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SUPPORTING INFORMATION

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

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Clinical and genetic features of hereditary angioedema with and without C1-inhibitor (C1-INH) deficiency in Japan

To the Editor,

Hereditary angioedema with normal C1-inhibitor (HAEnCI) is an umbrella term for several types of HAE that phenotypically resemble HAE but in which variants affecting function have been identified in genes other than the C1-INH gene (*SERPING1*).[1] In contrast to European patients with HAE, we suppose comprehensive clinical and genetic data of HAE are scarcely reported from Asian countries, including Japan. In particular, HAEnCI in Asia has not been clearly characterized. Considering ethnic differences, it would be important to clarify the features of HAE in the Asian population. Here, we report the clinical and genetic features of Japanese patients with 158 cases from 122 families with HAE-C1-INH and 21 cases from 21 families with HAEnCI. HAEnCI was defined as follows: normal C1-INH activity, no variants affecting function in the *SERPING1* gene, at least one relative with recurrent angioedema attacks (i.e., positive family history), no history of urticaria, and lack of efficacy by antihistamines or corticosteroids.

Table 1 shows clinical features of our Japanese patients with HAE-C1-INH and HAEnCI. When compared with HAE-C1-INH, HAEnCI was significantly more predominant among females (95.2% vs. 66.9%, $p = .008$), more frequently affected in the face (61.9% vs. 32.9%, $p = .009$) and pharynx/larynx (47.6% vs. 21.5%, $p = .009$), associated with more frequent exacerbations in the previous year (mean ± SD: 14.4 ± 27.3 vs. 3.66 ± 7.14, $p = .0001$), associated with higher prevalence of patients with more than six angioedema attacks in the previous year (52.4% vs. 16.5%, $p = .0001$), and more susceptible to triggers such as physical stress (38.1% vs. 12.7%, $p = .007$) and upper respiratory infections (URI) (19.0% vs. 5.1%, $p = .037$). The prevalence of having a positive family history experiencing angioedema attacks was significantly higher in HAEnCI (100%) than in HAE-C1-INH (81.6%) ($p = .027$). This is because the inclusion criteria of HAEnCI demand a family history of angioedema. There were no differences in most of the clinical features of male and female patients with HAE-C1-INH except the incidence of urological attacks and prodromal symptoms (Table S1).

It is of note that our patients with HAEnCI suffer from edema in the face and pharynx/larynx twice more frequently than those with HAE-C1-INH. In European patients with HAEnCI, facial and oropharyngeal swellings develop more predominantly than those with HAE-C1-INH as well.[2] On the other hand, there are a number of differences in the clinical characteristics such as frequency of attacks and triggers between Japanese and European patients with HAEnCI. In particular, hormonal perturbations induced by menstruation/pregnancy or oral contraceptives did not seem to influence attacks in our patients with HAEnCI. This finding is in contrast with European patients with HAEnCI whose angioedema symptoms are frequently deteriorated by oral contraceptives or pregnancies.[2]

In the case of HAE-C1-INH, the incidence of abdominal attacks was different between our patients and European patients. Abdominal attacks are common clinical manifestations of European patients with HAE-C1-INH, whose incidence reaches over 90% in these patients.[3] In contrast, the proportion of patients who had experienced intestinal attack was 35.4% in our 158 patients with HAE-C1-INH (Table 1). Other reports from Asia such as the one from Mainland China[4] have shown similar incidence of abdominal attacks with the present study.

Genetic analysis directed toward the entire exons for the known causative genes was performed for our Japanese HAEnCI patients

TABLE 1 Clinical features of the Japanese patients with HAE-C1-INH and HAEnCI

Variables	HAE-C1-INH % (no. affected/observed[a])	HAEnCI % (no. affected/observed[a])	*p*
Female	66.9 (105/157)	95.2 (20/21)	.008
Comorbid disease			
AID	3.2 (5/158)	0.0 (0/21)	1.000[b]
Urticaria	7.6 (12/158)	0.0 (0/21)	.365[b]
Bronchial asthma	5.1 (8/157)	9.5 (2/21)	.334[b]
CVD	3.2 (5/158)	9.5 (2/21)	.192[b]
Arthralgia/arthritis	3.2 (5/158)	0.0 (0/21)	1.000[b]
Any kinds	27.2 (43/158)	42.9 (9/21)	.138
Family history			
At least another patient in family member	81.6 (129/158)	100.0 (21/21)	.027[b]
Death probably by angioedema	7.6 (12/158)	4.8 (1/21)	1.000[b]
Age of onset			
Mean ± SD	23.0 ± 12.6	26.8 ± 22.7	.298
≤40	95.2 (120/126)	82.4 (14/17)	.075[b]
≤20	51.6 (65/126)	52.9 (9/17)	.916
Age of diagnosis			
≤40	69.9 (100/143)	70.0 (14/20)	.995
≤20	13.3 (19/143)	40.0 (8/20)	.007[b]
Site of attacks			
Extremities	39.2 (62/158)	47.6 (10/21)	.462
Tongue	0.6 (1/158)	4.8 (1/21)	.221[b]
Face	32.9 (52/158)	61.9 (13/21)	.009
Pharynx/Larynx	21.5 (34/158)	47.6 (10/21)	.009
Intestine	35.4 (56/158)	38.1 (8/21)	.812
Urogenital	6.3 (10/158)	9.5 (2/21)	.636[b]
Others	7.0 (11/158)	14.3 (3/21)	.216[b]
Frequency of attacks in the previous 1 year			
Mean ± SD	3.66 ± 7.14	14.4 ± 27.3	.0001
≧6 times	16.5 (26/158)	52.4 (11/21)	.0001
Prodromal symptom	13.3 (21/158)	28.6 (6/21)	.097[b]
Trigger			
Psychological	13.9 (22/158)	33.3 (7/21)	.051[b]
Physical	12.7 (20/158)	38.1 (8/21)	.007[b]
Menstruation/Pregnancy	19.0 (20/105)	20.0 (4/20)	1.000[b]
Dental procedure	13.9 (22/158)	14.3 (3/21)	1.000[b]
URI	5.1 (8/158)	19.0 (4/21)	.037[b]
Medication and acute attack[c]			
pdC1-INH	31.0 (49/158)	19.0 (4/21)	.259
Tranexamic acid	19.0 (30/158)	38.1 (8/21)	.083[b]
Anti-histamine	6.3 (10/158)	19.0 (4/21)	.064[b]
Prednisolone	3.2 (5/158)	14.3 (3/21)	.053[b]
Epinephrine	1.3 (2/158)	4.8 (1/21)	.314[b]
Danazol	2.5 (4/158)	0.0 (0/21)	1.000[b]
FFP	0.0 (0/158)	0.0 (0/21)	–
Intubation/Tracheostomy	3.8 (6/158)	9.5 (2/21)	.238[b]
Prophylaxis			
Tranexamic acid	31.0 (49/158)	33.3 (7/21)	.829
Danazol	5.1 (8/158)	4.8 (1/21)	1.000[b]
Others	6.3 (10/158)	9.5 (2/21)	.636[b]

Note: A total number of registered are 158 for HAE-C1-INH and 21 for HAEnCI.

Abbreviations: AID, autoimmune disease; CVD, cardiovascular disease; FFP, fresh frozen plasma; SD, standard deviation; URI, upper respiratory infection.

[a]Some of the data are lacking.

[b]Fisher's exact test.

[c]Icatibant was approved in Japan after the termination of this registration.

TABLE 2 *SERPING1* mutations in our Japanese 112 families of HAE-C1-INH

	Alteration (physical location on chromosome 11)[a]	cDNA numbering (NM_000062.2)	Location	Effect on protein	Families	Reference[b] (LOVD)
1	g.57365720A>G	c.-22-2A>G	Intron1	Splicing defect	1	*
2	g.57365746G>A	c.3G>A	Exon2	p.Met1Ile	1	*
3	g.57365748_57365749del	c.5_6delCC	Exon2	p.Ala2Valfs*17	1	
4	g.57365760_57365767dup	c.17_24dupCCCTGCTG	Exon2	p.Thr9Profs*3	1	
5	g.57365795G>T	c.51+1G>T	Intron2	Splicing defect	1	*
6	g.57367351G>A	c.52-1G>A	Intron2	Splicing defect	1	*
7	g.57367406_57367407del	c.106_107delAG	Exon3	p.Ser36Phefs*21	3	*
8	g.57367416_57367417insGGATC	c.116_117insGGATC	Exon3	p.Asp39Glufs*42	1	
9	g.57367438_57367507del	c.138_207del	Exon3	p.Thr47Glnfs*9	1	*
10	g.57367447del	c.147delT	Exon3	p.Ile50Serfs*29	1	
11	g.57367504del	c.204delC	Exon3	p.Asn69Thrfs*10	1	
12	g.57367526del	c.226delA	Exon3	p.Thr76Profs*3	1	
13	g.57367646C>T	c.346C>T	Exon3	p.Gln116*	2	*
14	g.57367700_57367704del	c.400_404delGAGAG	Exon3	p.Glu134Serfs*121	1	
15	g.57367703_57367704del	c.403_404delAG	Exon3	p.His136Phefs*120	1	*
16	g.57367749C>T	c.449C>T	Exon3	p.Ser150Phe	2	*
17	g.57367761_57367765delins TCAGGGAGGCTCTTCAA	c.461_465delACCACins TCAGGGAGGCTCTTCAA	Exon3	p.Tyr154_His155delins PheArgGluAlaLeuGln	1	
18	g.57367767C>A	c.467C>A	Exon3	p.Ala156Asp	2	*
19	g.57367775del	c.475delG	Exon3	p.Ala159Glnfs*2	1	
20	g.57367848T>C	c.548T>C	Exon3	p.Leu183Pro	2	*
21	g.57367850G>A	c.550G>A	Exon3	p.Gly184Arg	2	*
22	g.57369507G>C	c.551-1G>C	intron3	Splicing defect	1	*
23	g.57369500-57369511dup	c.554_555ins TGTTGCAGGGGC	Exon4	p.Ala185_Gly186ins ValAlaGlyAla	1	
24	g.57369510del	c.553delG	Exon4	p.Ala185Leufs*26	1	*
25	g.57369523C>A	c.566C>A	Exon4	p.Thr189Asn	1	*
26	g.57369586T>C	c.629T>C	Exon4	p.Leu210Pro	1	*
27	g.57369610T>A	c.653T>A	Exon4	p.Val218Asp	1	*
28	g.57369623_57369624del	c.666_667delTC	Exon4	p.Gln223Aspfs*33	1	*
29	g.57369631_57369632delinsAA	c.674_675delinsAA	Exon4	p.Phe225*	2	*
30	g.57373482G>T	c.686-1G>T	intron4	Splicing defect	1	
31	g.57373492T>A	c.695T>A	Exon5	p.Ile232Lys	1	*
32	g.57373549T>A	c.752T>A	Exon5	p.Leu251Gln	1	
33	g.57373617A>G	c.820A>G	Exon5	p.Ile274Val	3	*
34	g.57373649dup	c.852dupT	Exon5	p.Thr285Tyrfs*20	1	
35	g.57373687G>A	c.889+1G>A	intron5	Splicing defect	1	*
36	g.57373886T>G	c.895T>G	Exon6	p.Trp299Gly	1	*
37	g.57373928T>C	c.937T>C	Exon6	p.Phe313Leu	1	*
38	g.57373956T>G	c.965T>G	Exon6	p.Val322Gly	2	
39	g.57373962T>G	c.971T>G	Exon6	p.Met324Arg	1	*
40	g.57373989C>A	c.998C>A	Exon6	p.Ala333Asp	1	*
41	g.57379188A>G	c.1030-2A>G	intron6	Splicing defect	1	
42	g.57379189G>C	c.1030-1G>C	intron6	Splicing defect	1	*
43	g.57379189G>A	c.1030-1G>A	intron6	Splicing defect	1	*

(Continues)

TABLE 2 (Continued)

	Alteration (physical location on chromosome 11)[a]	cDNA numbering (NM_000062.2)	Location	Effect on protein	Families	Reference[b] (LOVD)
44	g.57379193G>A	c.1033G>A	Exon7	p.Gly345Arg	1	*
45	g.57379194G>A	c.1034G>A	Exon7	p.Gly345Glu	1	*
46	g.57379216dup	c.1056dup	Exon7	p.Leu353Serfs*16	1	*
47	g.57379241C>T	c.1081C>T	Exon7	p.Gln361*	1	*
48	g.57379279_57379302del	c.1119_1142del	Exon7	p.Leu374_Ala381del	1	
49	g.57379317del	c.1157delT	Exon7	p.Leu386Argfs*11	1	*
50	g.57379317_57379318del	c.1157_1158delTG	Exon7	p.Leu386Argfs*38	2	
51	g.57379344_57379345	c.1184_1185delTC	Exon7	p.Leu395Profs*29	3	
52	g.57379355C>T	c.1195C>T	Exon7	p.Pro399Ser	1	*
53	g.57379379C>T	c.1219C>T	Exon7	p.Gln407*	2	
54	g.57379395T>A	c.1235T>A	Exon7	p.Ile412Asn	1	*
55	g.57381820T>G	c.1269T>G	Exon8	p.Tyr423*	1	*
56	g.57381835T>A	c.1284T>A	Exon8	p.Cys428*	1	*
57	g.57381891T>C	c.1340T>C	Exon8	p.Leu447Pro	1	*
58	g.57381919_57381921del	c.1368_1370delGGC	Exon8	p.Ala457del	1	
59	g.57381920G>C	c.1369G>C	Exon8	p.Ala457Pro	1	*
60	g.57381947C>T	c.1396C>T	Exon8	p.Arg466Cys	7	*
61	g.57381947del	c.1396delC	Exon8	p.Arg466Alafs*110	2	
62	g.57381948G>T	c.1397G>T	Exon8	p.Arg466Leu	3	*
63	g.57381982C>G	c.1431C>G	Exon8	p.Phe477Leu	1	*
64	p.57381996G>A	c.1445G>A	Exon8	p.Trp482*	1	
65	g.57382026T>A	c.1475T>A	Exon8	p.Met492Lys	1	*
66	g.57382028G>A	c.1477G>A	Exon8	p.Gly493Arg	1	*
67	g.57382029G>A	c.1478G>A	Exon8	p.Gly493Glu	1	*
68	g.57382031C>T	c.1480C>T	Exon8	p.Arg494*	3	*
69	g.57382034_57382036del	c.1483_1485delGTA	Exon8	p.Val495del	1	
70	g.57382044C>A	c.1493C>A	Exon8	p.Pro498His	1	*
71	g.57382044C>T	c.1493C>T	Exon8	p.Pro498Leu	1	*
72	Deletion of exon 1 to 4				1	*
73	Deletion of exon 1 to 8				2	*
74	Deletion of exon 4				5	*
75	Deletion of exon 4 to 8				1	
76	Deletion of exon 5 to 6				1	*
77	Deletion of exon 5 to 8				3	*
78	Duplication of exon 3				1	
79	Duplication of exon 4				1	*

[a]GRCh37/hg19 genomic chromosomal coordinates are shown.

[b]The mutations previously reported in LOVD v.3.0 (https://databases.lovd.nl/shared/genes/SERPING1) as of April 2021 are shown (*).

($n = 21$). Targeted next-generation sequencing for the factor XII gene (*F12*), the plasminogen (*PLG*) gene, the angiopoietin-1 (*ANGPT1*) gene, the kininogen 1 (*KNG1*) gene, and the *SERPING1* gene revealed missense variant: p.Lys330Glu (c.988A>G) in the *PLG* gene in two patients[5] and p.Arg466Ser (c.1396C>A) in the *F12* gene in 1 patient. All of these variants were heterozygous. The p.Lys330Glu variant in the *PLG* gene was originally reported in Europe as causative for HAEnCI.[6] The p.Arg466Ser variant identified in the *F12* gene has been reported in a patient with factor XII deficiency.[7] It is likely that this variant does not have any relationship with angioedema. None of the known variants affecting function in the *F12* gene, the *ANGPT1* gene, and the *KNG1* gene responsible for HAEnCI was identified in our patients. The patients carrying variants affecting function in the *F12* gene (HAE-F12) account for about 30% of HAEnCI in European countries.[8] Lack of the *F12* gene findings in our Japanese HAEnCI patients might reflect ethnic difference, or limitation of our approach focused only on

small variants of the exons. In addition, the recently reported variants affecting function of the myoferlin (*MYOF*) gene (p.Arg217Ser) and of the heparin sulfate 3-*O*-sulfotransferase 6 (*HS3ST6*) gene (p.Thr144Ser) were not identified in our patients.

Ninety-two percent (112/122) of the studied families with HAE-C1-INH were positive for the *SERPING1* variant affecting function (Table 2). Missense variants were the most common (45 families), followed by small insertions/deletions (30 families), large insertions/deletions (15 families), nonsense variants (13 families), and splicing defects (nine families). Nineteen variants were recurrent, at least in two different families. Twenty-five variants affecting function had not been reported in LOVD v.3.0 Leiden Open Variation Database[9] (https://databases.lovd.nl/shared/genes/SERPING1) as of April 2021. All the novel sequence variants except two (#17, #23) were predicted to be pathogenic by in silico analysis (Table S2). The variants #17 (c.461_465delACCACinsTCAGGGAGGCTCTTCAA) and #23 (c.554_555insTGTTGCAGGGGC) were in frame insertion/deletion and were predicted as variants of uncertain significance (VUS). There were no correlations between the types of *SERPING1* variants (missense vs. other variant) and the markers of severity of disease, such as the age of onset, frequency of attacks, and C1-INH activity (Table S3).

In conclusion, we demonstrated the clinical and genetic features of the patients with HAE-C1-INH and HAEnCI in Japan. When compared with European patients with HAE-C1-INH, the clinical features of our Japanese patients with HAE-C1-INH were almost similar, but the frequency of abdominal pain in our patients was considerably low. The clinical features of our Japanese patients with HAEnCI were significantly different from those of HAE-C1-INH in a number of aspects such as female-male ratio and sites of attacks. Lack of the *F12* gene variants affecting function in our HAEnCI patients might indicate that the genetic background is different between Japanese and European patients with HAEnCI.

KEYWORDS

factor XII, hereditary angioedema, hereditary angioedema with normal C1-inhibitor, Japanese, plasminogen

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CONFLICT OF INTEREST

As for COI, Dr. Hashimura has nothing to disclose. Dr. Kiyohara has nothing to disclose. Dr. Fukushi has nothing to disclose. Dr. Hirose is a consultant for Takeda Pharmaceutical Company (without salary). Dr. Ohsawa reports personal fees from Takeda Pharmaceutical Company, personal fees from CSL Behring, personal fees from Torii Pharmaceutical Company, personal fees from BioCryst Pharmaceuticals, outside the submitted work. Dr. Tahira has nothing to disclose. Dr. Horiuchi reports personal fees from Takeda Pharmaceutical Company, personal fees from CSL Behring, personal fees from Torii Pharmaceutical Company, outside the submitted work.

AUTHOR CONTRIBUTIONS

CH contributed to conception, performed data acquisition and analysis, and wrote the initial draft of the manuscript. CK and TT performed data analysis and drafted the manuscript. THirose, JIF, and IO were involved in data collection. THoriuchi designed the study, performed data acquisition and analysis, and wrote the manuscript. All authors contributed to drafting the article, revised the manuscript critically for important intellectual content, and approved the final version.

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SUPPORTING INFORMATION

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

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T_H2 cytokines and *Staphylococcus aureus* cooperatively induce atopic dermatitis-like transcriptomes

To the Editor,

Atopic dermatitis (AD) is a common inflammatory skin disease associated with barrier disfunction and T_H2/T_H22 immune polarity.[1,2] Patients with AD are often colonized or infected with *Staphylococcus aureus* (*S. aureus*).[3] Recent large cohort studies and skin microbiome analyses have identified that AD is dominated by *S. aureus*.[4] However, the interaction of cytokines and bacteria in AD skin remains unclear. We sought to define the impact of AD-related polarizing cytokines and *S. aureus* on keratinocytes.

Normal human epidermal keratinocytes from adult (HEKa) were exposed to heat-killed *Staphylococcus epidermidis* (*S. epidermidis*) and *S. aureus* with and without T-cell derived cytokines such as IFN-γ (T_H1), IL-4/IL-13 (T_H2), and IL-22 (T_H22). *Staphylococcus epidermidis*, a commensal bacterium of the skin, was used as a control for *S. aureuS. a*D-related key immune biomarkers were measured by quantitative real-time PCR (RT-qPCR) or by protein assays. Further analysis was performed through RNA sequencing to define broader responses to both bacteria and cytokines. RNA-sequencing data were analyzed using gene set variation analysis (GSVA) with our previously defined gene lists for the skin from AD lesional/non-lesional or normal subjects.[5–8]

Quantitative real-time PCR showed that individual cytokine or bacterial exposure induced distinctly different gene expressions in HEKa. *IL36 γ*, *CXCL8*, *S100A7*, and *FLG* were upregulated by bacterial stimulation (Figure 1G–J,L). *CXCL9*, *CXCL10*, and *CCL22* were upregulated by IFN-γ treatment, and *CCL26* was upregulated by IL-4, IL-13, or IL-4 + IL-13 treatment (Figure 1B–E). *IL37* and *IL17C* were both downregulated by T_H2 cytokines (Figure 1A,F,K). IL-22 treatment induced a higher expression of *S100A7* than bacterial stimulation (Figure 1I). Interestingly, *IL36 γ* was also upregulated by IL-4/IL-13/IFN-γ treatment and that was additively upregulated by the combined exposure of *Staphylococcus* species (Figure 1G,L).

RNA-sequencing analysis showed the different transcriptome profiles in HEKa exposed to cytokines or bacteria (Figures S1 and S2A,C). Since the transcriptome profile induced by IL-4 or IL-13 exposure alone was similar to that induced by the combined exposure of IL-4/IL-13 (Figure S2B), we then compared the cytokine-treated transcriptome profiles of the three groups, IL-4/IL-13, IFN-γ, and IL-22. Interestingly, the combined exposure with *S. aureus* and IL-4/IL-13 cytokines induced synergistic gene upregulation distinctly different from cytokines/bacteria alone or combined exposure with *S. epidermidis* and cytokines (Figure 2A highlighted). Moreover, GSVA revealed that the addition of *S. aureus* induced higher expression of gene set that was upregulated in AD skin lesions than the addition of *S. epidermidis* (Figure 2B column i, row 1). The addition of polarizing cytokines without bacteria, IL-4/IL-13, or IL-22 induced GSVA scores higher than for *S. aureus* alone and consistent with known effects of these cytokines on keratinocytes in AD lesions vs. normal skin (Figure 2B column ii, iv; row 1). IFN-γ was associated with AD in several gene sets (Figure 2B column iii, row 1, 2 for AD acute-lesions/lesions vs. acute non-lesions/normal skin). For genes expressed in AD lesions vs. normal skin, the combination of *S. epidermidis* with IL-4/IL-13 reduced the GSVA score by 1.03 ($p < .001$), whereas *S. aureus* combined with IL-4/IL-13 increased the GSVA score by 5.13 compared with IL-4/IL-13 ($p < .001$; Table S2). The combined effect of IL-4/IL-13 with *S. aureus* was higher than for the combinations with IL-22 or IFN-γ (Tables S2).

In conclusion, our results establish that keratinocytes can respond to combined signals for *Staphylococcus* species and T-cell polarizing cytokines. *Staphylococcus aureus* infection potentially contributes to the exacerbation of AD-associated gene expression programs in cooperation with T_H2 cytokines. These data provide clues into molecular mechanisms characterizing AD flares, which might be associated with increased colonization or infection of the skin by *S. aureus*.