

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 $\pm$ 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 China⁴ 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 ($n = 21$). 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 patients⁵ 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.

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

Variables	HAE-C1-INH % (no. affected/observed ^a)	HAEEnCI % (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 $\pm$ SD	23.0 $\pm$ 12.6	26.8 $\pm$ 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 $\pm$ SD	3.66 $\pm$ 7.14	14.4 $\pm$ 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 HAEEnCI.

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

^aSome of the data are lacking.

^bFisher's exact test.

^cIcatibant 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 TGTTCAGGGGC	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	*

^aGRCh37/hg19 genomic chromosomal coordinates are shown.

^bThe mutations previously reported in LOVD v.3.0 (<https://databases.lovd.nl/shared/genes/SERPING1>) as of April 2021 are shown (*).

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 Database⁹ (<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.

Additional online supporting information

Materials and methods

Patients

We established the Center for Research, Education, And Treatment of angioEdema (CREATE), a non-profit organization on Jan, 2011 (<http://create2011.jp/index.html>) to conduct HAE patient registry, genetic analysis, and support for HAE patient association, kumimu. Kumimu is a play on the Japanese word *mukumi*, which means “swelling”. The registration was terminated in December 2017. This research was approved by the Institutional Review Board of Kyushu University (#586-00). After obtaining written consent from the HAE patients, the attending doctor would use his/her personal ID and password to enter patient’s clinical and laboratory information into an internet-based database. A total of 77 institutions from all over Japan participated in the study. A total of 158 cases from 122 unrelated families of HAE-C1-INH and 21 cases from 21 unrelated families of HAEnCI were registered in this study. The patients with HAEnCI in the present study were selected according to the criteria previously demonstrated^{S1, S2}.

Analysis of disease-related genes for HAE-C1-INH (*SERPING1* gene), and for HAEnCI (*F12*, *PLG*, *ANGPT1*, *KNG1*, *MYOF*, *HS3ST6* genes)

To date, 6 disease-related genes have been reported for HAEnCI^{S3, S4, S5}. Subsets of patients with HAEnCI carry variants affecting function either in the genes for *Factor XII* (named as HAE-FXII), plasminogen (*PLG*) (HAE-PLG), angiopoietin 1 (*ANGPT1*) (HAE-ANGPT1), kininogen 1 (*KNG1*) (HAE-KNG1), myoferlin (*MYOF*) (HAE-MYOF) or heparin sulfate 3-*O*-sulfotransferase 6 (*HS3ST6*) (HAE-HS3ST6). In the remaining HAEnCI patients, pathogenesis remains unknown and the condition is called HAE-UNK. These 6 disease-related genes and the gene for C1-INH (*SERPING1*) were studied for variants affecting function in our HAE patients.

DNA was extracted from peripheral blood mononuclear cells by using QIAamp DNA Blood Mini Kit (Qiagen). Targeted next generation sequencing was performed to detect variations in candidate genes in patients with HAE as previously described^{S6}. Briefly, the DNA was enzymatically fragmented to the size of 200-600 bp, ligated with indexing adapters and then PCR-amplified with Illumina P5 and P7 primers. The prepared libraries were pooled in equal amounts. For hybridization-based enrichment of the complete coding regions of the following genes, *SERPING1*, *F12*, *PLG*, *ANGPT1*, and *KNIG1*, a xGen predesigned gene capture pools were obtained from IDT (Coralville, IA; <https://sg.idtdna.com/pages/products/next-generation-sequencing/targeted-sequencing/hybridization-capture/custom-probes-panels/xgen-gene-capture-pools>). The pooled libraries were mixed with IDT xGen blocking oligonucleotide (IDT) and hybridized in solution to the gene capture probes basically as described in the manufacturer's standard workflow. The libraries were applied to the NextSeq 500 Mid-Output flow cell (Illumina) and run on a 75 bp paired read mode.

Variants in all protein coding exons and their intron boundaries (10 base of intron) were called after removal of low-quality reads. Sequence reads were aligned to the reference human genome (hg38) using the Burrows-Wheeler Aligner MEMalgorithm (BWA-MEM version 0.7.5a35) using default parameters^{S7}. Variants were also called using GATK's HaplotypeCaller under the default parameters (version 4.0.0.0) inputting each sample's final BAM files and the target region BED files (<https://gatk.broadinstitute.org/hc/en-us/articles/360036194592-Getting-started-with-GATK4>). Variant calling tools, VarScan2^{S8} and VarDict^{S9}, were complementarily used not to overlook mosaicism and multiple-nucleotide variants.

To detect large insertions/deletions of the *SERPING1* gene, multiplex ligation-dependent probe amplification (MLPA) kit P243 *SERPING1* (MRC-Holland, Amsterdam, The Netherlands) was used according to the manufacturer's instructions.

Sanger sequencing of the exon 7 of the *MYOF* gene and the exon 2 of the *HS3ST6* gene was performed to detect the recently identified mutations^{S4, S5}. A list of the primer sequences we used is available upon request from the corresponding author. As described previously^{S10}, PCR was performed in a 10-μl mixture containing 25 ng of genomic DNA, 1× PCR buffer for KOD FX Neo polymerase (Toyobo), a 0.4 mM concentration of each dNTP, a 0.3 μM concentration of each primer, and 0.2 units of KOD FX Neo polymerase for 35 cycles. The annealing temperature for PCR was 63°C. The PCR products were treated with exonuclease I (New England Biolabs) and rAPid alkaline phosphatase (Roche Diagnostics) and then sequenced using a BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems). Sequence products were purified with Sephadex G-50 and run on an ABI 3730 genetic analyzer (Applied Biosystems), and the data were analyzed using PolyPhred.

In silico analysis for variants affecting function was carried out using MutationTaster^{S11} (www.mutationtaster.org), SIFT 4G^{S12} (<https://sift.bii.a-star.edu.sg/sift4g/>), SIFT Indel^{S13} (https://sift.bii.a-star.edu.sg/www/SIFT_indels2.html) and PolyPhen-2^{S14} (<http://genetics.bwh.harvard.edu/pph2/>) software.

SERPING1 variants were also evaluated using public databases. Clinvar^{S15} (<https://www.ncbi.nlm.nih.gov/clinvar/>) data were downloaded on May 5, 2021 for a total of 200 unique *SERPING1* variants. Seven of variants identified in our study were registered in Clinvar (Table S2). We also accessed the *SERPING1* gene homepage - Global Variome shared LOVD (<https://databases.lovd.nl/shared/genes/SERPING1>) on April 29, 2021, where total of 862 unique *SERPING1* variants were registered (LOVD v.3.0 Build 26). , Fifty-four of our variants were already reported in LOVD, although it is unknown whether large insertion/deletion (#72 to #79) identified in our study are exactly the same defects as those in LOVD (Table 2 and Table S2).

Statistical analysis

Differences in patient characteristics were compared using the Student t-test. Categorical variables were compared using the Pearson chi-squared test or the Fisher exact test where appropriate. Comparisons of means and proportions were based on unpaired *t* test and chi-square test, respectively. C1-INH activity of the patients with HAE were classified into two [$\leq 25\%$ (below the detection limit and $>25\%$ normal range)]; and C4 into three (≤ 5 , 5–13 and >13 mg/dL). All P values were two-sided, with those less than 0.05 considered statistically significant. All statistical analyses were performed using the computer program STATA Version 14.2 (STATA Corporation, College Station, TX).

Additional Results

Clinical features of male and female patients with HAE-C1-INH (Table S1)

There were no differences in most of the clinical features of male and female patients with HAE-C1-INH. However, urogenital attacks were significantly more frequent in male patients (15.4%) than in female patients (1.9%) ($p=0.002$). In HAE-C1-INH patients, prodromal symptoms were more frequently observed in females than in males ($p=0.049$).

In silico prediction of pathogenicity of the *SERPING1* mutations (Table S2)

The pathogenicity of the 71 kinds of *SERPING1* variants affecting function other than large insertions/deletions identified in our patients with HAE-C1-INH were predicted in silico by using Mutation Taster, PolyPhen-2, SHIFT and SHIFT_indel. All the variants but two (#17, #23) were predicted to be pathogenic by at least one software package. “Clinical significance” of variants described in ClinVar database and “classification” in LOVD 3.0 database are shown.

Genotype and phenotype/C1-INH activity correlation (Table S3)

We studied the correlation of *SERPING1* gene variants with the clinical phenotypes that are supposed to reflect the severity of the disease in our 113 patients with HAE-C1-INH. The clinical manifestations of missense variants affecting function and those of defects other than missense (nonsense variants affecting function, splice defects, small insertions/deletions, large insertions/deletions) were compared. This differentiation of the variant types into two is similar to the previous reports^{S15}. A comparison of clinical features between the two variant types mentioned above seems to be reasonable because missense variants affecting function lead to only one amino acid substitution, while all other variants affecting function culminate in the gross change in amino acid sequence.

The mean ages ($\pm$ SD) of onset in patients with missense variants affecting function and all other variants affecting function were 23.80 ± 12.04 and 21.56 ± 11.25 years, respectively, which was not significantly different. The manifestations of HAE attacks before 10 years of age showed no significant difference in these two groups. The frequencies of angioedema attacks during the previous one year were not significantly different between patients with missense variants affecting function and those with all other variants. There was no relationship between C1-INH activity and C4 concentration. In addition, the number of families with mortalities from suspected HAE attacks was not different between the groups of missense variants affecting function and all other variants. It is therefore likely that at least in our HAE-C1-INH cohort in Japan, there were no apparent correlations between the type of *SERPING1* variants affecting function and severity of the disease.

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Author contributions

CH designed the study, performed data acquisition, and analysis, and drafted the manuscript. CK and TT performed data analysis, and drafted the manuscript. JIF, THirose, and IO were involved in the data collection and drafting the manuscript. THoriuchi conducted the entire study design, analysis of the data, and wrote the manuscript.

Conflict of Interest

I Ohsawa has received speaker/advisor honoraria from BioCryst, CSL Behring, and Takeda. T Hirose and T Horiuchi has received speaker/advisor honoraria from CSL Behring, and Takeda. The other authors have no conflicts of interest.

Table S1. Clinical features of male and female patients with HAE-C1-INH

		Male % (no. affected/ observed*)	Female % (no. affected/ observed*)	p
Comorbid disease	AID	1.9 (1/52)	3.8 (4/105)	1.000**
	Urticaria	7.7 (4/52)	7.6 (8/105)	1.000**
	Bronchial asthma	3.8 (2/52)	5.7 (6/105)	1.000**
	CVD	3.8 (2/52)	2.9 (3/105)	1.000**
	Arthralgia/arthritis	0 (0/52)	4.8 (5/105)	0.171**
	Any kinds	25.0 (13/52)	28.6 (30/105)	0.637
Age of onset	Mean $\pm$ SD	22.88 $\pm$ 14.26	23.09 $\pm$ 11.70	0.930
	≤ 40	92.9 (39/42)	96.4 (81/84)	0.399**
	≤ 20	57.1 (24/42)	44.0 (37/84)	0.166
Age of diagnosis	≤ 40	68.1 (32/47)	70.8 (68/96)	0.736
	≤ 20	14.9 (7/47)	12.5 (12/96)	0.692
Site of attacks	Extremities	42.3 (22/52)	38.1 (40/105)	0.611
	Tongue	1.9 (1/52)	0 (0/105)	0.331**
	Face	32.7 (17/52)	33.3 (35/105)	0.936
	Pharynx/Larynx	23.1 (12/52)	21.0 (22/105)	0.761
	Intestine	34.6 (18/52)	36.2 (38/105)	0.846
	Urogenital	15.4 (8/52)	1.9 (2/105)	0.002**
	Others	9.6 (5/52)	5.7 (6/105)	0.507**
Frequency of attacks in the previous 1 year	Mean $\pm$ SD	3.134 $\pm$ 5.185	3.952 $\pm$ 7.915	0.500
	≥ 6 times	19.2 (10/52)	15.2 (16/105)	0.526
Prodromal symptom		5.8 (3/52)	17.1 (18/105)	0.049
Trigger	Psychological	11.5 (6/52)	15.2 (16/105)	0.530
	Physical	15.4 (8/52)	11.4 (12/105)	0.484
	Dental procedure	13.5 (7/52)	14.3 (15/105)	0.889
	URI	5.8 (3/52)	4.8 (5/105)	1.000**

*Some of the data are lacking.

** Fisher's exact test

Table S3. Genotype-phenotype relationship in 113 patients with HAE-C1-INH

		Missense (n = 51)	Other than missense (n= 62)	p
Age of onset	Mean $\pm$ SD	23.80 $\pm$ 12.04	21.56 $\pm$ 11.25	0.310
	<10 years	6	7	
	$\geq$ 10	45	55	0.937
Frequency of attacks recent one year	Mean $\pm$ SD	4.02 $\pm$ 6.75	5.37 $\pm$ 9.04	0.379
	<6	7	12	
	$\geq$ 6	44	50	0.461*
C1-INH activity	$\leq$ 25%	38	44	
	>25%	6	16	0.108
C4	$\leq$ 5mg/dL	36	41	
	5–13	8	16	
	>13	1	1	0.562*
Families with dead members due to HAE**	% (no. affected/ observed*)	13.7% (7/51)	5.9% (3/52)	0.201*

* Fisher's exact test

**A total of 113 patients from 103 families are analyzed.