.[9] UP is the most common variant of CM, and is characterized by disseminated brown macules or papules.[10] Consistent with the histologic feature showing massive mast cell infiltration in the papillary dermis with epidermal hypermelanosis, scratching the lesions induces mast cell degranulation and causes local flare and wheal reaction. This phenomenon, called Darier's sign, is useful for the diagnosis of UP. Similarly, cutaneous symptoms such as urticarial rashes, edema, and pruritus can be triggered by mechanical and thermal stimuli. Mastocytoma of the skin usually presents a few brown or orange plaques or

Table 1
Hereditary diseases with urticaria

Designation		OMIM Number	Responsible Gene
Mastocytosis		#154800	*KIT*
Hereditary angioedema (HAE)	Types I and II	#106100	*CINH*
	Type III	#610618	*F12*
Cryopyrin-associated periodic syndrome (CAPS)	Familial cold-induced autoinflammatory syndrome (FCAS)	#120100	*NLRP3*
	Muckle-Wells syndrome (MWS)	#191900	
	Chronic infantile neurologic cutaneous articular (CINCA) syndrome	#607115	
NLRP12-associated periodic syndrome (NAPS12)		#611762	*NLRP12*
PLCG2-associated antibody deficiency and immune dysregulation (PLAID)		#614468	*PLCG2*
Aquagenic urticaria		191850	Unknown
Familial localized heat urticaria		191950	Unknown
Dermodistortive urticaria		125630	Unknown
Familial dermographism		125635	Unknown

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Table 2
Classification of mastocytosis

Variant	Subvariant	Prognosis (Expected Median Survival)
Cutaneous mastocytosis (CM)	Urticaria pigmentosa (UP)	Good
	Mastocytoma of the skin	
	Diffuse CM	
Systemic mastocytosis (SM)	Indolent SM	Good (>16 y)
	SM with an associated clonal hematologic non–mast cell lineage disease (SM-AHNMD)	Poor (2 y)
	Aggressive SM	Poor (3.5 y)
	Mast cell leukemia	Very poor (2 mo)
	Mast cell sarcoma	Very poor
	Extracutaneous mastocytoma	Good

nodules, larger than 1 cm in diameter.[11] Diffuse CM is an extremely rare variant of CM, in which the skin undergoes generalized infiltration and may even cause erythroderma. Because of the dense infiltration of mast cells, bullous lesions can appear after stimuli or even spontaneously, more commonly in the latter 2 variants of CM.[12] Although evidence of systemic involvement (eg, bone marrow infiltration) is common, systemic symptoms are rare (indolent systemic mastocytosis).[13] Rarely, florid systemic symptoms (diarrhea, wheezing, syncope, and even anaphylaxis) may occur (aggressive systemic mastocytosis) after extensive release of mast cell mediators.

Functional mutations in the *KIT* gene have been detected from mast cells in the lesional skin, and rarely in the blood or bone marrow, indicating the somatic occurrence of these mutations.[14] KIT (CD117) is a receptor for stem cell factor (SCF), the essential growth factor for mast cells and melanocytes, and functions as a receptor tyrosine kinase. The most common activating *KIT* mutation, D816V, can be identified in mast cells from more than 90% of SM cases in adults. By contrast, the same mutation has been found in mast cells from only about one-third of pediatric CM patients.[15] The remaining 5% of adult SM cases and more than half of pediatric CM cases predictably show other *KIT* mutations.[16,17] It should be noted that the presence of the mutation in D816V does not alone induce malignant transformation of mast cells, and pediatric CM cases with this mutation reportedly fail to segregate with progressive or persistent disease.[18,19] Most strikingly, as predicted by an early report of an occurrence of mastocytosis in familial traits and monogenic twins, germline *KIT* mutations have been identified in some familial cases, indicating that mastocytosis can be a hereditary disease with a KIT mutation.[20,21]

HAE

HAE (OMIM #106100) is a rare autosomal dominant disorder with recurrent attacks of nonpitting tissue edema, as a result of increased vascular leakage in subcutaneous or submucosal tissue.[3] Although a description of this disease originates from the late nineteenth century, the underlying deficiency of C1-INH was identified in the 1960s.[22,23] The tissue edema is self-limited, with a longer duration than the wheals of chronic spontaneous urticaria. However, swelling of the extremities, face, and genitals often interferes with daily life, and laryngeal and upper airway swelling is potentially life-threatening and needs emergency control. Abdominal pain with nausea, vomiting, and diarrhea caused by intestinal edema may be misdiagnosed as acute

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abdomen and treated surgically. Moreover, facial edema, especially affecting the eyelids or lips, can damage the patient's body image and impair the quality of life of patients and their families.[24]

HAE is classified into 3 types depending on the level and activity of C1-INH (Table 3). Type I, with low level and activity of CI-INH, accounts for approximately 85% of HAE cases. Type II, with normal or elevated CI-INH level and impaired functional activity, affects the remaining 15% or so. Both types are caused by heterozygous mutations in the *C1NH* (also called *SERPING1*) gene, possibly leading to haploinsufficiency of the C1-INH activity. More than 200 disease-related mutations, deletions, or insertions causing reduced production of the C1-INH protein are associated with type I HAE, whereas point mutations in the protease-binding region of the C1-INH protein are linked to type II HAE.[25] By contrast, type III HAE (OMIM #610618), with normal level and activity of C1-INH, is rare but restricted to females.[26] In 2006, a unique heterozygous missense mutation in the *F12* gene was identified in 4 families with a founder effect.[27,28] This gain-of-function mutation has been shown to increase the activity of factor XII (also called Hageman factor), which is involved in the generation of kinins and is regulated by estrogens.

C1-INH is a plasma protein belonging to a family of serine protease inhibitors (serpins) and plays a regulatory role in multiple steps of the complement, contact, and coagulation systems (Fig. 1). The effect of C1-INH deficiency in the complement system has a diagnostic value, because a low serum C4 level is highly suggestive of C1-INH deficiency. By contrast, the role of C1-INH in the contact system is more important for the pathogenesis of HAE. In the case of C1-INH deficiency, activation of this system is exaggerated by hyperactivation of factor XII and prekallikrein, leading to overproduction of bradykinin, a potent vasodilator and inducer of vasopermeability. In fact an increased plasma bradykinin level has been revealed in HAE patients, especially during attacks.[29]

Appropriate management of HAE requires urgent therapy, and short-term and long-term prophylaxis.[30] For acute attacks neither corticosteroid nor epinephrine is effective, and plasma-derived pasteurized C1-INH is most widely administered. Fresh frozen plasma is still used in cases of emergency, but should be used carefully because of a risk of worsening the attack. Recently, a kallikrein inhibitor (ecallantide) and a selective bradykinin B2 receptor antagonist (icatibant) have been approved in Europe and the United States for the treatment of acute attacks. For short-term prophylaxis before dental manipulation or surgery, danazol has been used and, recently, nanofiltered C1-INH has been used for adult and adolescent cases. The same regimens have been used for long-term prophylaxis.

CAPS

Familial cold urticaria (FCU), showing recurrent attacks of urticarial rashes after general exposure to the cold, was first described in 1940.[31] Arthralgia, myalgia, chills, and fever accompanied attacks. A related hereditary disease with recurrent episodes of urticarial

Table 3
Classification of HAE

	C4 Level	C1-INH Level	C1-INH Activity
Type I	Low	Low	Low
Type II	Low	Normal or elevated	Low
Type III	Normal	Normal	Normal

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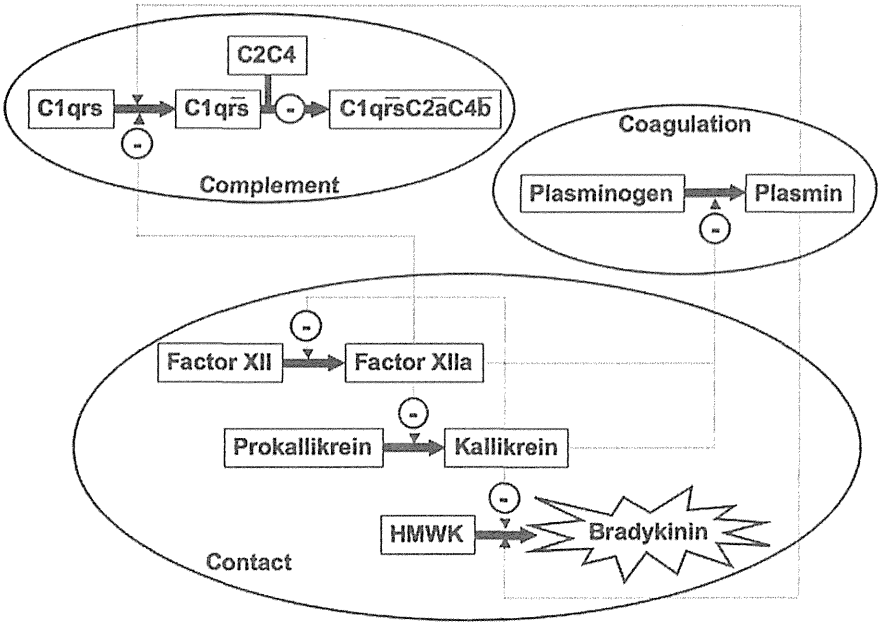

Fig. 1. The regulatory role of C1-INH in the complement, contact, and coagulation systems. Action points of CI-INH are indicated by dashes within circles. HMWK, high molecular weight kininogen.

rash with accompanying symptoms without cold exposure was first described in 1962.[32] Development of late-onset sensorineural deafness and renal amyloidosis was possible, and the disease was called urticaria-deafness-amyloidosis or Muckle-Wells syndrome (MWS; OMIM #191900). In 2001, a new gene in chromosome 1q44 was identified as being responsible for both diseases.[33] The identified gene product has been designated as cryopyrin, which means "cold-induced fever," because of its similarity to pyrin, the *MEFV* gene product responsible for familial Mediterranean fever.[34] Moreover, based on the genetic and clinical similarities to hereditary periodic fever syndromes, the name of the disease has been changed from FCU to familial cold-induced autoinflammatory syndrome (FCAS; OMIM #120100). In 2002, mutations in the same gene were further detected in more severe hereditary disorder, chronic infantile neurologic cutaneous and articular (CINCA) syndrome (OMIM #607115), characterized by a neonatal-onset triad of rash, chronic meningitis, and joint inflammation with recurrent fever.[35,36] Thus these 3 disorders sharing the same genetic origin are defined as forming a sequential spectrum of CAPS.[37] Although CAPS patients may show absence for *NLRP3* mutations, especially in the case of the severe variant, somatic mosaicism of the *NLRP3* mutation has globally been identified in some of these cases.[38]

Cryopyrin is composed of the N-terminal pyrin domain (PYD), central nucleotide oligomerization domain (NOD), and C-terminal leucine-rich repeats (LRR). This molecule is one of the most characterized NOD-like receptor (NLR) family molecules, and has formally been designated as NLRP3.[39] When stimulated with various danger signals, NLRP3 forms a pentamer and associates with procaspase-1 containing the caspase-recruitment domain (CARD), through an adaptor molecule, "apoptosis-associated speck-like protein with a CARD (ASC)," consisting of both PYD and CARD. This NLRP3–ASC–procaspase-1 complex, formed through homophilic interaction of each domain (PYD-PYD and CARD-CARD), has been designated as the NLRP3 inflammasome.[40] This complex works as a cytoplasmic platform activating caspases-1–mediated IL-1β/IL-18 secretion. In the case of CAPS, missense mutations in NLRP3 cause constitutive formation of the inflammasome complex and subsequent IL-1β secretion