

Cloning, expression, and immunological characterization of two novel profilins from *Artemisia annua*

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The incidence of allergic disease has increased rapidly in recent decades in both developed and developing countries.^[1] In China, pollen of *Artemisia* is one of the most important outdoor allergen as reported by the first national pollen survey across all provinces.^[2] *Artemisia* pollen has been found to be an important cause of seasonal asthma.^[3] *Artemisia vulgaris* and *Artemisia annua* are the most important *Artemisia* species in China. *A. vulgaris* mainly grows in western regions, while *A. annua* is abundant in the densely populated northern and eastern regions.^[4] Immunoglobulin E (IgE) antibodies play a key role in allergy diseases. They are synthesized and released by B cells and bind to high-affinity IgE receptors on mast cells or basophils.^[5] Once the patients are exposed to corresponding allergens, the effector cells will be triggered and then release its mediator, leading to an allergic inflammation.^[5] Allergen immunotherapy based on specific allergens to patients is considered a promising and effective treatment for allergic diseases.^[6,7] Therefore, identification of the IgE-binding allergens will improve the diagnosis of allergy diseases and application of immunotherapy. However, previous studies have mainly investigated pollen allergens from *A. vulgaris* (Art v), such as Art v 1–6. As for *A. annua* (Art an), only two allergens have been identified as Art an 1 (defensin-like protein) and Art an 7 (putative galactose oxidase).^[4] Thus, it is important to study allergens from *A. annua* pollens.

In the present study, we identified two profilins from *A. annua* by next-generation sequencing technology and the basic local alignment search tool. The complementary DNAs encoding profilin 1 and profilin 2 were amplified by polymerase chain reaction using primers designed from

non-coding region sequences of two profilins obtained by transcriptome sequencing. The two profilins from *A. annua* pollen exhibited open reading frames of 399 base pair encoding 133 amino acids. The sequence data of the profilins have been deposited in Genbank under the accession numbers of MN105099 and MN105100. The amino acid sequences of profilin 1 and profilin 2 were exactly the same as those of Art v 4.0101 and Art v 4.0201 from *A. vulgaris*, respectively. Moreover, profilin 1 and profilin 2 shared approximately 65% to 90% sequence identity with other profilins from different plant pollens [Supplementary Figure 1A, <http://links.lww.com/CM9/A422>]. A phylogenetic tree analysis revealed that profilin 1 forms a close cluster with Amb a 8, whereas profilin 2 is close to Hel a 2 [Supplementary Figure 1B, <http://links.lww.com/CM9/A422>], and all these profilins were clustered into the same group.

To isolate recombinant allergens for further analysis, profilin 1 and profilin 2 genes were subcloned into pET-28a vector and expressed in *Escherichia coli* BL-21. These two profilins were expressed as the inclusion bodies, which were purified by nickel affinity chromatography under denaturing conditions. The refolded proteins showed a single band with an approximate molecular weight of 12,000 Da as revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis [Supplementary Figures 1C and 1D, <http://links.lww.com/CM9/A422>].

The IgE-binding activity of the identified allergens is essential for the initiation of allergic inflammation. Two hundred patients with allergy reactions to *Artemisia* pollen

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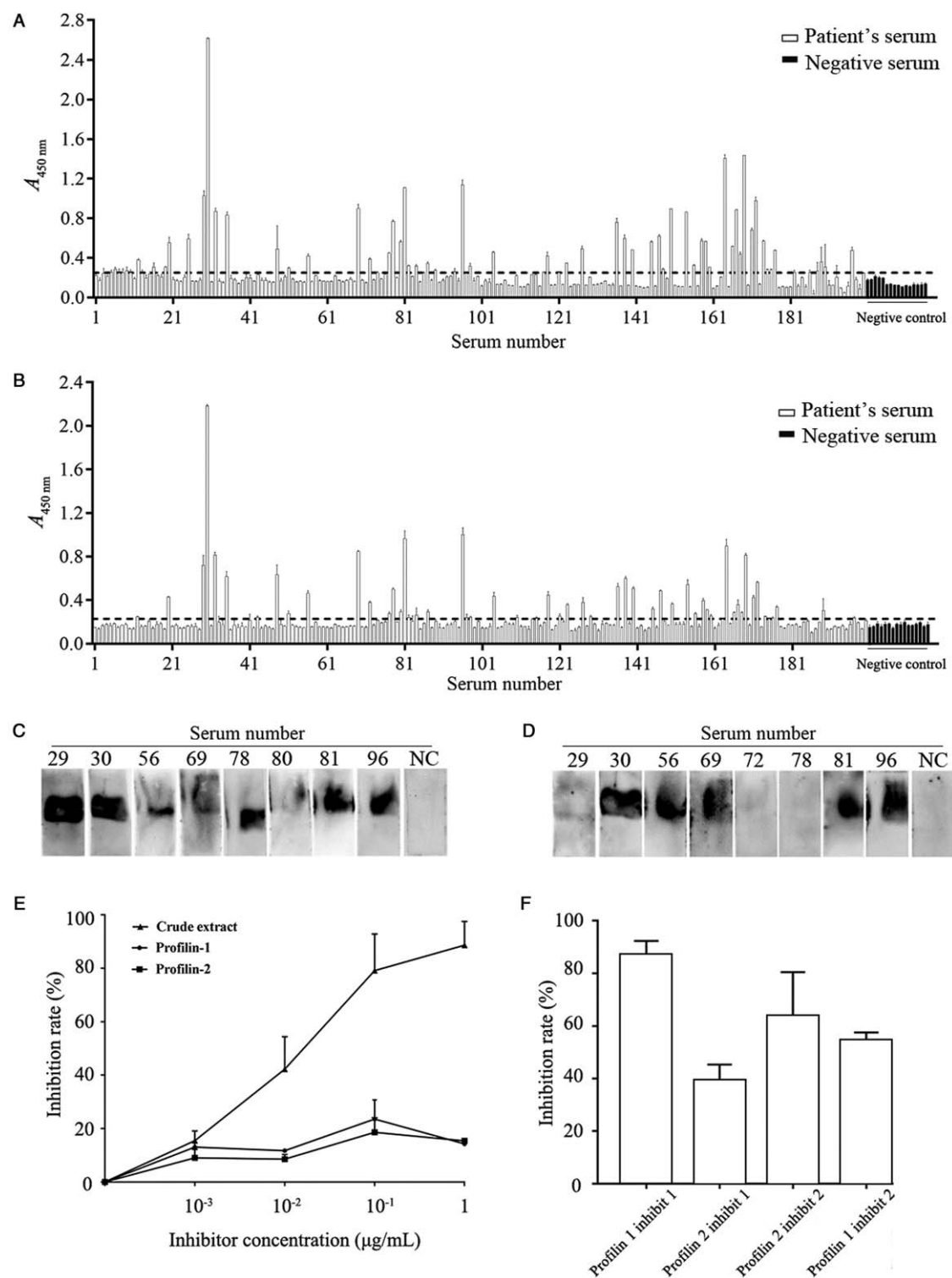


Figure 1: Immunoreactivity analysis of profilin 1 and profilin 2 by ELISA, Western blotting, and competitive inhibition experiment. ELISA analysis of profilin 1 (A) and profilin 2 (B). Two hundred *Artemisia* allergy serum samples and 16 negative control serum samples were used. The values were shown as mean $\pm$ SD. The dotted line represents the cut-off value, which was calculated as mean absorbance value of the negative control plus 3 SDs (mean + 3*SD) of the 16 negative control serum samples. Results of the Western blot analysis of profilin 1 (C) and profilin 2 (D). The serum number corresponds to ELISA serum number, which reacted to profilin 1 and/or profilin 2. NC represents negative control serum. (E) Inhibition of the IgE immunoreactivity to the *A. annua* extract by profilin 1, profilin 2, and crude extract. (F) Competitive inhibition experiment conducted to analyze the cross-reactivity between profilin 1 and profilin 2. *A. annua*: *Artemisia annua*; ELISA: Enzyme-linked immunosorbent assay; IgE: Immunoglobulin E; SD: Standard deviation.

and 16 healthy subjects were included in the present study. The overall clinical information of the patients is shown in Supplementary Table 1, <http://links.lww.com/CM9/A423>. All participants provided a written informed consent for the use of their blood samples for this study. Results of enzyme-linked immunosorbent assay (ELISA) showed that serum IgE reacted to profilin 1 in 65 of 200 (32.5%) patients [Figure 1A] and reacted to profilin 2 in 54 of 200

(27%) [Figure 1B]. The sensitization rates were similar to profilins from other pollens, such as Bet v 2 (22%, 55/242)^[8] and Hel a 2 (30.5%, 37/121),^[9] which might be due to their structural conservation. The IgE binding activities of the two profilins were further confirmed by Western blot using serum samples from eight representative patients and one negative control. Western blot showed that of the eight positive serum samples, seven showed strong bands in profilin 1 [Figure 1C] and five showed bands in profilin 2 [Figure 1D]. However, some ELISA-positive serum samples showed no bands in Western blotting. This is because some IgE-binding epitopes of profilin 1 and profilin 2 are conformational, which are easily disrupted during the Western blot test.

A competitive inhibition experiment was performed to evaluate the immunoreactivity of profilin 1 and profilin 2, in comparison to the crude extract of *A. annua* pollen. We found that the average inhibition rate of profilin 1 and profilin 2 was 23.6% and 18.6%, respectively [Figure 1E]. A further competitive inhibition experiment was conducted to analyze the cross-reactivity between profilin 1 and profilin 2. It was showed that 54.57% of the IgE from No. 30 serum bound to profilin 2 was inhibited by profilin 1, and 39.35% of that bound to profilin 1 was inhibited by profilin 2 [Figure 1F].

Comprehensive analysis of clinical symptoms and ELISA results revealed that there was no significant difference in the immunoreactivity characteristics of profilins in patients with different allergic symptoms (asthma, rhinitis, eczema, hay fever, dermatitis, or conjunctivitis) [Supplementary Figures 1E and 1F, <http://links.lww.com/CM9/A422>]. Similarly, there was no significant difference in the immunoreactivity characteristics of profilins between asthma and non-asthma patient groups, and rhinitis and non-rhinitis patient groups [Supplementary Figures 1G and 1H, <http://links.lww.com/CM9/A422>]. These indicated that sensitization to profilins from *A. annua* was not relevant to one or more specific clinical symptoms in Chinese *Artemisia* allergic patients, which was consistent with previous studies.^[10]

In summary, we identified two profilins named as profilin 1 and profilin 2 from *A. annua* pollen. These two profilins have identical amino acid sequences to that of Art v 4.0101 and Art v 4.0201 from *A. vulgaris*, respectively, but with different nucleotide sequences and non-coding regions. Moreover, alignment analysis showed that profilin 1 and profilin 2 shared high amino acid sequence identity to the previously reported profilin allergens from various species. This may contribute to cross-sensitization or polysensitization to different pollen or food allergens. The IgE-binding rates of profilin 1 and profilin 2 in *Artemisia* allergy patients were 32.5% and 27%, respectively, similar to that of pollen profilins from other species. We also found that the immunoreactivity of profilin 1 and profilin 2 in Chinese patients with *Artemisia* allergic decreased with increasing age, but this needs further confirmation in patients from different ethnicities and race. Finally, the

identified novel profilins had a non-negligible influence in Chinese *Artemisia* allergic patients and would be valuable to component-resolved allergy diagnostics in the future.

Ethical statement

The study protocol was approved by the Ethical Committee of the First Affiliated Hospital of Nanjing Medical University (Ethical Approval Number: 2018-SRFA-094). Written informed consent for the use of blood samples was obtained from all participants before study.

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Conflicts of interest

None.

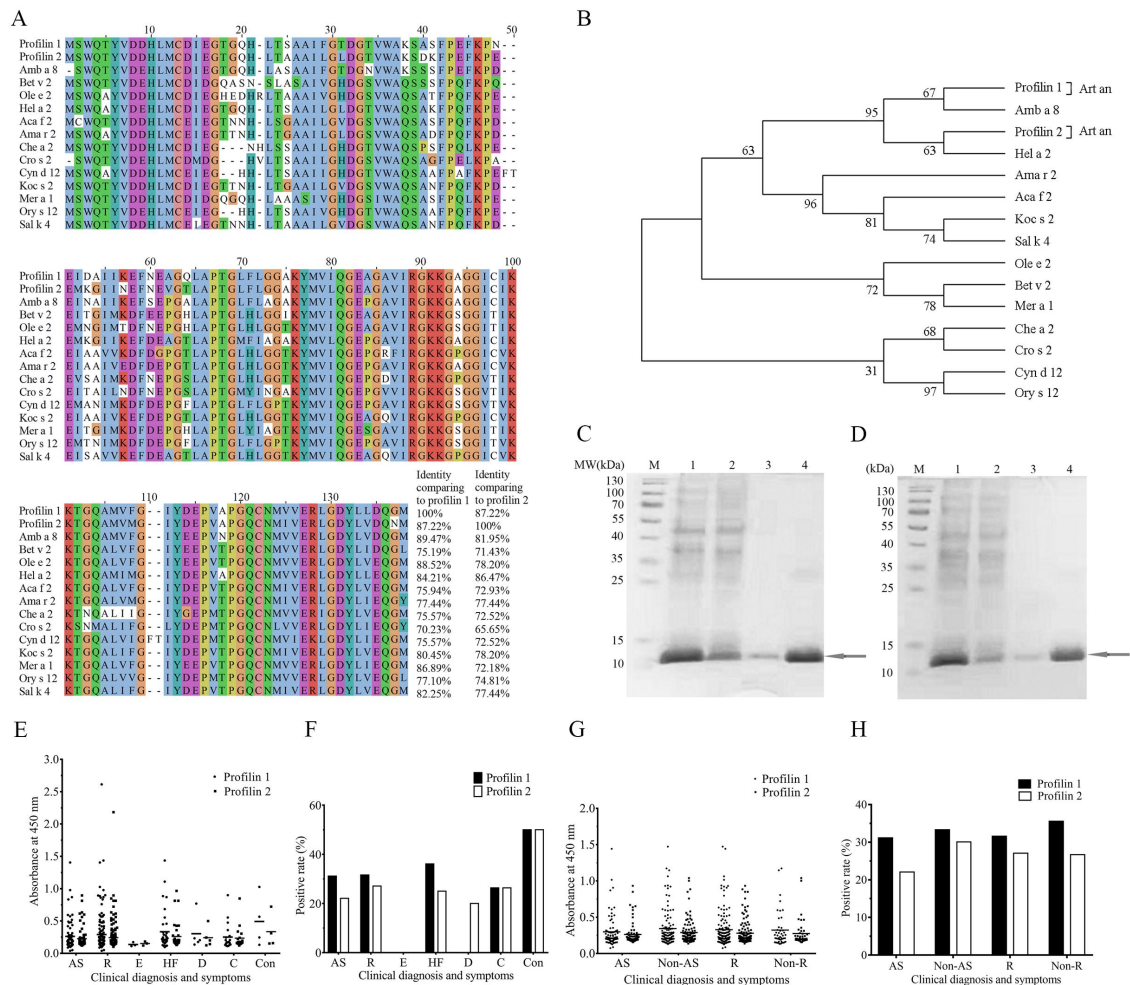
References

1. Lake IR, Jones NR, Agnew M, Goodess CM, Giorgi F, Hamaoui-Laguel L, *et al.* Climate change and future pollen allergy in Europe. *Environ Health Perspect* 2017;125:385–391. doi: 10.1289/EHP173.
2. Tang R, Sun JL, Yin J, Li Z. *Artemisia* allergy research in China. *Biomed Res Int* 2015;2015:179426. doi: 10.1155/2015/179426.
3. Gao Z, Fu WY, Sun Y, Gao B, Wang HY, Liu M, *et al.* *Artemisia* pollen allergy in China: component-resolved diagnosis reveals allergic asthma patients have significant multiple allergen sensitization. *Allergy* 2019;74:284–293. doi: 10.1111/all.13597.
4. Fu W, Gao Z, Gao L, Jin J, Liu M, Sun Y, *et al.* Identification of a 62-kDa major allergen from *Artemisia* pollen as a putative galactose oxidase. *Allergy* 2018;73:1041–1052. doi: 10.1111/all.13375.
5. Kounis NG, Konari L, Plotas P, Soufras GD, Tsigkas G, Davlouras P, *et al.* Kounis syndrome caused by double allergens: the more allergens present, the easier, the quicker, and the more severe the anaphylaxis. *Chin Med J* 2020;133:1889–1890. doi: 10.1097/CM9.0000000000000946.
6. Li Y, Yu SY, Tang R, Zhao ZT, Sun JL. Sublingual immunotherapy tablets relieve symptoms in adults with allergic rhinitis: a meta-analysis of randomized clinical trials. *Chin Med J* 2018;131:2583–2588. doi: 10.4103/0366-6999.244108.
7. Li LS, Zhang H, Wang M, Wang XJ, Guan K. Early transcriptomic profiling variation caused by cluster allergen immunotherapy. *Chin Med J* 2020;133:1366–1368. doi: 10.1097/CM9.0000000000000829.
8. Movérare R, Westritschnig K, Svensson M, Hayek B, Bende M, Pauli G, *et al.* Different immunoreactivity profiles in birch pollen-sensitive patients from six European populations revealed by recombinant allergens: an imprint of local sensitization. *Int Arch Allergy Immunol* 2002;128:325–335. doi: 10.1159/000063855.
9. Asturias JA, Arilla MC, Gómez-Bayón N, Aguirre M, Martínez A, Palacios R, *et al.* Cloning and immunological characterization of the allergen Hel a 2 (profilin) from sunflower pollen. *Mol Immunol* 1998;35:469–478. doi: 10.1016/s0161-5890(98)00036-4.
10. San Nicolás M, Braun T, Eder K, Berghaus A, Gröger M. Clinical relevance of IgE to profilin and/or polcalcin in pollen-sensitized patients. *Int Arch Allergy Immunol* 2016;169:101–107. doi: 10.1159/000444279.

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Supplementary Figure 1: (A) The amino acid sequence alignment of profilin 1 and profilin 2 with homologous allergens from different plant pollens. (B) Phylogenetic relationship of the amino acid sequences of these two profilins with other homologs. (C) Expression, purification, and SDS-PAGE analysis of profilin 1 and profilin 2. The arrow indicated purified profilin 1 (C) and profilin 2 (D). (E) Distribution of ELISA values in patients with different clinical symptoms. (F) Sensitization rates by ELISA in patients with different clinical symptoms. (G) Distribution of ELISA values in patients with asthma and non-asthma and rhinitis and non-rhinitis. (H) Sensitization rates by ELISA in patients with asthma and non-asthma and rhinitis and non-rhinitis.

Amb a 8: short ragweed; Bet v 2: European white birch; Ole e 2: olive; Hel a 2: sunflower; Aca f 2: needle bush; Ama r 2: redroot pigweed; Che a 2: white goosefoot; Cro s 2: saffron crocus; Cyn d 12: Bermuda grass; Koc s 2: burning bush; Mer a 1: annual mercury; Ory s 12: rice; Sal k 4: saltwort. Lane M: pre-stained protein marker 26616; Lane 1: the precipitant of cells lysate after ultrasonication; Lane 2: column flow-through fraction; Lane 3: elution fraction using 30 mmol/L imidazole; Lane 4: elution fraction using 100 mmol/L imidazole. AS: asthma; C:Cough; Con:Conjunctivitis; D:Dermatitis; E, Eczema; ELISA: Enzyme-linked immunosorbent assay; HF: Hay fever; R: Rhinitis.



Supplementary Table 1: Information of 200 patients allergic to *Artemisia* pollen, specific IgE level and clinical symptoms.

Patient s	Sex	Age	Immunocap (ku/L)	Class	Clinical symptoms
1	M	52	45.2	4	Hay fever
2	M	60	32.6	4	Chronic rhinitis
3	M	38	9.13	3	Allergic rhinitis and asthma
4	F	44	39.4	4	Asthma; allergic rhinitis
5	F	24	96.2	5	Allergic rhinitis and asthma
6	F	50	12.5	3	Allergic rhinitis
7	F	32	20	4	Hay fever; food allergic reaction
8	M	57	92.7	5	Hay fever
9	F	50	61.4	5	Allergic rhinitis; allergic asthma
10	M	36	23.1	4	Allergic asthma
11	F	53	>100	6	Allergic rhinitis; hay fever; asthma
12	M	40	10.3	3	Allergic rhinitis
13	F	53	51.2	5	Allergic rhinitis; allergic conjunctivitis
14	M	17	>100	6	Urticaria; rhinitis; severe allergic reaction

15	F	33	27.2	4	Allergic rhinitis
16	F	45	37	4	Allergic rhinitis
17	F	62	30.1	4	Allergic rhinitis; allergic asthma
18	F	49	32.2	4	Allergic rhinitis; allergic asthma
19	F	42	36	4	Allergic rhinitis
20	F	47	>100	6	Cough; hay fever
21	F	25	20.7	4	Allergic rhinitis
22	M	4	>100	6	Cough; hay fever
23	F	21	34.6	4	Allergic rhinitis; allergic asthma
24	F	34	83	5	Allergic rhinitis; cough
25	F	22	20.2	4	Allergic rhinitis; asthma
26	M	16	54.1	5	Allergic rhinitis; allergic asthma; severe allergic reaction
27	M	27	8.6	3	Cough; allergic rhinitis
28	F	22	>100	6	Urticaria; severe allergic reaction
29	M	5	69.1	5	Rhinitis; conjunctivitis
30	F	20	>100	6	Allergic rhinitis
31	F	41	>100	6	Allergic rhinitis; asthma
32	F	35	74	5	Allergic rhinitis; allergic asthma; hay fever

33	F	22	42.8	4	Hay fever; allergic asthma
34	F	38	4.78	3	Allergic rhinitis; cough
35	M	43	24.9	4	Allergic rhinitis and asthma
36	F	43	7.97	3	Allergic rhinitis
37	F	29	4.55	3	Allergic rhinitis; asthma
38	F	53	7.67	3	Bronchial asthma; allergic rhinitis
39	F	34	28	4	Allergic rhinitis; bronchial asthma
40	M	42	5.75	3	Allergic rhinitis
41	F	38	33.9	4	Allergic rhinitis; asthma
42	M	41	4.69	3	Allergic rhinitis
43	M	16	71.3	5	Allergic rhinitis; dermatitis
44	M	39	36.7	4	Allergic dermatitis
45	F	8	8.28	3	Acute urticaria
46	F	40	4.7	3	Allergic rhinitis
47	F	37	37.2	4	Allergic rhinitis
48	F	33	10.1	3	Allergic rhinitis and asthma
49	F	35	53.3	5	Allergic rhinitis; cough
50	F	37	27.7	4	Hay fever
51	F	62	22	4	Severe allergic reaction; allergic

					rhinitis
52	M	37	10.8	3	Bronchial asthma; allergic rhinitis
53	F	43	13.9	3	Allergic rhinitis; food allergic reaction
54	F	23	25.4	4	Hay fever
55	F	23	32.6	4	Allergic rhinitis
56	F	37	10	3	Allergic rhinitis and asthma
57	M	16	58.3	5	Bronchial asthma; allergic rhinitis
58	F	33	32.6	4	Hay fever
59	M	36	7.21	3	Allergic rhinitis and asthma
60	F	53	6.63	3	Allergic rhinitis; bronchial asthma; severe allergic reaction
61	F	45	>100	6	Allergic asthma; allergic rhinitis
62	F	59	17.1	3	Allergic rhinitis; bronchial asthma
63	F	40	36.6	4	Allergic rhinitis; hay fever
64	M	12	32.7	4	Allergic rhinitis
65	F	46	51.4	5	Allergic asthma
66	F	25	30.4	4	Eczema; chronic rhinitis

67	F	5	7.98	3	Allergic rhinitis; bronchial asthma
68	F	29	22.1	4	Bronchial asthma; allergic rhinitis
69	F	11	16.9	3	Allergic rhinitis; cough
70	M	15	38.7	4	Bronchial asthma; allergic rhinitis
71	F	38	5.4	3	Hay fever
72	F	40	4.99	3	Rhinitis; asthma
73	F	45	9.53	3	Allergic rhinitis; hay fever
74	F	58	68	5	Allergic rhinitis; bronchial asthma
75	F	30	8.2	3	Rhinitis; asthma
76	F	42	6.85	3	Rhinitis; asthma
77	F	32	29.9	4	Hay fever
78	F	20	39.4	4	Dermatitis
79	F	30	24.0	4	Rhinitis/allergic rhinitis; food allergic reaction
80	M	35	13.8	3	Severe allergic reaction; food allergic reaction
81	M	26	86	5	Hay fever
82	F	40	16	3	Allergic rhinitis

83	F	58	49.4	4	Allergic rhinitis; hay fever
84	F	45	7.95	3	Allergic rhinitis
85	M	41	52.8	5	Allergic rhinitis; bronchial asthma
86	M	64	5.42	3	Allergic rhinitis
87	F	26	11.5	3	Allergic rhinitis; severe allergic reaction
88	M	44	4.04	3	Rhinitis; cough
89	M	49	32.5	4	Hay fever
90	M	39	44.8	4	Bronchial asthma; allergic rhinitis
91	M	51	31.3	4	Hay fever
92	M	34	8.81	3	Allergic rhinitis; hay fever
93	F	52	>100	6	Allergic rhinitis; allergic asthma
94	F	29	35.9	4	Allergic rhinitis; bronchial asthma
95	F	69	10.3	3	Dyspnea; food allergic reaction; severe allergic reaction
96	F	9	98.4	5	Severe allergic reaction
97	F	28	28.9	4	Allergic rhinitis; cough; severe allergic reaction
98	F	40	4.82	3	Asthma

99	F	33	89	5	Allergic rhinitis; ulcerative colitis
100	F	15	26.9	4	Hay fever
101	M	42	>100	6	Allergic rhinitis and asthma; severe allergic reaction
102	F	45	28	4	Rhinitis; asthma
103	F	55	55.6	5	Bronchial asthma; allergic rhinitis
104	M	48	33.3	4	Hay fever
105	F	45	>100	6	Allergic rhinitis; bronchial asthma
106	F	35	59.6	5	Allergic rhinitis; asthma
107	M	41	49.9	4	Allergic rhinitis and asthma; hay fever
108	F	47	81.4	5	Allergic rhinitis
109	F	45	>100	6	Allergic rhinitis and asthma
110	F	8	18.2	4	Rhinitis
111	F	36	78.4	5	Allergic rhinitis
112	M	32	50.9	5	Asthma
113	F	55	34.8	4	Allergic rhinitis
114	F	43	51.1	5	Hay fever

115	M	22	35.9	4	Allergic rhinitis
116	F	31	47.5	4	Hay fever
117	F	24	32.3	4	Allergic rhinitis; asthma
118	F	14	>100	6	Allergic rhinitis
119	M	44	28.4	4	Allergic rhinitis
120	F	38	30.3	4	Allergic rhinitis
121	F	31	30.4	4	Allergic rhinitis
122	M	33	64	5	Allergic rhinitis; allergic asthma
123	M	22	19.2	4	Allergic rhinitis
124	M	9	67	5	Bronchial asthma; eczema; cough
125	F	17	48.6	4	Dermatitis; rhinitis
126	F	35	43	4	Allergic rhinitis; allergic conjunctivitis
127	F	16	15.1	3	Allergic rhinitis
128	F	10	41.6	4	Urticaria
129	F	22/2 3	55.6	5	Allergic rhinitis and asthma
130	F	7	23.5	4	Rhinitis; cough; chest tightness
131	F	13	67.3	5	Bronchial asthma; allergic rhinitis

132	F	51	28.1	4	Allergic rhinitis; bronchial asthma
133	F	25	30.5	4	Allergic rhinitis
134	F	32	>100	6	Rhinitis; cough; chest tightness
135	F	70	37.9	4	Hay fever
136	F	10	>100	6	Allergic rhinitis
137	F	28	49.3	4	Hay fever
138	M	5	60.6	5	Bronchial asthma; allergic rhinitis
139	M	32	56.2	5	Eczema; nodular pruritus
140	M	27	52.2	5	Allergic rhinitis; allergic asthma
141	F	10	None	None	Cough
142	F	26	77.7	5	Rhinitis
143	M	29	37.3	4	Severe allergic reaction
144	M	33	74.7	5	Allergic rhinitis
145	M	62	11.1	3	Suffocated
146	F	44	69.2	5	Allergic rhinitis and asthma
147	M	50	61.3	5	Allergic rhinitis
148	M	47	>100	6	Hay fever
149	F	22	85.7	5	Hay fever
150	M	5	>100	6	Allergic rhinitis

151	F	60	58.4	5	Allergic rhinitis
152	F	10	41.4	4	Allergic rhinitis and asthma
153	F	38	44.9	4	Allergic rhinitis; urticaria
154	M	6	21.1	4	Allergic rhinitis
155	M	35	>100	6	Severe allergic reaction; food allergic reaction
156	F	32	57.5	5	Hay fever
157	F	60	>100	6	Allergic rhinitis; cough
158	F	26	45.5	4	Allergic rhinitis; allergic urticaria
159	M	36	>100	6	Allergic rhinitis; allergic conjunctivitis
160	F	26	16.5	3	Rhinitis
161	M	17	9.44	3	Allergic rhinitis
162	M	36	5.96	3	Allergic rhinitis
163	M	9	38.9	4	Rhinitis; cough; asthma
164	M	11	>100	6	Rhinitis; asthma
165	F	32	83.5	5	Bronchial asthma; allergic rhinitis
166	M	30	74.6	5	Rhinitis; asthma
167	F	25	96.4	5	Allergic rhinitis; hay fever

168	F	30	>100	6	Allergic rhinitis; bronchial asthma
169	F	12	>100	6	Allergic rhinitis; hay fever
170	F	25	17.1	3	Allergic rhinitis
171	M	13	82.6	5	Hay fever
172	M	13	>100	6	Allergic rhinitis; asthma
173	F	35	51.5	5	Allergic rhinitis; cough
174	F	29	>100	6	Bronchial asthma; allergic rhinitis
175	F	35	7.38	3	Bronchial asthma; allergic rhinitis
176	F	10	6.14	3	Asthma
177	M	14	>100	6	Cough; asthma; allergic rhinitis
178	M	16	>100	6	Bronchial asthma; allergic rhinitis
179	F	28	33.9	4	Severe allergic reaction; food allergic reaction; hay fever
180	M	30	>100	6	Allergic rhinitis
181	F	19	18	4	Allergic rhinitis
182	M	45	24.3	4	Allergic rhinitis
183	F	44	49.2	4	Bronchial asthma; allergic rhinitis

184	F	34	15.4	3	Allergic rhinitis; dermatitis
185	F	61	35.8	4	Rhinitis
186	F	24	30.5	4	Allergic rhinitis
187	F	24	92	5	Allergic rhinitis; bronchial asthma
188	F	35	18.6	4	Rhinitis; cough; asthma
189	F	7	67.3	5	Allergic rhinitis; cough
190	M	27	34.7	4	Allergic rhinitis; allergic asthma; hay fever
191	M	21	56	5	Bronchial asthma; allergic rhinitis
192	M	31	>100	6	Allergic rhinitis; asthma
193	M	54	37	4	Allergic rhinitis
194	M	11	>100	6	Hay fever
195	F	22	67.7	5	Allergic rhinitis; asthma
196	M	31	23.7	4	Allergic rhinitis
197	F	44	92.7	5	Allergic rhinitis
198	M	34	5.53	3	Bronchial asthma; allergic rhinitis
199	M	33	18.8	4	Allergic rhinitis; allergic asthma
200	F	47	23.9	4	Rhinitis

M: Male; F:Female.