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Prevalence of the intron 22 inversion of the factor VIII gene and inhibitor development in Polish patients with severe hemophilia A

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
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Summary

Introduction:

Patients with severe hemophilia A often develop inhibitors (antibodies) against transfused factor VIII.

Materials and Methods:

One hundred thirteen Polish patients with severe hemophilia A, who had been treated on demand with cryoprecipitate until 1992 and exclusively with factor VIII concentrates after 1995, were examined for intron 22 inversion by Southern blotting and the presence and magnitude of inhibitor activity in blood as determined by the Bethesda assay. The patients' ages ranged 4–67 years (mean: 33.7±12.4 years, median: 32 years).

Results:

The number of patients with the inversion amounted to 57, while in 56 patients the mutation types were unknown; 47 patients had a distal and 10 patients a proximal type of inversion. Thirteen patients with inversions (22.8%) were found to have inhibitor in their blood. Most patients (14 out of 15) who developed inhibitors in the course of cryoprecipitate therapy were high responders. Conversely, 4 of 5 patients treated between 1992 and 1995 with both cryoprecipitate and intermediate-purity factor VIII concentrates were low responders. One multitransfused patient who had remained inhibitor-free on cryoprecipitate therapy developed inhibitor after receiving a large dose of factor VIII concentrate during surgery. None of these 5 patients developed inhibitors during their 12–40 years of treatment with cryoprecipitate, suggesting that it was less immunogenic than factor VIII concentrates.

Conclusion:

The prevalence of the intron 22 inversion mutation of the factor VIII gene in Polish hemophiliacs is similar to that in other European countries. Treatment regimens with either cryoprecipitate or virus-inactivated plasma-derived factor VIII concentrates may affect inhibitor formation in hemophilia A patients.

Key words:

hemophilia A • cryoprecipitate • factor VIII concentrates • intron 22 inversions • inhibitor in hemophilia

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INTRODUCTION

Inversion mutations of intron 22 of the factor VIII gene are responsible for up to half (34–50%) of all cases of severe hemophilia A^{1, 8, 9, 14, 17, 19, 20}. These inversions are referred to as type 1 or type 2 depending on whether they are caused by homologous recombination between intron 22 of the factor VIII gene and its distal or proximal extragenic copy, respectively. The third type of intron 22 inversion (3A or 3B) is mediated by a rare, third extragenic copy of factor VIII^{1, 24}. Its prevalence in severe hemophilia is about 0.5%¹ and is hence usually unreported. Another, more recently described mutation is an inversion involving a 1041-bp sequence inside intron 1 of the factor VIII gene and an reversely oriented extragenic duplcon located 140 kb upstream of the gene towards the telomeric side^{3, 5}. The prevalence of this type of mutation accounts for about 5% of patients with severe hemophilia^{3, 30}.

Intron 22 inversions, together with nonsense mutations and large deletions (also assigned as null mutations), are associated with a high risk of developing inhibitors when such patients are treated with preparations containing factor VIII^{19, 20, 28}. The inhibitor prevalence in patients bearing an intron 22 inversion is 7–10 times higher than in those with missense mutations or small deletions¹⁸.

It is generally assumed that patients who develop inhibitors either lack factor VIII or harbor it in a curtailed form, with essential antigenic epitopes missing^{20, 25}. Additional risk factors include genetic constitution, in particular race and the presence or absence of specific MHC class I and II genes, the regimen of factor VIII therapy, and immunological challenges such as vaccination or viral infection^{18, 19, 20}. This suggests that environmental factors play a role in inhibitor development. A discordant antibody response to factor VIII in two pairs of monozygotic hemophilic twins strengthens this opinion⁷. Inhibitor prevalence ranges 21–88% of all severe hemophilia A patients²⁰, depending on the mutation type.

Poland is one of the few European countries in which the prevalence of intron 22 inversion in hemophilia was subjected to scrutiny, apart from a preliminary report carried out in 10 patients²⁶. The present study included 113 patients with severe hemophilia treated on demand only, and thus less exposed to factor VIII than patients on a prophylactic regimen in other countries. Taking into account a recent finding that intensive exposure to factor VIII concentrates is a risk factor for inhibitor development even in patients with mild hemophilia²⁹, we also report the inhibitor prevalence in our sample of patients.

MATERIALS AND METHODS

The patient group consisted of 113 Polish hemophiliacs aged 4–67 years (mean: 33.7±12.4 years, median: 32 years). They were selected from a cohort of unrelated patients regularly attending the Institute of Hematology and Blood Transfusion who suffered from severe hemophilia A (factor VIII<1%), had been under observation for a long time, and were available at the time of the mutation study. Bleeding episodes in the two youngest patients were treated exclusively with lyophilized factor VIII concentrates. Until 1992 the replacement therapy in the remaining 111 patients consisted of cryoprecipitate; from 1992 to 1995, both cryoprecipitate and lyophilized factor VIII concentrates were administered, and since 1995 only lyophilized factor VIII concentrates have been administered. Only plasma-derived factor VIII concentrate products of intermediate, high, and ultra-high purity were used. The patients were treated under an on-demand regimen and received many different factor VIII products, so switching from one brand to another was rather frequent. Factor VIII inhibitors were measured by the Bethesda assay¹⁰. Patients were regarded as low-responding if their peak titer was ≤5 Bethesda units (BU) and high-responding if it was >5 BU. Determination of inhibitors was carried out on the day of drawing blood for the mutation study. Dates on which inhibitors had appeared for the first time were taken from the patients' records. This part of the study was retrospective in character.

Intron 22 inversion mutations were determined by Southern's method employing the EcoRI/SstI fragment of the genomic DNA probe from intron 22, p482.6⁹, that we had obtained by courtesy of Prof. I. R. Peake. All studies were performed in accordance with Polish law and the Declaration of Helsinki.

RESULTS AND DISCUSSION

Table 1 shows the details of the inversion mutations in our patients, including the presence of inhibitor and the magnitude of the immune response to factor VIII. The prevalence of patients with inversions, including the ratio of the distal to proximal types, as well as the prevalence of inversions with and without inhibitors are quite similar to those reported by an international consortium study of 2093 patients with severe hemophilia from 22 different centers; 42% of those patients had intron 22 inversions, with 35% and 7% being of the distal and proximal types, respectively¹. Twenty percent of the patients with inversions also had inhibitor in their blood, whereas only 16% of patients with other types of mutations had inhibitor.

Table 1. Intron 22 inversions and inhibitors prevalence in patients with severe hemophilia A

Inversions	Number of patients		Total
	with inhibitor	without inhibitor	
No	8 (14.3%) 5HR, 3LR	48 (85.7%)	56 (49.5%)
Proximal	1 (10%) HR	9 (90%)	57 (50.5%)
Distal	12 (25.5%) 10 HR; 2 LR	35 (74.5%)	
Total	21 (18.6%)	92 (81.4%)	113

HR – high responders, LR – low responders.

In our study, 22.8% of patients with inversions were found to have inhibitor. Other researchers have reported similar findings. Thus, intron 22 inversions accounted for 33% of 533 patients with hemophilia (severe and non-severe) and 21% patients with inversions had inhibitor²⁰. According to data obtained from smaller samples of patients, the prevalence of inhibitor in patients with intron 22 inversions was as high as 27.3%, and even 42.4%²⁸, but in a later report the authors admitted that the prevalence might have been overestimated because patients with the inhibitor might have been tested for a causative mutation with a higher priority¹⁸. The present study shows that in Poland, as in other countries, intron 22 inversion is probably the most common mutation in severe hemophilia A. Another conclusion is that the on-demand therapy used in Poland, and prophylactic therapy with factor VIII carried out in many European countries, produce similar results with respect to inhibitor development. However, Ghosh et al.⁸ reported a much lower inhibitor prevalence of 8.2% in patients with severe hemophilia in India who, like patients in the present study, were treated on demand, though less frequently (about once a year) than in Poland (about 20–30 times a year). Ghosh et al.⁸ assumed that infrequently treated patients do not develop the inhibitor. It is also possible, however, that infrequent therapy may allow for the disappearance of the inhibitor from the circulation between treatment episodes, thus lowering the prevalence estimate, especially for transient inhibitors. Another difference between the Indian and Polish patients was that the former received mainly cryoprecipitate, blood plasma, and even whole blood, whereas the latter had been treated exclusively with factor VIII concentrates since 1995.

With respect to inhibitor development, the cryoprecipitate may be a safer product than the factor VIII concentrate for two reasons: first, factor VIII in cryoprecipitate and in blood plasma is complexed with

Table 2. Distribution of high and low responders among recipients of cryoprecipitate and factor VIII concentrates

	Number of patients		Total
	HR	LR	
Cryoprecipitate	14	1	15
Factor VIII concentrate	1	–	1
Cryoprecipitate and factor VIII concentrate	1	4	5

HR – high responders, LR – low responders.

the von Willebrand factor, which may shield the immunogenic epitopes of factor VIII^{2, 12}. This view is supported by experimental evidence showing that a purified human factor VIII protein is more immunogenic to factor VIII knock-out mice than the same protein infused in admixture with the von Willebrand factor⁴. Furthermore, when cryoprecipitate was still widely used the inhibitor prevalence in hemophilia A was reported to be lower than today's^{11, 27}. A previously lower inhibitor incidence in hemophilia A was also reported in controlled studies on patients treated exclusively with cryoprecipitate^{6, 22}. Secondly, the method of isolation of factor VIII may also affect its immunogenicity. According to two reports from the Netherlands and Belgium, the incidence of inhibitor in hemophiliacs increased after the introduction of intermediate-purity, fluid-phase virus-inactivated factor VIII concentrates^{21, 23}. The inhibitor developed in a number of inhibitor-tolerant patients who had been previously multitransfused with either a dry-heat-inactivated lyophilized cryoprecipitate or dry-heat-inactivated intermediate-purity factor VIII concentrates. German researchers reported similar experience with solvent-detergent or solvent-detergent pasteurized factor VIII concentrates¹⁵. As stipulated in “Materials and Methods”, our patients received only cryoprecipitate until 1992 and since 1995 exclusively factor VIII concentrates; among the patients reported in the present study was one high responder who had remained inhibitor-free for 40 years of treatment with cryoprecipitate, but who developed an inhibitor after the first massive infusion of an intermediate-purity concentrate (solvent detergent-treated) during surgery (see Table 2). Four previously treated patients without inhibitor development, multitransfused with cryoprecipitate for 12–17 years, developed an inhibitor when their treatment was switched from cryoprecipitate to factor VIII concentrates. Unfortunately, it was not possible to establish with certainty which of the two preparations was the culprit. We assume, however, that the factor VIII concentrate was the more likely immunogen. Interestingly, almost all the patients who developed inhibitor after treatment exclusively

with cryoprecipitate or factor VIII concentrate were high responders, whereas four out of the five patients treated with cryoprecipitate and subsequently with factor VIII concentrates were low responders. In all four low-responding patients, inhibitors occasionally disappeared and later reappeared. This suggests that the patients responded weakly to the novel antigenic epitopes not present or exposed in the cryoprecipitate.

It is obvious that more studies are required before the role of the treatment regimen and that of factor VIII itself on inhibitor development can be established with confidence. The recent introduction of recombinant factor VIII into the therapy of hemophilia does not alter this conclusion, as the recombinant and highly purified plasma-derived products seem to carry a similar risk of inhibitor development^{13, 16}. Obviously, recombinant factor VIII concentrates are, however, superior to plasma-derived preparations with respect to safety from viral contamination.

REFERENCES

1. Antonarakis S. E., Rossiter J. P., Young M., Horst J., de Moerloose P., Sommer S. S. and Ketterling R. P. (1995): Factor VIII gene inversions in severe hemophilia A: results of an international consortium study. *Blood*, 86, 2206–2212.
2. Auerswald G., Spranger T. and Brackmann H. H. (2003): The role of plasma-derived factor VIII/von Willebrand factor concentrates in the treatment of hemophilia A patients. *Haematologica* 88, EREPO5.
3. Bagnall R. D., Waseem N., Green P. M. and Giannelli F. (2002): Recurrent inversion breaking intron 1 of the factor VIII gene is a frequent cause of severe hemophilia A. *Blood*, 99, 168–174.
4. Behrmann M., Pasi J., Saint-Remy J. M., Kotitschke R. and Kloft M. (2002): Von Willebrand factor modulates Factor VIII immunogenicity: comparative study of different Factor VIII concentrates in a haemophilia A mouse model. *Thromb. Haemost.*, 88, 221–229.
5. Brinke A., Tagliavacca L., Naylor J., Green P., Giangrande P. and Gianelli F. (1996): Two chimaeric transcription units result from an inversion breaking intron 1 of the factor VIII gene and a region reportedly affected by reciprocal translocations in T-cell leukaemia. *Hum. Mol. Genet.*, 5, 1945–1951.
6. El Alfy M. S., Tantawy A. A., Ahmed M. H. and Abdin I. A. (2000): Frequency of inhibitor development in severe hemophilia A children treated with cryoprecipitate and low-dose immune tolerance induction. *Haemophilia*, 6, 635–638.
7. European Study Group of Factor VIII Antibody (1979): Development of factor VIII antibody in haemophilic monozygotic twins. *Scand. J. Haematol.*, 23, 64–68.
8. Ghosh K., Shetty S., Kulkarni B., Nair S., Pawar A., Khare A., Baidur S. and Mohanty D. (2001): Development of inhibitors in patients with haemophilia from India. *Haemophilia*, 7, 273–278.
9. Goodeve A. C., Preston F. E. and Peake I. R. (1994): Factor VIII gene rearrangements in patients with severe hemophilia A. *Lancet*, 343, 329–330.
10. Kasper C. K., Aledort L., Aronson D., Counts R., Edson J. R., van Eys J., Frattantoni J., Green D., Hampton J., Hilgartner M., Levine P., Lazerson J., McMillan C., Penner J., Shapiro S. and Shulman N. R. (1975): Proceedings: A more uniform measurement of factor VIII inhibitors. *Thromb. Diath. Haemorrh.*, 34, 612.
11. Kreuz W., Escuriola-Ettingshausen C., Martinez-Saguer I., Güngör T. and Kornhuber B. (1996): Epidemiology of inhibitors in haemophilia A. *Vox Sang.*, 70, (suppl. 1), 2–8.
12. Kreuz W., Ettingshausen C. E., Auerswald G., Saguer I. M., Becker S., Funk M., Heller C., Klarmann D., Klingebiel T. and GTH PUP Study Group (2003): Epidemiology of inhibitors and current treatment strategies. *Haematologica*, 88, EREP04.
13. Kreuz W., Ettingshausen C. E., Zyschka A., Oldenburg J., Saguer I. M., Ehrenforth S. and Klingebiel T. (2002) Inhibitor development in previously untreated patients with hemophilia A: a prospective long-term follow-up comparing plasma-derived and recombinant products. *Semin. Thromb. Hemost.*, 28, 285–290.
14. Lakich D., Kazazian H. H. Jr., Antonarakis S. E. and Gitschier J. (1993): Inversions disrupting the factor VIII gene are a common cause of severe haemophilia A. *Nat. Genet.*, 5, 236–241.
15. Laub R., Di Giambattista M., Fondu P., Brackmann H. H., Lenk H., Saenko E. L., Felch M. and Scandella D. (1999): Inhibitors in German haemophilia A patients treated with a double virus inactivated factor VIII concentrate bind to the C2 domain of FVIII light chain. *Thromb. Haemost.*, 81, 39–44.
16. Lusher J. M. (2002): First and second generation recombinant factor VIII concentrates in previously untreated patients: recovery, safety, efficacy, and inhibitor development. *Semin. Thromb. Hemost.*, 28, 273–276.
17. Naylor J., Brinke A., Hassock S., Green P. M. and Giannelli F. (1993): Characteristic mRNA abnormality found in half the patients with severe haemophilia A is due to large DNA inversions. *Hum. Mol. Genet.*, 2, 1773–1778.
18. Oldenburg J., Brackmann H. H. and Schwaab R. (2000): Risk factors for inhibitor development in hemophilia A. *Haematologica*, 85 (suppl. 10), 7–14.
19. Oldenburg J., El-Maarri O. and Schwaab R. (2002): Inhibitor development in correlation to factor VIII genotypes. *Haemophilia*, 8 (suppl. 2), 23–29.
20. Oldenburg J., Schröder J., Brackmann H. H., Müller-Reibe C., Schwaab R. and Tuddenham E. (2004): Environmental and genetic factors influencing inhibitor development. *Semin. Hematol.*, 41 (suppl. 1), 82–88.
21. Peerlinck K., Arnout J., Gilles J. G., Saint-Remy J. M. and Vermeylen J. (1993): A higher than expected incidence of Factor VIII inhibitors in multitransfused hemophilia A patients treated with an intermediate purity pasteurized Factor VIII concentrate. *Thromb. Haemost.*, 69, 115–118.
22. Peerlinck K., Rosendaal F. R. and Vermeylen J. (1993): Incidence of inhibitor development in a group of young hemophilia A patients treated exclusively with lyophilized cryoprecipitate. *Blood*, 81, 3332–3335.
23. Rosendaal F. R., Nieuwelhuis H. K., van den Berg H. M., Heijboer H., Mauser-Bunschoten E. P., van der Meer J., Smit C., Strengers P. F. and Briet E. (1993): A sudden increase in factor VIII inhibitor development in multitransfused hemophilia A patients in The Netherlands. Dutch Hemophilia Study Group. *Blood*, 81, 2180–2186.
24. Rossiter J. P., Young M., Kimberland M. L., Hutter P., Ketterling R. P., Gitschier J., Horst J., Morris M. A., Schaid D. J. and de Moerloose P. (1994): Factor VIII gene inversions causing severe hemophilia A originate almost exclusively in male germ cells. *Hum. Mol. Genet.*, 3, 1035–1039.
25. Saenko E. I., Ananyeva N. M., Kouivaskia D. V., Khrenov A. V., Anderson J. A., Shima M., Qian J. and Scott D. (2002): Haemophilia A: effects of inhibitory antibodies on factor VIII functional interactions and approaches to prevent their action. *Haemophilia*, 8, 1–11.
26. Sawecka J., Skulimowska J. and Kościelak J. (2000): Inversion mutations in patients with severe hemophilia A. *Acta Haematol. Pol.*, 31, 47–50.

27. Scharer I., Bray G. L. and Neutzling O. (1999): Incidence of inhibitors in haemophilia A patients – a review of recent studies of recombinant and plasma-derived factor VIII concentrates. *Haemophilia*, 5, 145–154.
28. Schwaab R., Brackmann H. H., Meyer C., Seehafer J., Kirchgesser M., Haack A., Olek K., Tuddenham E. G. and Oldenburg J. (1995): Haemophilia A: mutation type determines risk of inhibitor formation. *Thromb. Haemost.*, 74, 1402–1406.
29. Sharathkumar A., Lillicrap D., Blanchette V. S., Kern M., Leggo J., Stain A. M., Brooker L. and Carcao M. D. (2003): Intensive exposure to factor VIII is a risk factor for inhibitor development in mild hemophilia A. *J. Thromb. Haemost.*, 1, 1228–1236.
30. Tizzano E. F., Cornet M. and Baiget M. (2003): Inversion of intron 1 of the factor VIII gene for direct molecular diagnosis of hemophilia A. *Haematologica*, 88, 118–120.

