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BRIEF COMMUNICATION

Novel duplication in the *F12* gene in a patient with recurrent angioedema



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Abstract Edema formation is mediated by histamine or bradykinin release and may have several hereditary and acquired causes. In hereditary forms of bradykinin-mediated angioedemas, mutations in the genes encoding C1-inhibitor (*SERPING1*) as well as coagulation factor XII (*F12*) have been described. We present a novel *F12* gene mutation, a duplication of 18 base pairs (c.892_909dup) in a 37-year-old woman with recurrent angioedema and normal C1-inhibitor level. A single episode of facial edema in the family of the patient showed co-segregation with the mutation. This duplication is causing the repeated presence of 6 amino acids (p.298–303) in the same region of factor XII, as those three mutations described previously in cases of hereditary angioedema with normal C1-INH function. These results may confirm the importance of the proline-rich region of factor XII protein in edema formation.
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1. Introduction

Angioedema is a localized swelling of the subcutaneous or submucosal tissue, caused by dilatation and increased vascular permeability leading to plasma flow from the intravascular to the extracellular space [1]. Angioedema developed by either acquired or hereditary causes can be

classified as histamine- or bradykinin-mediated [2]. In bradykinin-mediated edemas excessive release of bradykinin, a potent endothelium-dependent vasodilator induces edema formation. In these forms, mutations in components of the bradykinin-forming cascade have been described such as in the gene encoding C1-inhibitor (*SERPING1*) [3,4]. Moreover in some cases mutations have been found in the gene of factor XII (*F12*), that activates kallikrein, the catalyst of bradykinin release; however the pathogenesis is not yet clarified in this form of angioedema [5,6].

We present a case of recurrent angioedema bearing a previously not identified mutation of the blood coagulation factor XII (Hageman factor).

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2. Case report

A 37-year-old Caucasian woman has had recurrent angioedema three times during the past two years. Symptoms included swelling of the face and pharynx (with globus sensation), dyspnea, along with colicky abdominal pain, and nausea. On the first two occasions, airway and abdominal symptoms resolved within hours after the administration of calcium gluconate, whereas facial edema disappeared in a day. During the latest attack, itching of the palm and a map-like, erythematous rash accompanied the above symptoms. The patient received a systemic antihistamine with a corticosteroid; mucosal changes disappeared 1–2 h later, whereas cutaneous manifestations resolved within a day. These episodes occurred in summer, during or after meals, however, food allergy could not be proven as a contributing factor. There was no association with trauma, stress or physical exertion. The patient had never taken oral contraceptives; she had two uneventful pregnancies with delivery by cesarean section. Her medical history included tonsillectomy, appendectomy, Hashimoto's thyroiditis with hypothyroidism treated with L-thyroxine, as well as mild seasonal rhinitis. Skin and specific IgE tests (nutritive and inhalative series) were positive only for ragweed.

There was a family history of allergic reactions. Her mother and 10-year-old daughter have pollen allergy. Furthermore, her daughter had a single episode of facial edema at the age of eight, but it has not recurred since then.

Although no further members of the family with angioedema could be identified, the clinical picture (recurrent angioedema without wheals) suggested hereditary angioedema (HAE) [2].

3. Methods and results

The plasma levels of the C1-inhibitor (C1-INH) and C4 were measured during remission, by radial immunodiffusion. Functional activity of C1-INH was determined with immunoassay. Both the plasma antigenic concentration and the functional activity of C1-INH, as well as C4 levels were all within the normal range. These findings ruled out HAE types I and II caused by mutations leading to the reduced synthesis or impaired function of C1-INH.

Hereditary angioedema with normal C1-INH function (HAE with normal C1-INH), potentially associated with mutations of the gene (*F12*) encoding blood coagulation factor XII (FXII), was also considered. Screening for mutations in the *F12* gene was done by DNA sequencing, after PCR amplification of coding exons using primers described previously [7]. A novel variation – duplication of 18 base pairs (c.892_909dup) – was identified in heterozygous form (Fig. 1). The duplication, located in exon 9, causes the repeated presence of the amino acids p.298–303 in the proline-rich region of the protein (p.Pro298_Pro303dup).

To investigate the effect of this duplication, FXII plasma coagulant activity was determined by the one-stage clotting assay using FXII-deficient plasma (Siemens Healthcare Diagnostics). Activated partial thromboplastin time (aPTT) was measured with a BCS-XP automated coagulometer (Siemens). Both parameters were within the normal range in the patient and in the family members studied (III/1, IV/1 and IV/2, Fig. 2).

In order to estimate the frequency of this novel sequence variation in healthy individuals, 109 blood donors (43 males/66 females) were screened by PCR–RFLP. The c.892_909dup was missing in controls, it was not reported by international databases (dbSNP, dbVar, or Exon Variant Server release ESP6500SI containing data from 6503 samples), and therefore, it was considered a new mutation. Sequencing the complete coding region of the *F12* gene in this patient revealed only one additional exonic variation (rs17876030), causing the change of the p.207 amino acid (proline) to alanine. According to the dbSNP, this variation is a rare polymorphism with a minor allele frequency of 0.054 (based on 1000Genome phase 1 data).

Available members of the patient's family were surveyed, and a detailed family history was recorded, but no further members with recurrent angioedema could be identified. Screening for the duplication revealed that four family members, including the patient's daughter with a history of facial edema, are also carrying this mutation (Fig. 2). The carrier state of Pro207Ala and rs1801020 (c.46C/T), reported as the only common variation accounting for the diversity of factor XII level, was also determined in all family members.

4. Discussion

Hereditary angioedema with normal C1-inhibitor affecting mainly women was described in 2000 [8,9]. Its clinical symptoms are similar to those of types I and II; however, plasma C4 and C1-inhibitor level and activity are normal. In contrast to the latter HAE types (caused by C1-inhibitor deficiency), factor XII-dependent overproduction of bradykinin is held responsible for edema formation in HAE with normal C1-INH.

In the gene encoding FXII two missense mutations, affecting the same residue (Thr328), were identified in about 15–30% of HAE cases with normal C1-INH [5,10,11]. Recently, a deletion occurring in the same region was described in a family [6], but no other genetic variation predisposing to the disease has yet been reported. These alterations are supposedly gain-of-function mutations; however, their effect on coagulation was ambiguous [12]. To date, mutations are identified in less than one third of cases [5,10,11] implying the presence of further genetic risk factors.

In our patient with recurrent angioedema, we found a novel duplication in the *F12* gene, located in the same region of FXII as the formerly described mutations. The functional relevance of this new mutation is not apparent as FXII coagulant activity was normal in two carriers, showing that c.892_909dup does not alter coagulation activity. Among the family members available, one mutation carrier had angioedema symptoms. However, the existence of additional three symptom-free mutation carriers suggests that even if the identified mutation is pathogenic, further genetic or environmental risk factors may also influence disease development.

A frequently described precipitating factor of HAE with normal C1-INH is exposure to estrogens. This form of angioedema was primarily reported as estrogen dependent [9,13] and connection with exogenous or endogenous estrogens is known in most cases with *F12* mutations [14]. However, later several affected women and also men with *F12* mutations have been reported, in whom no relation

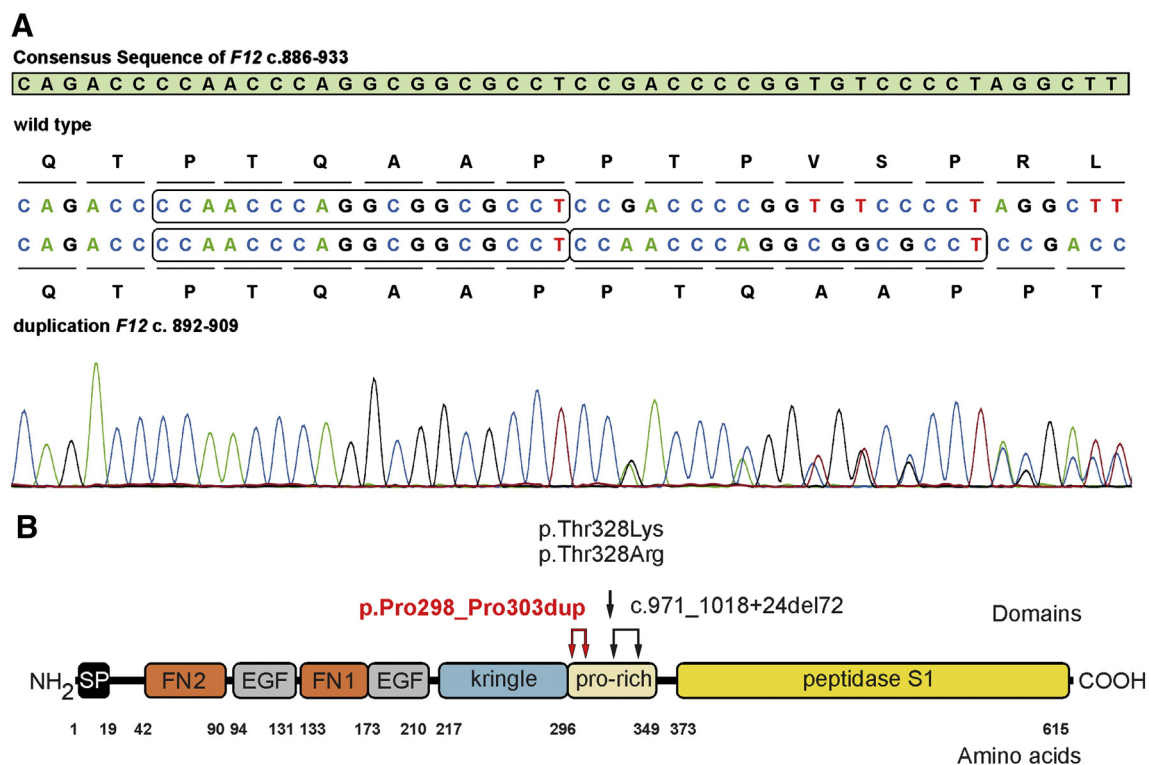


Figure 1 Mutations in the *F12* gene. A. Part of the sequencing chromatogram showing the newly identified *F12* gene mutation in exon 9 (numbering refers to the cDNA position of bases) B. FXII protein domains (according to <http://www.uniprot.org/uniprot/P00748>) with localization of the p.Pro298_Pro303dup and the previously described mutations associated with HAE with normal C1-INH.

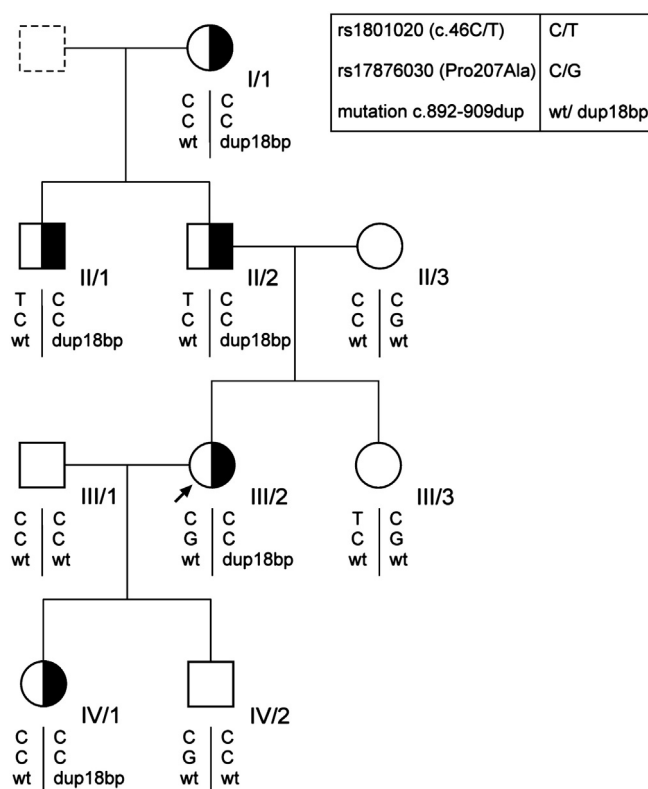


Figure 2 Pedigree of the family with the novel *F12* c.892_909dup mutation.

with increased estrogen level could be found [11,15–18]. In the index case, there was no direct evidence of high estrogen levels before symptom onset.

Allergens or foods may also trigger edema attacks in patients with HAE with normal C1-INH [14], as activated mast cells release heparin, which activates FXII, and increases vascular permeability *in vivo* [19]. Our patient and her mutation carrier daughter both had a history of pollen allergy and edematous attacks appeared to be related to meals in the index patient. Furthermore, the persisting autoimmune disease may also have influenced the occurrence of symptoms in the patient.

As the genetic risk factors of recurrent angioedema with normal C1-INH are known only in a portion of cases, detailed investigation of patients without the reported mutations is of high importance. The newly identified mutation causes the duplication of six amino acids in FXII, which may influence the molecular structure and proper functioning of the protein; however, further studies are needed to confirm this assumption.

Conflict of interest statement

The authors disclose no conflict of interest.

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