A Compound Mutation (c.953C<G and c.49G<A) Aggravates Functional Impairments of C1-INH in Hep G2 Cells

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To the editor,

Hereditary angioedema (HAE), an autosomal dominant disease, shows considerable heterogeneity of manifestations, ranging from being asymptomatic or frequent episodes of edema of the extremities to life-threatening upper airway edema. In China, 60.8% of HAE patients experience airway mucosal swelling; of these patients, 9% die from it.¹ Unfortunately, there are no effective biomarkers or modifiers that can predict the severity of HAE. Several studies have focused on the relationship between the genotype and the phenotype in HAE.²⁻⁵ We published nonsense and frameshift mutation, and mutations in Arg466 that affected the antigenic level of C1 esterase inhibitor (C1-INH) but were not associated with the severity of HAE.³ A previous study reported that c.-21T>C could impact C1-INH mRNA levels and might influence disease severity,² but another study demonstrated that c.-21T>C was not associated with a more severe manifestation.⁵ Our previous work on the C1-INH gene in a single HAE family showed that some subjects carried not only the hereditary mutation but also an extra mutation or single nucleotide polymorphism. Whether these compound mutations aggravate C1-INH deficiency and the severity of HAE is unknown. Therefore, we selected 2 types of compound mutations and compared their impact on C1-INH deficiency *in vitro*. The methods were described in Supplementary Material.

Four patients with type I HAE originated from 2 families. Their clinical information is shown in Table. Four genotypes were identified (Table); c.1328A<G+c.167T<C (A1), c.1328A<G (A2), c.953C<G+c.49G<A (B1), and c.953C<G (B2). Compared with healthy control subjects, the C1-INH mRNA of patients A1, A2, B1, and B2 was 0.5%, 0.7%, 0.5%, and 8.1% of normal levels, respectively. By ligating C1-INH cDNA to M2 plasmid, transfecting them into Trans1-T1 cells, and picking single clone for sequencing, we detected both the compound mutations were located in the same mRNA transcript, namely, in *cis* to each other. Recombinated plasmid carrying 4 C1-INH genotypes were

expressed in the Hep G2 cell line. Functional analysis showed that all genotypes expressed lower functional C1-INH compared with the normal control. The functional levels of C1-INH in genotypes in A1, A2, B1, and B2 were 0.34 ± 0.13 , 0.24 ± 0.02 , 0.44 ± 0.06 , and 0.11 ± 0.03 U C1-INH/mL, respectively. By statistical analysis, c.49G<A+c.953C<G resulted in significantly lower function of recombinant C1-INH than c.953C<G ($P<0.05$). However, this difference was not observed between c.167T<C+c.1328A<G and c.1328A<G. Thus, it is inferred that c.49G<A might aggravate the dysfunctional level of C1-INH caused by c.953C<G. In terms of clinical symptoms, patient B1 expressed a severe phenotype, experiencing recurrent episodes that involved the upper airway, extremities, and face, whereas patient B2 presented only with several attacks hands edema during pregnancy and face swelling after trauma. However, since only 1 case with additional c.49G<A had severe symptoms in this study, it was hard to draw a conclusion that c.49G<A is associated with severe HAE phenotype in c.953C<G carriers. More proof studies are needed to confirm our results.

In summary, the mutant genotypes that we identified in this study impaired C1-INH function in Hep G2 cells, and the level of functional C1-INH with the compound mutation c.953C<G+c.49G<A was significantly lower compared with c.953C<G alone.

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Table. Clinical and genetic information of the patients

Patients' number	Gender	Age (yr)	C1-INHa (g/L)	C4 (g/L)	Manifestations	Mutations	Molecular consequence	mRNA level*
A1	Male	33	0.09	0.061	UAE (2–3 episodes/year), extremities	c.1328A<G c.167T<C	p.H443R SNP	0.51
A2	Female	11	0.1	0.081	Twice hands edema	c.1328A<G	p.H443R	0.76
B1	Female	41	0.06	0.05	UAE (once per month), face	c.953C<G c.49G<A	p.S318× p.G17R	0.5
B2	Female	31	0.04	0.018	Hands edema during pregnancy, face swelling after head trauma	c.953C<G	p.S318×	8.13

C1-INHa, C1 esterase inhibitor antigen level; UAE, upper airway edema.

*C1-INH mRNA relative quantity by real-time polymerase chain reaction.

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