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# NIVELI GJENETIK I PENETRIMIT DHE EKSPRESIVITETIT NË FAKTORËT PËR HEMOFILIN E TIPIT A, HEMOGLOBINË, aPTT DHE GJENOTIP

# ГЕНЕТСКО НИВО НА ПЕНЕТРАЦИЈА И ЕКСПРЕСИВНОСТА ВО ФАКТОРИТЕ ЗА ТИПОТ А НА ХЕМОФИЛИЈА, ХЕМОГЛОБИН, aPTT И ГЕНОТИПОТ

# GENETIC LEVEL OF PENETRATION AND EXPRESSIVITY IN FACTORS FOR TYPE OF HEMOPHILIA, HEMOGLOBIN, aPTT AND GENOTYPE

**Abstract**

This study investigates the penetration and genetic expression of factor VIII of hemophilia to individuals who are in the fourth degree of kinship from the mother's line. So it comes to two hemophiliac brothers and a hemophiliac cousin whose mothers are sisters but who are married in different families. For these cases of hemophiliacs were performed laboratory, biochemical and genetic analyzes to observe the level of penetration and expression of factor VIII. We will present with the help of the genetic tree the origins or gene genealogies of the gene for this disease. Based on biochemical and genetic analysis using PCR, we will present factor I, IX, XI, aTTP, vWF-Factor Von Willebrandov, locus Xq28, ccddee genotype, fibrinogen, blood group, rhesus factor in hemophiliacs involved in this study. We will likewise compare the laboratory analysis of individuals and look at the similarities or differences in the values gained. Based on these results, doctors can determine doses

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about these cases with hemophilia. In this case it is suggested that the heparin dose should be in correlation with the aPTT activity to avoid thrombin.

## Introduction

The work was carried out in the Presevo population stretching east of Kosovo and south-east of Serbia in the E75 valley. Hemophilia A is inherited through the X sexual chromosome. Women have 50% chance of transmitting factor VIII mutation to each pregnancy. The males who inherit from the mother factor VIII will be affected by hemophilia. Men with factor VIII transmit mutation to all their daughters and never to their sons. According to genealogy analysis and interviews in families made and related to this case of hemophilia A, we will parse the track of this gene. With this study we can comment on the inheritance of hemophilia A through generations and we will see that this mutation comparing the obtained results has or does not have any correlation between the individuals in this paper.

## Material and methods

The material for this paper is collected in the form of a questionnaire in the families in Presevo and in the villages around Presevo. During this questionnaire we have had cases with hemophilia type A, where from 6000 inhabitants we identified 4 cases of male hemophilia A but one of them died. From the detailed analysis we saw that it is about three first cousins from the maternal line that are in the genetic vicinity at the 4th degree of kinship. For this study, we conducted genome genealogical research that was affected by factor VIII of hemophilia.

Genetic trees have been used as a method by which we can become familiar with the history or genealogy of hemophilia.

To become familiar with the level of hemophilic factors and to know their genotype for this disease, laboratory, biochemical and genetic analyzes have been done at the Nis institute. For molecular assays we have the results from the PCR with which the genotype for hemophilia ccddee is located, the location of the hemophilic gene locus in the sex X chromosome Xq28. The analysis of the aTTP metacondition factors, factor VIII factor, vWF-factor Von Willebrandov and other analyzes presented in Table 1 were performed.

**Analysis and discussion of results**

**a) Genealogical analysis of Hemophilus A**

Genealogical analysis of cases with hemophilia A in three families shows that the source of this disease is from the female of generation III-8. This is ascertained by the analysis of previous generations where we have no cases with this disease. The analysis clearly shows that we have a dead hemophilia that is in the IV-generation and the ordinal number 10 (IV-10). Of the three cases with hemophilia we have also the son with hemophilic disease in the V band and with ordinal number 9 (V-9) and two hemophiliacs in the V band with ordinal number 11 (V-11) who is dead and the Vlach of the same generation V with ordinal number 12 (V-12) alive being hemophilic.

From genealogical analysis (Fig.1) it is clear that factor VIII factor for type A hemophilia is the individual in generation III with ordinal number 8 (III-8) which is inherited from her mother's stock exchange II with ordinal number 3 (II-3). The person of the generation IV-10 presented as a target with the arrows is a dead man.

According to genealogical cases, this dead brother IV-10 has two sisters carrying type A hemophilia that are underlined with the arrows in IV band with ordinal numbers 14 and 15. Both married sisters (14 and 15) in different families have three dead hemophilic boys in V band with ordinal number 11 (V-11). While two other hemophiliacs are alive of the V-9 and V-12 generation. These are in the 4th stage of kinship 1/16. If they make a marriage in this family with IV-degree relatives every 16th born will be hemophiliac.

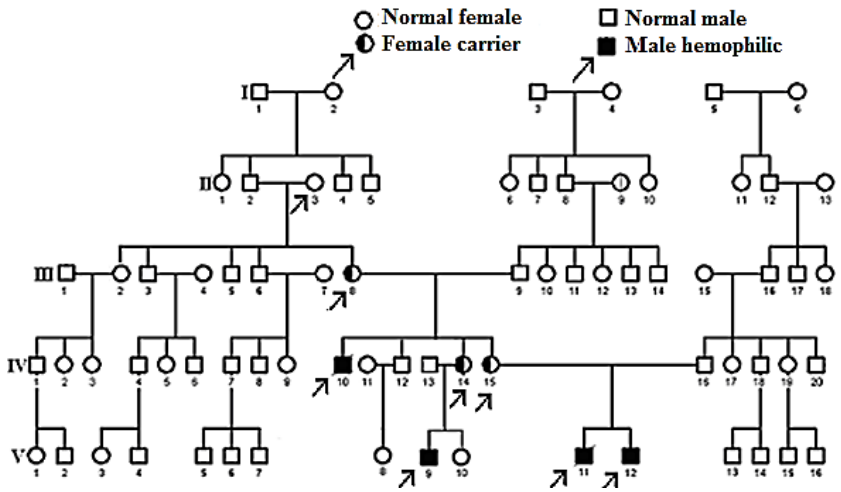


**Figure 1. The genetic tree of hemophilia disease in cats type A-VIII**

**b) Biochemical and genetic analysis of hemophiliacs**

From the PCR analysis it is found that the factor VIII of hemophilia A. The results obtained in the case of hemophilic A-VIII result in the average type of homophiles with these values of 1.5%, 1.7% and 2%.

The hemoglobin level of hemophilia type A-VIII is 10.4, 10.6, and 10.7.

The time of thromboplastin activation (aPTT) is clearly seen to be much delayed 71.7s, 68s and 75s.

The prothrombin test specifically evaluates the presence of factors VII, V, and X, prothrombin and fibrinogen. A prothrombin time within the 11-15 second interval (dependent on the source of the thromboplastin used) indicates that the patient has a normal amount of upper coagulation factors.

With aPTT ratios of 1.5 to 2.5, in the therapeutic range used by many labs, variable levels of anti-factor Xa heparin have been achieved. According to this process it turns out that the defect has to do with the reversible negative link that will lead to decreased heparin and thromboplastin growth.

These three cases with hemophilia have the Rh-negative blood group and according to hemophilia analysis there is a correlation with the negative factor of the genotype dd. We base this on the basis of the similarities of the rhesus negative genotype dd with the genotype dd of hemophiliacs. Therefore, we think that these diseases are caused by a correlation between the genes in the X chromosome and the gene in the autosomal chromosome number 1.

**Table 1. Laboratory, biochemical and genetic results of hemophiliacs**

| Three cases with hemophilia | First person (first brother) | Second person (second brother) | Third person (the cousin of the two brothers) |
|---|---|---|---|
| **Type of hemophilia** | **Hemophilia type A** | **Hemophilia type A** | **Hemophilia type A** |
| **Blood group** | **A, Rh negative** | **A, Rh negative** | **A, Rh negative** |
| **Factor VIII level** | **1.7% (preferably 50-150%)** | **1.5% (preferably 50-150%)** | **2%( preferably 50-150%)** |
| **Factor I level** | **338,5%** | **338%** | **339%** |

| | | | |
|---|---|---|---|
| **Factor IX level** | **91,5% (66)** | **91%** | **91%** |
| **Factor XI level** | **82.8% (117)** | **83%** | **83%** |
| **Fibrinogen** | **4.01% (130-300 mg/dL)** | **4% (130-300 mg/dL)** | **4% (130-300 mg/dL)** |
| **Genotype** | **ccddee** | **ccddee** | **ccddee** |
| **Intron 22** | **Region Xq28** | **Region Xq28** | **Region Xq28** |
| **aPTT(Partial Thromboplastin Time)** | **71.7s (preferably 25-35s)** | **68s (preferably 25-35s)** | **75s (preferably 25-35s)** |
| **Leukocytes** | **16.5** | **17** | **17.1** |
| **Erythrocytes** | **4.20** | **4.18** | **4.10** |
| **Hemoglobin** | **10.4 (12-15-g/dL)** | **10.6** | **10.7** |
| **vWF- Factors Von Willebrandov** | **46%** | **46%** | **45%** |

Analyzing the average values in Table 2 it is clear that factor VIII has a low level compared to the reference value. Therefore, we say that the type of hemophilia in these 3 cases is the average type because the average for these is; 1.37% reference is = 1-5% (F VIIIC> 5 **iu** dL-international unit-iu).

**Table 2. Laboratory status based on the median values investigated**

| Test | Average | Reference range | Units |
|---|---|---|---|
| **Factor VIII level** | **1.37%** | **50-150%** | **%** |
| **Heavy** | **<1%(F VIIIC(1 iu dL)** | **50-150%** | **iu-** international unit |
| **Average** | **1-5%(F VIIIC> 5 iu dL)** | **50-150%** | **iu-** international unit |
| **Easy** | **> 5% (F VIIIC>iu dL)** | **50.150%** | **iu-** international unit |
| **Genotype** | **ccddee** | **ccddee** | **Dominant** |
| **aPTT (Partial Thromboplastin Time)** | **71.56-s** | **25-36-s** | **Seconds -s** |
| **Hemoglobin** | **10.6** | **12-15 (120-150g/L)** | **g/dL or g/L** |
| **vWF- Factors Von Willebrandov** | **46%** | **50-160%** | **%** |

Therefore, we can conclude that the inheritance of these factors has been done with a high stability because the expression of the genes has given the same results in these cases. It is thought that factor VIII

interference which results from mutation in intron 22 in Xq28 region of chromosome X has had the same penetration.

According to the genetic-PCR analysis it is clear that the three cases of hemophilia A are ccddee. The locus of the gene in the chromosome has the region on the Xq-28 arm.

## Conclusion

Based on the comparison of the hemoglobin factor in the three cases with Type A hemophilia, it is clear that there is a correlation between penetration and expression between the hemophilic factor and the hemoglobin factor. The activation of thromboplastin for baiting is approximately the same as it appears: aPTT-71.7s (25-35s), 68s (25-35s) and 75s (25-35s). According to the E-cadherin contact protein analysis there is a filamentous deformation which slows thromboplastin activity. Also, the level of factor VIII in the three cases has a very low penetration and therefore the expression is lower and thus does not stop the flow of blood. Vitamin K has a very low level in the cases mentioned. Factor VIII values are as follows: Case 1. Vllau 1.7% but (preferbly 50-150%); Case 2. Vllau 1.5% (Preferably 50-150%) and Case 3. Conical 2% (Preferably 50-150%).

## Resume

**a)** Based on the analysis of the laboratory results it is concluded that it comes to type A Hemophilus. From the obtained laboratory and genetic results, it is seen that the gene penetrance is very much the same as the factor VIII and the aPTT factor. They belong to the middle Hemophilus because they have a mean value of <1.37.

**b)** Other research has shown that the sensitivity of an APTT reagent to heparin depends on its phospholipid content and the nature of the activator present. Therefore, we say that in this case we have an average of these two factors because the penetration and expressiveness of the genes is such.

**c)** We can say that the negative resection factor with genotype dd in autosomal chromosome 1 has an epigenetic effect on the genotype dd of the X chromosome (ccddee). This means that hemophilia is a hereditary disease of the correlation type but also epistatic because the phenotype is not the only result of the sex X chromosome gene.

**d)** According to genetic-biochemical analysis it is found that factor VIII results from mutation in intron 22 in Xq28 region of chromosome X and their genotype is: ccddee. Genealogical analysis of cases with hemophilia A in three families shows that the source of this disease is from the female of generation III-8.

**e)** It is evident that the number of hemophiliacs is constantly increasing and that the cause is radioactive pollution of the environment, chemical, physical, biological, etc.

**f)** According to the average knowledge of hemophilia arise 1: 5000 - 1: 10000 but from 6000 individuals interviewed there are 4 cases with type A hemophilia.

Therefore, there is an appeal to international hemophilia societies to have a genetic care and consultation of populations at risk of these hemophilic factors.

Reviewed by:
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**Literature**

**1.** Ahrens, P., T.A. Kruse, M. SchWarty, P.B. Rasmussen & N. Din. 1987. A ne W HindIII restriction fragment length polymorphism in the Hemophilia A locus. Hum. Genet.76: 127-128.

**2.**Anand SGinsberg JSKearon CGent MHirsh J The relation between the activated partial thromboplastin time response and recurrence in patients with venous thrombosis treated with continuous intravenous heparin. *Arch Intern Med.* 1996;1561677- 1681.

**3**. Basu DGallus AHirsh JCade J A prospective study of the value of monitoring heparin treatment with the activated partial thromboplastin time. *N Engl J Med.* 1972;287324- 327.

**4.** Chan, V., T.N. Tong & T.P. Chan. 1989. Multiple XbaI polymorphisms for carrier detection and prenatal diagnosis of haemophilia A. Br. J. Haematol. 73: 497-500.

**5**. Gawoski JArkin CFBovill TBrandt JTRock Jr WATriplett DA The effects of heparin on the activated partial thromboplastin time of the College of American Pathologists survey specimens:

responsiveness, precision, and sample effects. *Arch Pathol Lab Med.* 1987;111785.

**6.** Gitschier, J., W.I. Wood, T.M. Goralka, K.L. Wion, E.Y. Chen, D.H. Eaton, G.A.Vehar, D.J. Capon & R.M. LaWn. 1984. Characterization of the human factor VIII gene. Nature 312:326-330.

**7**. Herrmann, F.H., M. Wehnert, W. Schröder & K. Wulff. 1990. Genomic diagnosis of hemophilia A and B. Thromb. Haemorrh. Disorders 2(1): 11-15

**8.** Lakich, D., H. Kazazian, S.E. Antonarakis & J. Gitschier. 1993 Inversions disrupting the factor VIII gene are a common cause of severe haemophilia A. Nature Genet. 5: 236-241.

**9**. Miller, S.A., D.D. Dykes & H.F. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res. 16: 1215. Oberle I., G. Camerino, R. Heilig, L. Grunebaum, J.P. Cazenave, C. Crapanzano, P.M. Mannucci & J.L. Mandel. 1985. Genetic screening for haemohilia A With a polymorphic DNA probe. N. Engl. J. Med. 312: 682-686.

**10.** Strmecki L, Benedik-Dolnièar M, Komel R. A novel mutation Q602STOP in exon 12 of the FVIII gene. Human Heredity 1998; 48: 119–20.

**11.**Van den Besselaar AMHPMeeuwisse-Braun JBertina RM Monitoring heparin therapy: relationships between the activated partial thromboplastin time and heparin assays based on ex-vivo heparin samples. *Thromb Haemost.*1990;6316- 23.