

# Prevalence of inversions in introns 1 and 22 of the factor VIII gene and inhibitors in patients from southern Brazil

## *Prevalência das inversões nos íntrons 1 e 22 do gene do fator VIII e inibidores em pacientes do sul do Brasil*

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### ABSTRACT

**Objectives:** The development of antibodies (inhibitors) against exogenous factors is the main complication in the treatment of hemophilia. Both genetic and non-genetic factors are related to inhibitor development. Among the genetic factors, the type of mutation that caused the disease is one of the most important. The objectives of the present study were to establish the prevalence of inversions in introns 1 and 22 of the factor VIII gene in patients with severe hemophilia A, correlating these with inhibitor development, and to compare the results with data from the literature. **Method:** Unrelated severe hemophilia A patients were analyzed for the presence of inversions in intron 1 ( $n = 77$ ) and intron 22 ( $n = 39$ ) by polymerase chain reaction (PCR). Detection of the inhibitor was performed by the mixing test and its quantification was performed by the Bethesda method. **Results:** The prevalence of inversions in introns 1 and 22 was 2.6% and 41%, respectively. No patient with inversions in intron 1 had inhibitors, whereas 26.3% of patients with inversions in intron 22 developed inhibitors. **Conclusion:** Due to the small number of patients with inversions in intron 1, it was not possible to perform a statistical test for the correlation with risk of inhibitor development. Inversions in intron 22 of the factor VIII gene were not associated with an increased risk of inhibitor development in the analyzed samples ( $p = 1$ ).

**Key words:** hemophilia A; blood coagulation factor inhibitors; factor VIII; mutation; molecular biology.

### RESUMO

**Introdução:** O desenvolvimento de anticorpos (inibidores) contra o fator exógeno é a principal complicação do tratamento de hemofílicas. Tanto fatores genéticos quanto não genéticos estão relacionados com o surgimento dos inibidores. Entre os fatores genéticos, o tipo de mutação que originou a doença é um dos mais importantes. Os objetivos do presente estudo foram estabelecer a prevalência das inversões nos íntrons 1 e 22 do gene do fator VIII em pacientes com hemofilia A grave, correlacionando-a com o desenvolvimento de inibidores, bem como comparar os resultados encontrados com dados da literatura mundial. **Método:** Foram analisados pacientes hemofílicos A graves não aparentados quanto à presença da inversão no íntron 1 ( $n = 77$ ) e da inversão no íntron 22 ( $n = 39$ ), utilizando a técnica de reação em cadeia da polimerase (PCR). A detecção do inibidor foi realizada pelo teste de mistura; a sua quantificação, pelo método de Bethesda. **Resultados:** As prevalências das inversões nos íntrons 1 e 22 foram de 2,6% e 41%, respectivamente. Nenhum paciente com a inversão no íntron 1 apresentou inibidores, enquanto 26,3% dos pacientes com a inversão no íntron 22 desenvolveram os anticorpos. **Conclusão:** O número reduzido de pacientes com a inversão no íntron 1 não permitiu a aplicação de teste estatístico para a correlação com o risco de desenvolvimento de inibidores. A inversão no íntron 22 do gene do fator VIII não se associou ao maior risco de desenvolvimento de inibidores na amostra analisada ( $p = 1$ ).

**Unitermos:** hemofilia A; inibidores dos fatores de coagulação sanguínea; fator VIII; mutação; biologia molecular.

## RESUMEN

**Introducción:** El desarrollo de anticuerpos (inhibidores) contra el factor exógeno es la principal complicación del tratamiento de hemofilias. Tanto factores genéticos como no genéticos están relacionados con la aparición de los inhibidores. Entre los factores genéticos, el tipo de mutación que originó la enfermedad es uno de los más importantes. El objetivo de este estudio fue establecer la prevalencia de las inversiones en los intrones 1 y 22 del gen del factor VIII en pacientes con hemofilia A severa, relacionándola con el desarrollo de inhibidores, así como comparar los resultados encontrados con datos de la literatura en el mundo. **Método:** Pacientes con hemofilia A severa no emparentados fueron analizados cuanto a la presencia de inversión en el intrón 1 ( $n = 77$ ) y de la inversión en el intrón 22 ( $n = 39$ ), usando la técnica de reacción en cadena de la polimerasa. La detección del inhibidor fue realizada por el estudio de mezclas; su cuantificación, por el método Bethesda. **Resultados:** La prevalencia de las inversiones en los intrones 1 y 22 fueron 2,6% y 41%, respectivamente. Ningún paciente con la inversión en el intrón 1 presentó inhibidores, mientras 26,3% de los pacientes con la inversión en el intrón 22 desarrollaron anticuerpos. **Conclusión:** El pequeño número de pacientes con inversión en el intrón 1 no permitió la aplicación de la prueba estadística para correlación con el riesgo de desarrollo de inhibidores. La inversión en el intrón 22 del gen del factor VIII no se asoció a un mayor riesgo de desarrollo de inhibidores en la muestra analizada ( $p = 1$ ).

**Palabras clave:** hemofilia A; inhibidores de factor de coagulación sanguínea; factor VIII; mutación; biología molecular.

## INTRODUCTION

Hemophilia treatment involves the intravenous replacement of the deficient factor. The formation of inhibitors [polyclonal antibodies of the immunoglobulin class G (IgG)] against the exogenous factor is the most serious complication of replacement therapy in patients with hemophilia<sup>(1)</sup>. The affected patients stop responding to infusion of the deficient factor and exhibit hemorrhagic episodes that are difficult to control.

Between 10% and 30% of patients with hemophilia A develop inhibitors to treatment, while the incidence among patients with hemophilia B is much lower, about 1%-5%<sup>(2)</sup>. The production of inhibitors is influenced by genetic as well as non-genetic factors<sup>(3,4)</sup>. The major genetic factors are the type of mutation that caused the disease and the genetic susceptibility of cell surface molecules involved in the immune response, such as major histocompatibility complex molecules, T-lymphocyte receptors, and cytokine receptors, as well as several immunomodulatory molecules<sup>(5)</sup>.

Mutations associated with the highest risk of inhibitor development are those that prevent the synthesis of endogenous protein (null mutations) and are associated with the severe disease phenotype. These are the large deletions, nonsense mutations, and (for hemophilia A) intron 1 and 22 inversion mutations<sup>(5,6)</sup>.

It is estimated that more than 40% of severe cases of hemophilia A are caused by inversions in the gene encoding factor VIII<sup>(7)</sup>.

## OBJECTIVES

This study aimed to establish the prevalence of inversions in introns 1 and 22 of the gene encoding factor VIII in a sample of patients with hemophilia A from the state of Paraná, Brazil, correlating these mutations with the presence of inhibitors against factor VIII and its quantification in the same population.

## METHOD

The subjects who participated in the study had hemophilia A and were enrolled in the Hematology and Hemotherapy Center of the state of Paraná. Patients from different families were generally selected because hemophilic individuals from the same family exhibit the same disease-causing mutation. This was an observational, analytical, and cross-sectional experimental study.

Inhibitors were detected through the mixing test. The Bethesda method was used for quantifying inhibitors and classifying samples as low/high response; that is, when inhibitors were persistently quantified at  $\leq 5$  Bethesda units (BU)/ml or above 5 BU/ml in response to exogenous factor exposure, respectively. This classification correlates with the clinical severity, since patients with  $\leq 5$  BU/ml usually respond to an increase in the dose of the infused factor, whereas those with more than 5 BU/ml require the use of a bypass agent to control their bleeding episodes<sup>(2)</sup>.

Polymerase chain reaction (PCR) amplification using reverse transcription was used to identify the inversion in intron 22<sup>(8)</sup>; and fast PCR, to identify the inversion in intron 1<sup>(9)</sup>.

The prevalence frequencies obtained in this study were compared with those from articles appearing in PubMed over the last 10 years, by selecting studies with the largest number of participants. The chi-square frequency test ( $\chi^2$ ) was applied with a 5%  $\alpha$ , and random Monte Carlo simulations were performed when the assumptions of the frequency test were not met. The tests were performed through the R platform. The estimated difference between categorical variables was performed using Fisher's exact test, with a minimum level of significance of 5%.

The research was conducted in accordance with the 2008 Revised Declaration of Helsinki and was approved by the Research Ethics Committee. Patients signed a free and informed consent form prior to their participation.

## RESULTS AND DISCUSSION

A total of 45 patients with severe hemophilia A were tested for intron 22 inversion, of which 39 were unrelated; 84 participants were tested for intron 1 inversion, of which 77 were unrelated. The prevalence for intron 22 inversion among unrelated individuals was 41% and did not exhibit a significant difference ( $\chi^2 = 1.13$ ;  $p = 0.3$ )<sup>(10)</sup>. The prevalence for intron 1 inversion among unrelated patients was 2.6% and also did not show a significant difference ( $\chi^2 = 0.05$ ;  $p = 1.18$ )<sup>(11)</sup>.

Leiria *et al.* (2009)<sup>(12)</sup> studied 107 patients with hemophilia A also in southern Brazil, but from the state of Rio Grande do Sul, and reported a prevalence of 46% and 3% for inversions in introns 22 and 1, respectively.

The prevalence of inhibitors in related and non-related patients with the inversion in intron 22 was 26.3% and did not show a significant difference ( $\chi^2 = 0.01$ ;  $p = 0.9$ )<sup>(10)</sup>. However, a significant difference ( $\chi^2 = 26$ ;  $p < 0.01$ ) was observed among patients with an inversion in intron 1<sup>(11)</sup>.

Although not used in this study, applications for the development of genograms, such as the Genogram Development and Management System (GDMS), can facilitate the classification in related and unrelated patients and make the collection of unrelated samples more effective, because in the presence of multiple affected family members, the adequate collection of family history is a laborious task<sup>(13)</sup>.

Regarding the behavior of inhibitors in the 45 patients tested for intron 22 inversion, the prevalence of inhibitors in patients

with and without inversion (19 and 26 patients, respectively) was very similar: 26.3% ( $n = 5$ ) and 26.9% ( $n = 7$ ), respectively. Thus, no difference was observed in the intron 22 inversion distribution according to the presence of inhibitor ( $p = 1$ ) (Figure 1), nor with respect to the degree of response ( $p = 0.41$ ) (Figure 2). The two patients with an inversion in intron 1 showed no inhibitor.

The causes underlying inhibitor development remain elusive with respect to the various possible etiological factors.

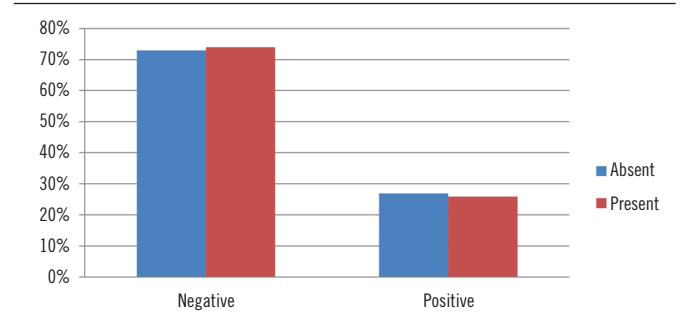


FIGURE 1 – Intron 22 inversion and inhibitor behavior against factor VIII (positive/negative)

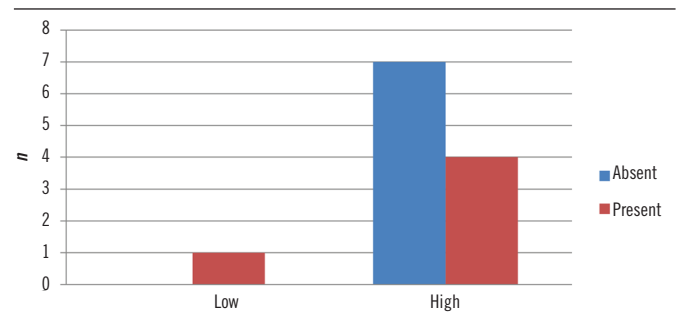


FIGURE 2 – Intron 22 inversion and inhibitor response rate (high/low)

## CONCLUSION

Inversion in intron 22 of the gene encoding factor VIII was not associated with a higher risk of inhibitor development in the sample analyzed, whereas the small number of patients with the intron 1 inversion did not allow the application of a statistical test for determining its association with the risk of inhibitor development.

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