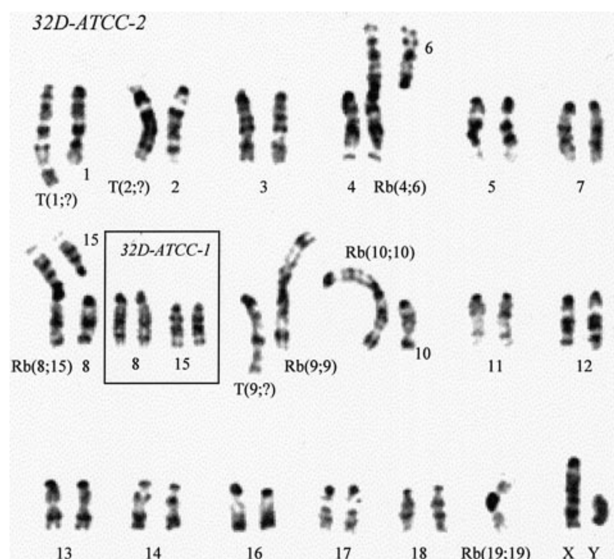

Figure 2. G-banded karyotype of 32D Cl3 cells from American Type Culture Collection (ATCC). 32D-ATCC-1 and 32D-ATCC-2 indicate the 2 equally represented cell populations in the cell line.

modified in a different way from the original one in each laboratory. In this view we performed a cytogenetic analysis of a 32D Cl3 clone from the American Type Culture Collection (ATCC) (ATCC number CRL-11346) and of a 32D Cl3 clone with the same source as our clone (Dr Rovera) but cultured in another laboratory. ATCC 32D Cl3 metaphase analyses showed the presence of 2 equally represented cell populations (here, 32D-ATCC-1 and 32D-ATCC-2). In 32D-ATCC-1 we identified 38 chromosomes: 33 telocentric and 5 chromosomes with 2 arms. G-banding showed chromosome pairs 3, 5, 7, 8, 11, 12, 13, 14, 15, 16, 17, 18, and XY as standard mouse chromosomes, while the others displayed Rb(4;6), Rb(9;9)+9, Rb(10;10)+10, Rb(19;19), and some additions of unidentified genetic materials (Figure 2). The 32D-ATCC-2 (2n = 37 chromosomes, of which 6 have 2 arms) possess the same chromosomal characteristics with the addition of an Rb(8;15).

The 32D Cl3 cell line with the same source as our cells displayed the presence of at least 5 different cell populations characterized by a variety of Rb translocations, most frequently involving chromosome 6 and various additions of unidentified chromosomic materials.

In conclusion, although all of the 32D Cl3 cells we examined were strictly IL-3 dependent for growth in culture and were able to differentiate into mature granulocytes in the presence of G-CSF, they showed a highly variable karyotype characterized by several complex chromosomal rearrangements. Our findings lead us to consider the 32D Cl3 cell line chromosomically very unstable and thus induce the speculation that each laboratory may possess its own clone of 32D Cl3 genetically different from the original one and from other clones of other laboratories, even if all clones maintain at least the 2 phenotypic characteristics that represent the peculiarity of this cell line. Nevertheless, other phenotypic features not yet considered could be different in different clones, and some metabolic pathways could be altered as a result of chromosomal alterations. We wish to remind all those utilizing this cell line for scientific research of the possible consequence of chromosomal instability.

In general, in matters of cell culture, the possibility of selection in vitro of cellular clones with different genotypic characteristics should always be evaluated (even if the original karyotype is conserved).

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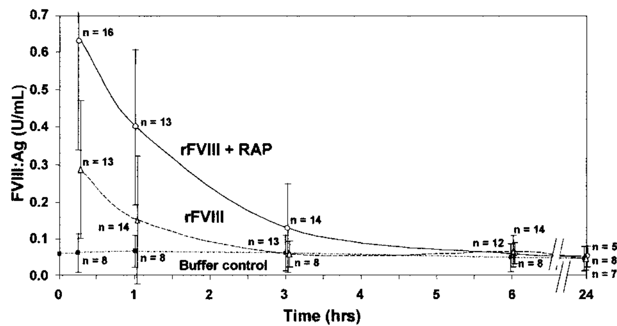
To the editor:

In vivo inhibition of low density lipoprotein receptor–related protein improves survival of factor VIII in the absence of von Willebrand factor

We recently reported that administration of recombinant 39 kd receptor–associated protein (RAP) to von Willebrand factor (vWf) knockout mice induced a significant, sustained rise in endogenous murine factor VIII levels comparable to that induced by the administration of recombinant vWf.[1] RAP is an antagonist[2] of low density lipoprotein receptor–related protein (LRP), a ubiquitously expressed multifunctional endocytic receptor.[3] In vitro studies show that factor VIII binds to LRP and that, by specifically blocking LRP, RAP prevents the binding of factor VIII to LRP.[4] Our in vivo data indicated that LRP facilitates the clearance of endogenous murine FVIII when vWf is absent. The report also included preliminary data suggesting that preadministration of RAP slows elimination of infused recombinant human FVIII (rFVIII) in vWf knockout mice, but the number of animals tested was too small to allow statistically relevant analysis of the data. We therefore extended the infusion study to further investigate the effect of LRP inhibition by RAP on recovery and half-life of infused human rFVIII in vWf-deficient knockout mice.

vWf knockout mice (with undetectable vWf and FVIII levels of about 20% due to secondary FVIII deficiency[5]) were treated with



Inhibition of LRP by RAP improves survival of infused human recombinant FVIII in vWf knockout mice. Animals were treated with 200 U/kg human rFVIII with and without preadministration of RAP (40 mg/kg). FVIII antigen (FVIII:Ag) was measured by an ELISA specific for human FVIII. Group means are shown ± SD.

buffer, human rFVIII (Baxter Hyland Immuno, Thousand Oaks, CA), and RAP (made as a recombinant fusion protein with glutathione S-transferase), as described in our original study.[1] Briefly, 3 groups were tested: (1) a control group received 40 mL/kg buffer followed 15 minutes later by a second injection of 20 mL/kg buffer (n = 11); (2) the rFVIII group received 40 mL/kg buffer followed by 200 U/kg human rFVIII in a volume of 20 mL/kg (n = 15); (3) the RAP preadministration group received 40 mg/kg RAP in a volume of 40 mL/kg followed by 200 U/kg rFVIII in a volume of 20 mL/kg (n = 18). In vivo recovery was determined at 15 minutes as described,[1] and FVIII levels were measured with an ELISA that is specific for human FVIII (Immunozym FVIII:Ag, Baxter, Vienna, Austria). Statistical comparisons were based on repeated measure analysis of variance. Because of the difficulty in drawing blood at frequent intervals from vWf knockout mice, sufficient material for analysis was not available from each animal at each data point and the number of data points was limited, which allowed only fitting of a 1-compartment model for calculation of the half-life of rFVIII.[6]

As expected for detection of a human protein in mice, FVIII levels were below the limit of detection in all animals before infusion of rFVIII and in the control group at all time points. Mean recovery (±SD) was only 5.8% (±3.7%) in the rFVIII group (6 males, 7 females), but 12.6% (±5.9%) in the group with preadministration of RAP (8 males, 8 females), $P = .0023$. There was neither an effect of sex ($P > .1$) nor an interaction between sex and group.

FVIII was maintained at higher levels and was detectable in plasma for a longer period of time with preadministration of RAP (6 hours) than without preadministration of RAP (3 hours) (Figure). Blocking LRP by preadministration of RAP prolonged the half-life of the infused rFVIII from only 42 minutes with rFVIII alone to 67 minutes with preadministration of RAP.

Saenko et al[7] reported that RAP slows the clearance of infused plasma-derived FVIII/vWf in normal mice. Our extended study now demonstrates that inhibition of LRP by a single bolus administration of RAP has a significant inhibitory effect on the clearance of infused FVIII in the absence of vWf, thus further supporting the involvement of LRP in the clearance mechanisms of FVIII.

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To the editor:

A novel missense mutation (C329Q) in *factor VII* gene

FVII is a vitamin-K-dependent plasma glycoprotein. It is synthesized in the liver and circulates in the blood as an inactive zymogen.[1] Upon vascular injury and the presence of tissue factor (TF), FVII is complexed to TF and is cleaved to its active form, FVIIa. The VIIa/TF complex then cleaves and activates both factors X and IX to initiate the coagulation process. Deficiency of factor VII results in a defect in the initiation of coagulation by the extrinsic pathway. Hereditary factor VII deficiency is a rare autosomal recessive bleeding disorder with variable clinical expressions.[2] It is estimated to occur in 1 out of 500 000 persons. The severity of bleeding is variable. The patients may have symptoms such as easy bruising, epistaxis, gingival hemorrhage, increased menstrual blood loss, and cerebral hemorrhage. The clinical symptoms of patients can be improved by transfusion of fresh plasma or blood but not by the administration of vitamin K or hemostat. The *FVII* gene is located on 13q34-qter and contains 9 exons and 8 introns.[3,4] Characterization of *FVII* gene mutations will provide insight into the understanding of function/structure correlation of FVII and the heterogeneity of the deficiency. More than 50 mutations responsible for FVII deficiency have been identified so far.[5] Those include missense mutation, base deletion, splicing site mutation, and promotor mutation. In this short report, we describe a novel missense mutation from a Chinese patient of FVII deficiency with mild symptoms.

The female patient, a 53-year-old, was born of parents with a known consanguinity in Zhousan, Zhenjian, China. She has been easy to bruise and has had gingival hemorrhaging since her childhood, with no liver disease but a history of increased