not match that of the patient. Tiede et al.[2] observed that some patients with acquired hemophilia have been exposed to a factor VIII protein that does not match their own. Specifically, there are polymorphisms of factor VIII at amino acids 1241 (Asp→Glu) and 2004 (Glu→Lys). In patients with certain HLA-DRB1 alleles, exposure to a "foreign" factor VIII variant protein may result in a major-histocompatibility-complex class II presentation of novel T-cell epitopes.

It was not clear whether the patient had been transfused before hemophilia A developed. It would be interesting to know whether the patient was transfused before ipilimumab therapy and thereby possibly exposed to a foreign factor VIII protein.

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No potential conflict of interest relevant to this letter was reported.

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THE AUTHORS REPLY: Lozier reports that exposure to factor VIII with blood transfusion may induce the formation of a factor VIII inhibitor in patients with a variant factor III genotype, causing acquired hemophilia A. In our case, the patient's factor III genotype was not determined, but he did not receive any blood or blood component during the months before the ipilimumab therapy was begun. Moreover, the activated partial-thromboplastin time was normal (26 seconds; international normalized ratio [INR], 0.9) until the third administration of ipilimumab and increased significantly just before the fourth infusion (66 seconds; INR, 2.2), concomitant with the onset of hematuria. These findings strongly support our suggestion that development of the factor VIII inhibitor was due to ipilimumab therapy.

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Since publication of their article, the authors report no further potential conflict of interest.

Factor XIII in the Treatment of Hemophilia A

TO THE EDITOR: Patients with hemophilia A (a deficiency of factor VIII [FVIII]) have spontaneous bleeding because of abnormal thrombin generation, which results in the formation of weak, unstable clots.[1] The formation of these weak clots is also the result of delayed and reduced activation of coagulation factor XIII (FXIII).[2] The standard treatment is based on FVIII substitution to control and prevent bleeding, but this process is expensive and time-consuming. We hypothesized that supraphysiologic levels of FXIII would normalize clot stability at low levels of FVIII.

Clot stability was recorded by means of the changes in plasma turbidity after the simultaneous addition of tissue factor (dilution, 1:40,000) and tissue plasminogen activator (0.75 nM). FVIII-deficient, platelet-poor plasma, spiked with increasing concentrations of recombinant FVIII plus plasma-derived FXIII or buffer, was compared with normal control plasma. The primary end point was the area under the curve (AUC) for turbidity.

The addition of FVIII improved the AUC (Mann–Whitney test, $P<0.005$), but the maximum concentration (1 IU per milliliter, 100% of normal levels) failed to normalize clot stability (Fig. 1A). Normal clot stability was achieved at very low concentrations of FVIII in the presence of supraphysiologic levels of FXIII (10 μg per milliliter equals a 50% increase in plasma levels, which should be achievable through intravenous infusion of 25 IU per kilogram of body weight) (Fig. 1A).

Calibrated automated thrombin generation (at 1 pM of tissue factor) was measured after the addition of recombinant FVIII, with and without plasma-derived FXIII (pdFXIII). The speed and quantity of thrombin generation was not altered

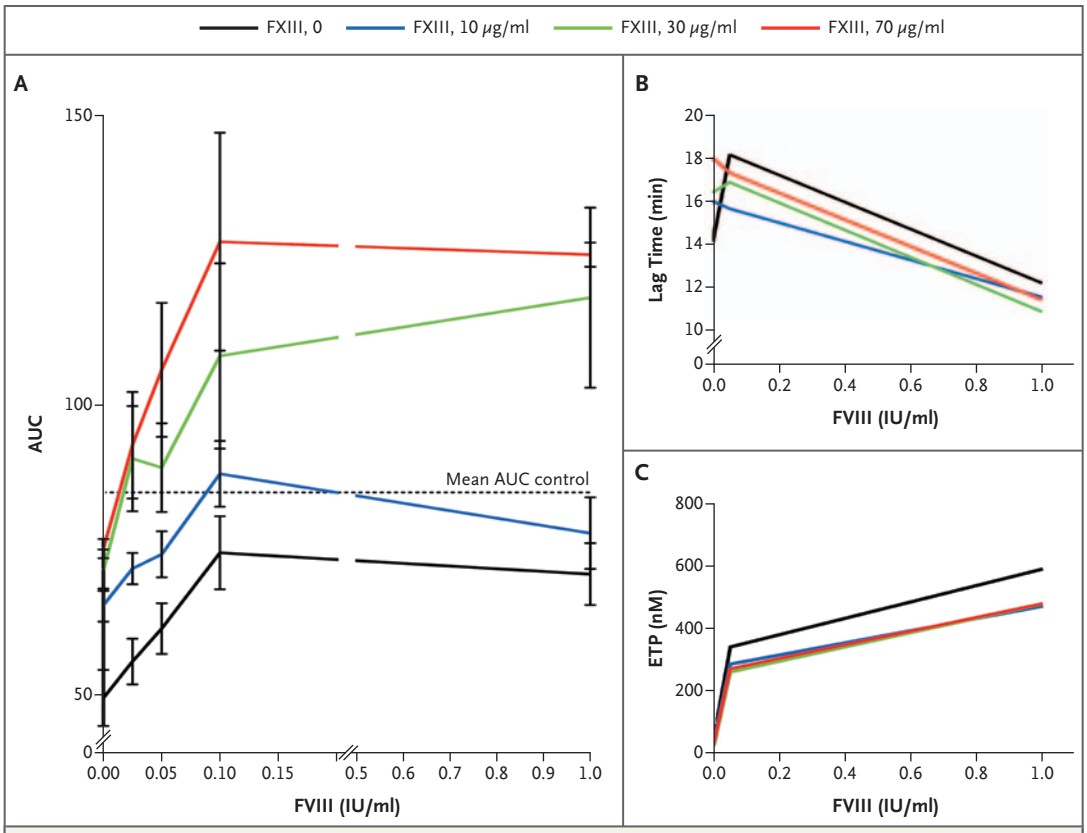

Figure 1. Clot Stability and Thrombin Generation with Increasing Concentrations of Factors VIII and XIII.

Panel A shows changes in the area under the curve (AUC) for turbidity after activation with tissue factor and Ca^{2+} and lysis with tissue plasminogen activator as a means of assessing clot stability. The data show that there is a response to increasing concentrations of factor VIII (FVIII), alone and in combination with factor XIII (FXIII). Panel B illustrates the effect of increasing concentrations of FVIII alone and in combination with varying concentrations of FXIII on thrombin generation (lag time). Panel C shows the effect of increasing concentrations of FVIII alone and in combination with varying concentrations of FXIII on end thrombin potential (ETP). The data show that FXIII does not affect thrombin generation.

by the addition of pdFXIII (Mann–Whitney test, $P>0.1$ for all comparisons) (Fig. 1B and 1C).

We have shown that clot stability in patients with hemophilia A can be normalized with the addition of pdFXIII, even at very low levels of FVIII. Data suggest that pdFXIII may correct the imbalance between fibrin formation and FXIII activation in the blood of persons with hemophilia.[2] The K_m of thrombin-dependent FXIII activation is higher[3] than the plasma concentration of FXIII. Hence, the rate of FXIII activation by thrombin should increase if the plasma concentration of FXIII is increased.

These observations suggest that FXIII therapy may be useful as an adjunct in factor-sparing and cost-effective regimens. Because FXIII has a half-life of 9 days,[4] dosing would be infrequent, improving convenience and limiting expense. Since FXIII fully corrects plasma clot stability at low concentrations of FVIII, it could be useful in the treatment of bleeding events or in supporting hemostasis during surgery in patients with nonsevere hemophilia. Finally, adjunct FXIII may prove helpful when used in combination with new long-acting FVIII concentrates as a bridging therapy, when FVIII levels are low between doses.

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Repeat Expansion in *C9ORF72* in Alzheimer's Disease

TO THE EDITOR: Alzheimer's disease is the most common progressive neurodegenerative disorder[1] and a leading cause of dementia in the elderly. The genetic causes of Alzheimer's disease are complex, and only four mendelian genes have indisputably been associated with the disease.[2] Mutations in genes encoding the amyloid precursor protein and presenilin 1 and 2 underlie rare, early-onset mendelian forms of the disorder. In sporadic Alzheimer's disease, a common genetic variant (*APOE4*) encoding apolipoprotein E is associated with a high risk of the disease. In contrast, the *APOE2* allele is thought to lower the risk.

We recently found that a large hexanucleotide repeat expansion (GGGGCC) within *C9ORF72* on chromosome 9p21 accounts for approximately 40% of cases of familial amyotrophic lateral sclerosis (ALS) and 30% of cases of familial frontotemporal dementia.[3] In contrast, the repeat expansion was not detected in 709 unaffected persons of European, African, or Asian ancestry (of these persons, 409 were of European descent). Given the clinical and pathologic overlap between familial frontotemporal dementia and Alzheimer's disease, we tested the hypothesis that the *C9ORF72* hexanucleotide expansion may also be associated with susceptibility to Alzheimer's disease.

Using samples obtained from the National Institute of Mental Health Alzheimer's Disease Genetics Consortium, we screened samples from 342 families with members affected by late-onset Alzheimer's disease for the presence of the pathogenic expansion by means of a repeat-primed polymerase-chain-reaction method.[3] The series included 771 subjects who had received a probable diagnosis of Alzheimer's disease (on the basis of criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association) and 223 siblings who were assessed as being unaffected at the time of collection.[4] The subjects with Alzheimer's disease, mainly sibling pairs, had a mean age at onset of 74 years (range, 60 to 97).

We found that *C9ORF72* large repeat expansions were present in 3 of 342 families (<1%) apparently affected with Alzheimer's disease. The hexanucleotide expansion was seen in 6 of 771 subjects (<1%) in whom probable Alzheimer's disease was diagnosed and in 2 of 223 unaffected siblings (<1%). The 2 unaffected carriers were siblings of carriers with probable Alzheimer's disease and were the youngest members of their respective family units. It is therefore possible that Alzheimer's disease developed in these subjects after recruitment to the series.

The three families whose members carried the *C9ORF72* repeat expansion were of European descent. The first family was composed of four sisters, each of whom carried the expansion. The three oldest sisters had received an initial diagnosis of probable Alzheimer's disease on the basis of their clinical symptoms (with ages at onset ranging from 61 to 63 years). Postmortem analyses of two of the affected sisters showed neuropathological findings consistent with a primary diagnosis of frontotemporal dementia with ubiquitin-positive, tau-negative neuronal inclusions. One sister (with an *APOE2/3* genotype) had no lesions that were typical of Alzheimer's disease; the other (with an *APOE3/3* genotype) had moderate numbers of neuritic plaques and neurofibrillary tangles. The second family was a sibling pair of brothers in whom probable Alzheimer's disease had been diagnosed at the ages of 65 and 71 years. The third family consisted of four siblings,