

## Fibrinolysis and bradykinin formation: From *in vitro* observations to human disease in “just” 45 years

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Hereditary angioedema (HAE) with normal C1 inhibitor (HAE-N), previously known as type III HAE, was described by Bork et al<sup>1</sup> and Binkley and Davis<sup>2</sup> as a new entity predominantly affecting women that is strikingly estrogen dependent. A defining mutation was found in a subpopulation of patients with an incidence that varies greatly from region to region but averages about 30% to 40%. Threonine 309 of Factor XII is replaced by lysine, most commonly,<sup>3</sup> or arginine as an alternative; rarely, it is replaced by a deletion/insertion. Most patients are heterozygotes (ie, with 50% normal Factor XII), but rare exceptions with homozygous mutations have been reported.

Because Factor XII is the initiating protein of the plasma bradykinin-forming pathway, it seemed likely that bradykinin would be the mediator of the angioedema. The responsiveness to agents used to treat types I and II HAE (C1 inhibitor deficiencies) seemed consistent with this idea, but these were limited to case reports rather than controlled trials. Thus acute attacks appeared responsive to icatibant, a bradykinin receptor antagonist; ecallantide, a plasma kallikrein inhibitor; or even, surprisingly, C1 inhibitor infusion.<sup>4</sup>

Nevertheless, it has been difficult to identify a molecular mechanism that links the mutation to bradykinin formation. The first reported observation was that dextran sulfate activation of plasma from patients with Factor XII mutation led to augmented kallikrein formation, suggesting that Factor XII is either more easily activated or has a higher specific activity than normal.<sup>5</sup> However, this became controversial because activation of plasma from patients with the Thr 309 Lys mutation, using dextran sulfate at 10 to 20 µg/mL as an activator, behaved normally.<sup>6</sup>

The next report increased our understanding substantially<sup>7</sup> because of the following observations. First, Factor XII knockout mice given replacement of cloned mutant Factor XII had increased vascular permeability relative to knockout mice given cloned normal Factor XII. Second, SDS gels of mutant Factor XII had a lower molecular weight than normal Factor XII because of the absence of carbohydrate, which is normally attached to the threonine that has been replaced. Third, dextran sulfate activation of Factor XII-deficient plasma reconstituted with mutant Factor XII is more readily activated to convert prekallikrein to kallikrein.

and then kallikrein cleaves high-molecular-weight kininogen to produce bradykinin. However, the last observation was made only with low doses of dextran sulfate (0.1 or 1  $\mu\text{g/mL}$ ), whereas dextran sulfate at 10  $\mu\text{g/mL}$ , which is typically used, did not distinguish the function of mutant Factor XII from normal. The report concluded that absence of carbohydrate at this site results in facilitated autoactivation of Factor XII (an assumption) on interaction with initiating surfaces but did not prove missing carbohydrate to be the cause because the effect of having lysine or arginine at that position was not considered. Nevertheless, the animal studies documented the functional relevance of the mutation, regardless of mechanism.

In this issue of the *Journal*, de Maat et al.<sup>8</sup> demonstrated that the lysine substitution for threonine creates a site that is more readily cleaved by plasmin and also leads to an increased rate of conversion of Factor XII to activated Factor XII (Factor XIIa). This superb study represents a major step forward in our understanding of how Factor XII, whether normal or mutated, can be activated in human disease. In general, activation of Factor XII can occur in 3 ways: autoactivation, in which Factor XIIa activates surface-bound Factor XII; feedback activation by kallikrein; or activation by plasmin.<sup>9</sup> Although autoactivation was not addressed, the authors clearly demonstrated that kallikrein activation of mutant Factor XII is normal, which is why activation with 10 µg/mL dextran sulfate is also normal. The activation by kallikrein is so fast that any difference in factor XII autoactivation is unlikely to be perceived. Thus plasmin now emerges as a focus of research linking fibrinolytic mechanisms to bradykinin formation and angioedema.

Activation of Factor XII by plasmin was discovered in 1971 by Kaplan and Austen,<sup>10</sup> and 4 activated forms of Factor XII were defined, 2 of which are commonly observed: Factor XIIa (80,000 kDa), which activates both prekallikrein and Factor XI, and Factor XIIf (30,000 kDa), which activates prekallikrein readily but loses 96% to 98% of the ability to activate Factor XI (ie, it is not a clotting factor). Its formation might relate to circumstances in which Factor XII is clearly activated yet kinin formation proceeds in the absence of discernable clotting.

Although activation of Factor XII by kallikrein was reported to yield either Factor XIIa or Factor XII<sub>f</sub> (renamed  $\alpha$  Factor XIIa and  $\beta$  Factor XIIa), because of 2 alternative bond cleavages,<sup>11</sup> this thesis turned out to be incorrect. Subsequent studies definitively demonstrated that the process is sequential (ie, Factor XII<sub>f</sub> is derived from Factor XIIa and not Factor XII),<sup>12</sup> and the same is true when plasmin activates Factor XII. **Fig 1** depicts Factor XII autoactivation on a surface, which can initiate the process of bradykinin formation (**Fig 1, A**) and the far more rapid enzymatic activation to form Factor XIIa and then Factor XII<sub>f</sub> (**Fig 1, B**). **Fig 1, C**, indicates the functions attributable to each form of activated Factor XII.

With the discovery that plasmin activation of mutant Factor XII is enhanced, the formation of plasmin is likely an important

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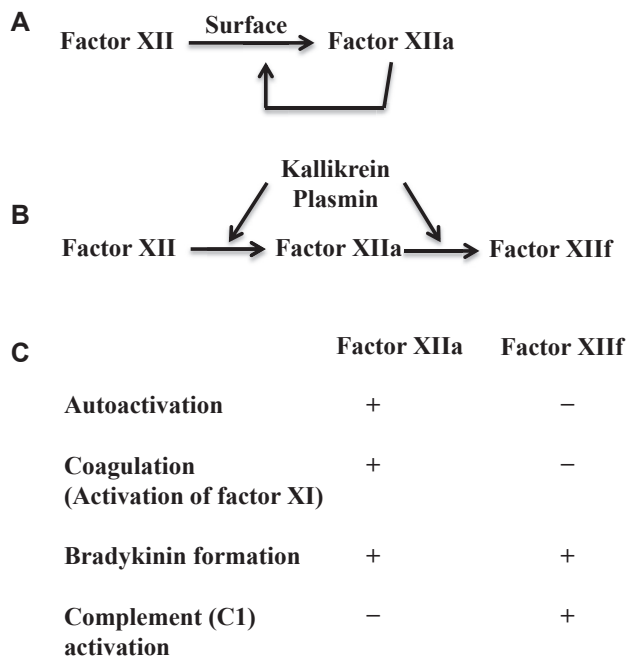


**FIG 1.** Formation and functions of activated factor XII. **A**, Autoactivation on an initiating surface. **B**, Activation of factor XII to factor XIIa and then factor XIIIf by plasma kallikrein and plasmin. **C**, Functions of 2 forms of activated factor XII.

contributor to the development of the angioedema, as seen in patients with this unusual form of HAE, but much work still needs to be done. We have recently reported that plasma levels of plasminogen activator inhibitor 2 in baseline samples of patients with HAE-N are strikingly low (21 samples from Germany and 1 from the United States), irrespective of whether the Factor XII mutation is present,<sup>13</sup> but more recent observations suggest this can vary depending on medication taken, country of origin, or both. This protein inhibits urokinase and tissue plasminogen activator (urokinase preferentially), and thus low levels can lead to augmented formation of plasmin and, presumably, activation of Factor XII. However, a source of these plasminogen activators is required. Cytokines, such as IL-1 or TNF- $\alpha$ , can stimulate endothelial cells to secrete both heat shock protein 90, an activator of the prekallikrein–high-molecular-weight kininogen complex, and urokinase, a plasminogen activator (manuscript in preparation). Estrogen is clearly the main precipitant of attacks of angioedema in patients with HAE-N,<sup>2</sup> but its connection to bradykinin formation is not yet clear, although it does cause an increase in Factor XII blood levels and enhances secretion of heat shock protein 90<sup>14</sup> but not urokinase. It should be noted that there is an “intrinsic” pathway for conversion of plasminogen to plasmin in plasma once Factor XII has been activated. Although plasma kallikrein, Factor XIa, and Factor XIIa can all convert plasminogen to plasmin directly (in descending order), kallikrein

activation of a trace amount of prourokinase in plasma is likely primary. These plasma fibrinolytic reactions might be responsible for the augmented activation of the kinin cascade in patients with HAE-N with the Factor XII mutation when the dose of initiator is particularly low.<sup>7</sup> The efficacy of tranexamic acid, a fibrinolytic inhibitor, in patients with HAE-N might also relate to these observations.

The majority of patients with HAE-N do not have a mutated Factor XII, yet their clinical presentation is strikingly similar to those with the mutation. Thus far, there is no information regarding the pathogenesis of this subpopulation of patients, just as idiopathic angioedema remains an enigma. There are no laboratory tests that assist diagnosis for the latter patients with recurrent angioedema, normal C1 esterase inhibitor levels, and absent family history. All these issues will undoubtedly be addressed in short order as research in this area accelerates and novel therapeutic approaches emerge.

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