

ing is supported by several other observational studies that used different sampling procedures. Storms presents an adjusted estimate of the efficacy of circumcision for HPV prevention, assuming differential HPV detection in circumcised and uncircumcised men. However, this adjustment for the biologic effect of circumcision on HPV conflates cause and effect and results in an uninterpretable underestimate of efficacy.

With regard to Storm's argument that adjustment for postsurgical sexual abstinence could have reduced the efficacy of circumcision for the prevention of HSV-2 infection: it is unclear how these estimates were derived. Wound healing was certified in 85.0% of men 4 weeks after surgery and in 95.8% of men 6 weeks after surgery. Men were to refrain from intercourse until certification of wound healing, so adjusting for this period of sexual abstinence, we estimate that the incidence of HSV-2 infection would be 4.2 per 100 person-years (114 of 2714 person-years) in the intervention group and 5.4 per 100 person-years (153 of 2851 person-years) in the control group ( $P=0.02$ ). These results, in conjunction with the trial in South Africa, which showed a reduction in HSV-2 acquisition,<sup>4</sup> indicate that circumcision is efficacious for the prevention of heterosexually acquired HSV-2 infection.

As noted by Ridzon and Singh, the evidence from three male-circumcision trials showing reduced HIV acquisition and from two trials showing reductions in the acquisition of HSV-2 and HPV provides support for the public health benefits of the procedure.

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## Inhibitors of Factor VIII in Hemophilia

**TO THE EDITOR:** Viel et al. (April 16 issue)<sup>1</sup> propose that treatment of hemophilia A with factor VIII that is matched for polymorphic variants may reduce the risk of the development of factor VIII inhibitors. Their study included patients with different types of causative mutations and disease severity. We believe that stratification of patients according to the presence or absence of factor VIII protein, whether it is functional or not, may be more suitable than evaluation of mismatched factor VIII as a risk factor for the development of inhibitors.

Mismatched replacement therapy appears to be a risk factor in patients with cross-reactive factor VIII. In these patients, the immune system is immunologically tolerant of endogenous factor VIII. Hence, matched exogenous therapeutic factor VIII may be identified as "closer to self" and

better tolerated, whereas mismatched factor VIII is identified as "closer to foreign" and more immunogenic. However, the immune systems of patients without cross-reactive factor VIII, which has never had contact with endogenous factor VIII, should recognize all polymorphic variants of factor VIII as being foreign and consider them to be immunogenic.<sup>2</sup>

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**TO THE EDITOR:** Viel et al. report a risk of the formation of factor VIII inhibitors that was three times higher among black patients with hemophilia with the H3 or H4 haplotype than among black patients with the H1 or H2 haplotype.

Since patients with mild or moderate hemophilia only need factor VIII replacement after trauma or surgery, these patients may not have received sufficient amounts of factor VIII concentrates for the development of an inhibitor. The article provides no data on exposure. We observed a median of only 10 cumulative days of exposure in a cohort of patients with mild or moderate disease; these patients were considerably older than those in the study by Viel et al. (median age, 41.0 years; vs. mean age, 17.5 years).<sup>1</sup>

The reported nonsignificant association between disease severity and haplotype may be due to a lack of statistical power and does not rule out confounding. In this study population, 27 of 57 patients with an H1 or H2 (the H1+H2 group) haplotype (47%) appear to have a missense mutation (i.e., moderate or mild hemophilia) as compared with 5 of 19 patients with an H3 or H4 (the H3+H4 group) haplotype (26%) (Table 1 of the article). Consequently, the reported association between the H3 and H4 haplotypes and inhibitors in black patients with hemophilia may be caused by overrepresentation of patients with severe hemophilia in this latter group — that is, confounding.

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**TO THE EDITOR:** Viel et al. should consider whether the 11 mutation types they identified influence the open reading frame of the supposed R484H and M2238V amino acid substitutions. Patients with an open reading frame shift should be excluded from the association analyses. In addition, although the proportion of patients with higher-risk mutation types does not differ significantly between the H1+H2 and the H3+H4 haplotype

groups, the subtypes of the high-risk mutation might be different in these two groups.

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**TO THE EDITOR:** Viel et al. have not adequately considered the type and effect of different factor VIII gene (*F8*) mutations. They include patients with *F8* mutations that abolish FVIII translation (inversions in introns 1 and 22)<sup>1,2</sup> or result in the truncation of FVIII (i.e., p.R336X). In these patients, the effect of *F8* haplotypes cannot be evaluated. A subgroup analysis involving patients with missense mutations — the most suitable subjects for this evaluation — showed no significant association between *F8* haplotypes and the development of inhibitors. Finally, the different risk of inhibitors associated with different types of *F8* mutations is not accounted for in the regression analysis.

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**TO THE EDITOR:** The compelling data in the article by Viel et al. indicate that the high incidence of inhibitors among black patients with hemophilia A is related to the discrepancy between their *F8* haplotypes and the haplotype of their replacement products. In Brazil, patients with hemophilia A are treated with plasma-derived FVIII concentrates, and the incidence of inhibitors among Brazilian patients of African descent with hemophilia A (31%) is higher than the incidence among white patients (20%). However, unlike the population with hemophilia A in the United States, there is no significant difference in the distribution of the H2 FVIII haplotype between these Brazilian racial and ethnic groups (Table 1 in the

Supplementary Appendix, available with the full text of this letter at NEJM.org). Furthermore, among Brazilians of African descent, the high-risk haplotype (H3) is uncommon and is not associated with the formation of inhibitors.

The genetic backgrounds of black Americans and black Brazilians are distinct.<sup>1</sup> In Brazil, the African ancestry originates from sub-Saharan Africa, whereas the ancestry of black Americans originates from West Africa,<sup>2</sup> which can partly explain the discrepancy. It may be that other polymorphisms in *F8*, other genes related to immune responses, or both are also implicated in the higher susceptibility to the development of inhibitors in patients of African origin with hemophilia A.

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**THE AUTHORS REPLY:** Two issues predominate in the comments from all the correspondents: adequate control of confounding and inclusion of patients in the analysis based on the extent to which endogenous FVIII protein is secreted. The odds ratio that we reported, 3.4, is not modest; to have resulted from confounding, either factors that suppressed or promoted inhibitor development were strongly associated with the H1+H2 or the H3+H4 haplotype groups, respectively, or the effect of weakly (but differentially) associated factors was large. One might reasonably expect no strong association in the target population between *F8* haplotype and mutation type or disease severity, days of exposure, age at first infusion, or variants in genes that are not on the X chromosome (allelic association occurs only between syntenic loci).<sup>1</sup> Nonetheless, it is entirely possible that this odds ratio was the result of confounding by unidentified factors or a quirk of sampling.

We have difficulty defending the inclusion of patients with mutations that might have introduced errors in the open reading frame, though this number was small (five patients), was distrib-

uted in both groups, and included no patients with an inhibitor. Because the vast majority of mutant *F8* genes have not been investigated functionally, the extent of their transcription and translation is not definitely known. A cross-reactive material-negative status in plasma does not necessarily indicate the absence of transcription and translation. For example, despite gross gene disruption in patients with intron-22 inversions, both relevant *F8* promoters remain intact and functional.<sup>2,3</sup> The presence of cross-reactive material intracellularly may be sufficient to induce immunologic tolerance to FVIII, even in patients with plasma deficiencies that are cross-reactive material-negative.

An appropriate analytic progression would be to perform analyses within subgroups. We attempted this analysis for the largest subgroup of patients, those with missense mutations. As Peyvandi et al. observed, the result was not significant, but the sample size was small. The estimate, however, was consistent with the overall result. Further study is required to confirm this finding.

The data reported by Santos et al. are intriguing. As they note, the distribution of genotypes is quite different from what we expected,<sup>4</sup> and the frequency of inhibitors differs from what we observed. Due to the replication and stringency of our sequencing strategy,<sup>4</sup> we identified the alleles of both known and unknown nonsynonymous single-nucleotide polymorphisms, and we ruled out the possibility that other coding-sequence bases were variants.

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