

# Using Factor VII in Hemophilia Gene Therapy

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## 1. Introduction

Human blood at physiological conditions is kept as fluid through precise system called homeostasis, if damage to the vessel, causing the system will be restored by vessel wall. Cases no regulation or homeostasis disorders, thrombosis (intravascular coagulation) or bleeding occur. In normal conditions, the secretion of vascular endothelial heparin-like and trmbomodulin molecules prevent blood coagulation and secretion of nitric oxide and prostacyclin prevent platelet aggregation and blood brings the liquid keeps. Homeostasis has three stages: vasoconstriction, platelet plug formation and blood coagulation, blood coagulation are reactions in which plasma zymogens become active enzymes that create the clotting reaction. Coagulation reactions will be set with inhibitory and stimulatory mechanisms. Coagulation is a regulatory process that keeps the blood flowing. Blood coagulation has two external and internal pathways (Figure 1), tissue factor and FVII form the external pathway, internal pathway is formed of FVIII, FIX and FXI (Ramanarayana et al., 2011; Ellison, 1977).

## 2. Hemophilia

Hemophilia had recognized in the fifth century BC, first the Jews law passed that when a woman has two dead boys doing the circumcision her third son should not be circumcised, they showed the mother will transmit the disease to her sons (History of hemophilia, 2011). Genetic and hereditary pattern of hemophilia was carefully described in 1803 by the American physician John Conrad Otto. He supposed that the bleeding was occurring due to lack of blood anti hemophilic factor (Cahill & Colvin, 1997). Glossary of hemophilia was developed for this disease in 1828 at the University of Zurich. Anti-hemophilia globin was discovered in 1937 by Patek and Tylor at Harvard University (History of Hemophilia Disease, 2011). The two forms of hemophilia A and B were distinguished in 1952 by Pavlosky, the Brazilian physician. Both diseases are sex-dependent and occur in males (Cahill & Colvin, 1977).

## 3. Causes hemophilia

Hemophilia is a genetic disorder happens in coagulation FVIII (hemophilia A) or FIX (hemophilia B) and are related to the X chromosome. Hemophilia A is a disease due to genetic defects in coagulation FVIII (Furie et al., 1994; White & Shoemaker, 1989) It is identified by Hoyer and Breckenridge (Hoyer & Breckenridge, 1968) and then by Denson for the first time (Denson et al., 1969). They showed that there was not FVIIIa in the plasma of

the most people with hemophilia. Hemophilia B caused by genetic defects occur in the coagulation FIX; the FIX deficiency will inhibit the activation of FX by FVIIa through external coagulation pathway (Furie et al., 1994; Thompson, 1986). About half The patients who suffer from severe hemophilia A there is a large inversion in intron 22 of their FVIII mRNA (Figure 2) which it is repeated (Arruda et al., 1995; Deutz-Terlouw et al., 1995; Okamoto et al., 1995; Pieneman et al., 1995; Van de Water et al., 1995; Goodeve et al., Jenkins et al., 1994; 1994; Naylor et al., 1993; Naylor et al., 1992). Different alleles of the VNTR (di nucleotides) have observed in intron 13 of FVIII in people with hemophilia A (Kochhan et al., 1994).

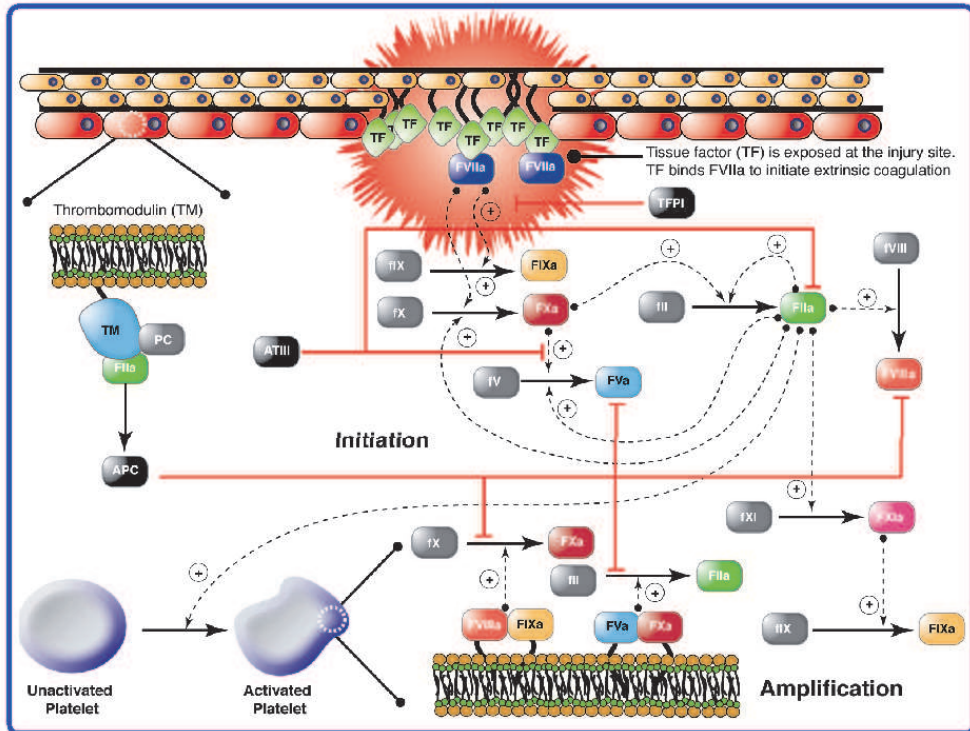


Fig. 1. External and internal pathways of blood coagulation process. (Reference <http://www.varnerlab.org/coagulation>) Read phonetically

Hemophilia A is occurring one for 5000-10000 birthday boys and hemophilia B one for 20,000 to 34,000 birthday boys (Dimitrios et al., 2009). The bleeding in joints of hemophilia patients the wound bleeding is longer continued (Petkova et al., 2004). The position of FVIII gene is Xq28 and of FIX is Xq27.1 location on distal long arm of chromosome X (Figure 3). The FVIII gene has 186 kb organized in 26 exons (about 9 kb) (Figure 4). There are detected some gene mutations on FVIII as insertion, deletions or point mutations which involved in the reduced or cut up in FVIII activation (Ramanarayana et al., 2011; Salviato et al., 2002; Cahill & Colvin, 1997; Arruda et al., 1995; Naylor et al., 1991; Higuchi et al., 1989; Youssofian et al., 1987; Gitschier et al., 1985). The FVIII organized in A, B and C domains

(Figure 5), which B domain is highly glycosylated and do not involve in FVIII activities (Eaton et al., 1986).

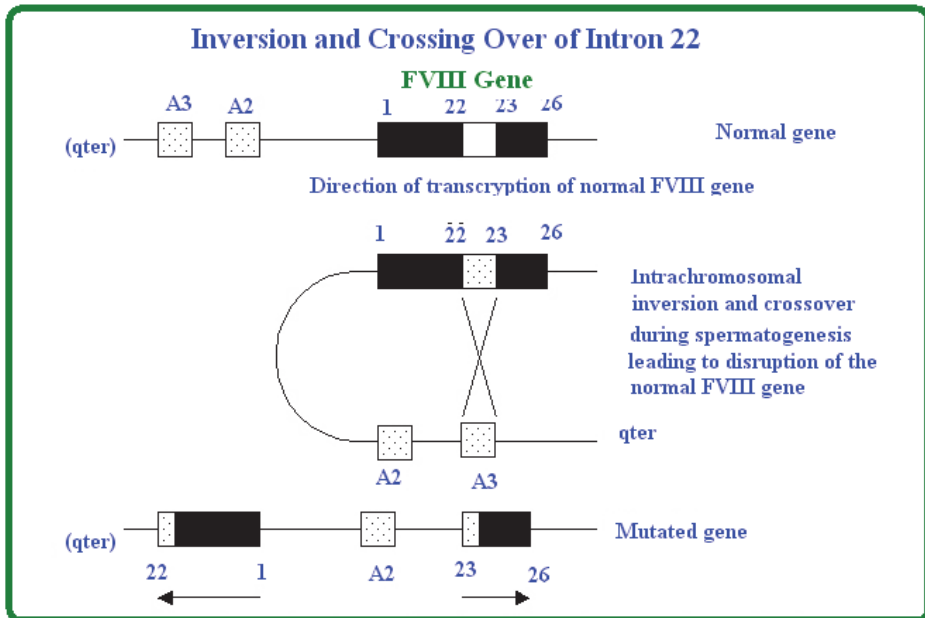


Fig. 2. Genetic mutation in intron 22 of FVIII (Schwartz et al., 2011)

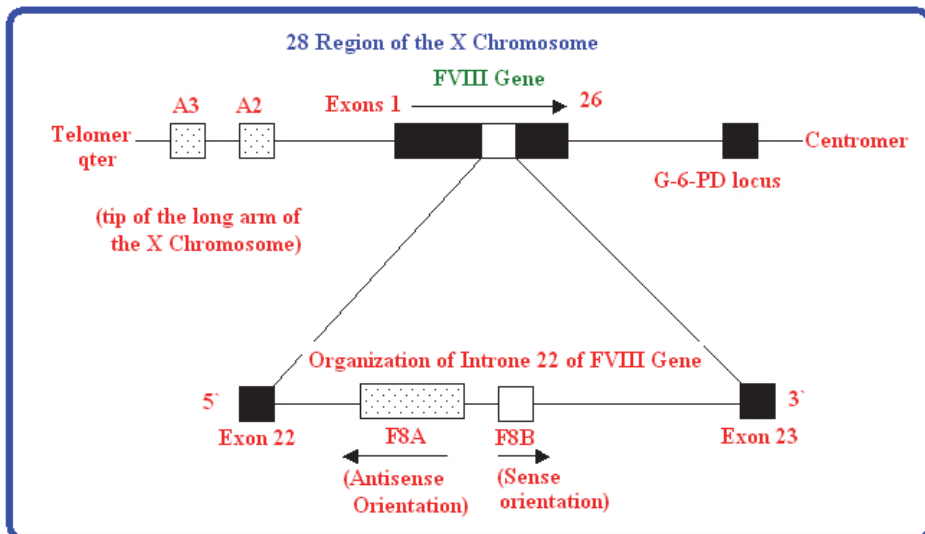


Fig. 3. The Map of FVIII on the long arm of chromosome X (Schwartz et al., 2011)

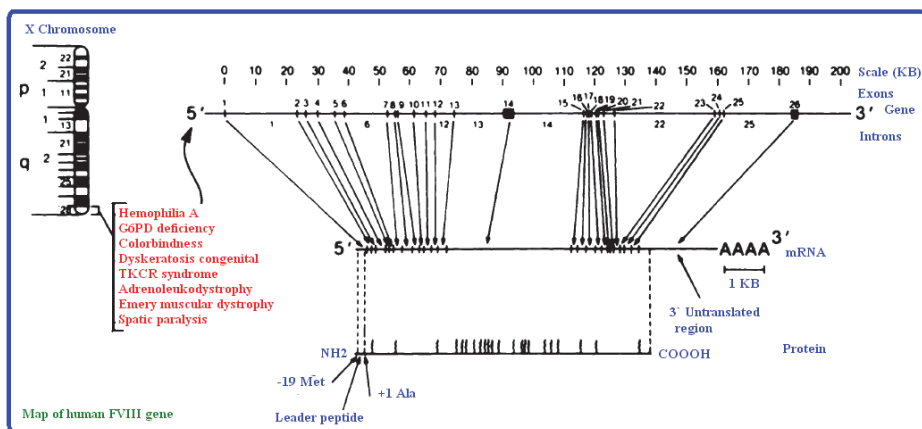


Fig. 4. Genetic map of FVIII (White & Shoemaker, 1989)

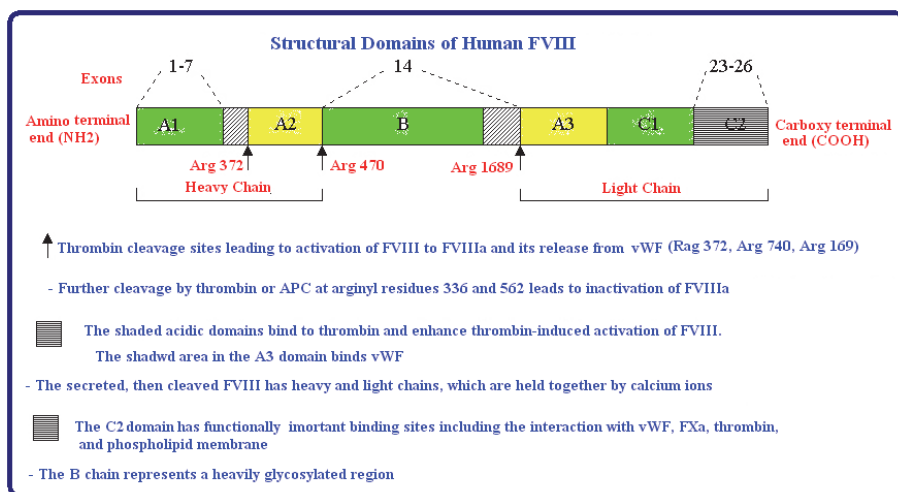


Fig. 5. The domains organization of FVIII (Schwartz et al., 2011)

#### 4. Diagnosis of hemophilia

Laboratory diagnosis of hemophilia is done based on activated partial thromboplastin time (aPTT), prothrombin time (PT), platelet count and bleeding time. There is an abnormality in the initial section of internal coagulation pathway at the prolonged aPTT and normal PT. The normal aPTT should not reject the FVIII deficiencies (hemophilia A), the aPTT is not enough sensitive too reduced amount of FVIII C. Prolonged PT alone, or PT and aPTT do not specify of hemophilia A, the liver diseases, overdose of warfarin or heparin and the distribution intravascular coagulation (DIC) can cause this coagulopathy. Thrombocytopenia alone cannot cause of hemophilia A. The nature and severity of bleeding is performed with cell blood counts (CBC) and differentiation also check for blood in the

stool and urine (Schwartz et al., 2011). Knights and Ingram in 1967 were used thromboplastin time assay for hemophilia A and B differentiation. Based on their test when alumina is added to normal plasma do not see the harm of FVIII, but FIX is deleted, remove the alumina from plasma FIX will re- back up. If thromboplastin time is increased in males with a history of prolonged bleeding, test is repeated after adding or removing alumina from the plasma. If thromboplastin time is shorter than the control, the patient is suffering from hemophilia A, but if thromboplastin time is shortened after the removal of the alumina, patient is suffering from hemophilia B (Knights & Ingram, 1967). Stites et al (1971) and Essien and Ingram (1967) were distinguished hemophilia A and B by FVIII inhibitory antibodies.

The test results in children and adults are different. Clotting Index and coagulability in hemophilia patients significantly lower than non-hemophilia one. In this test, coagulability of FVIII treated blood varies by the replaced FVIII type. rFVIII clotting index is lower than of derived plasma one (Goldenberg et al., 2006). Firshein and colleagues were diagnosed prenatal hemophilia A using radioimmunometric by fetal plasma and fetoscope by amniotic fluid at second trimester in pregnancy women (Firshein et al., 1979), other researchers were developed radioimmunometric method for measurements of FVIII antibody (Hoyer et al., 1985; Hellings et al., 1982; Ljung R, Holmberg, 1982). Antonarakis et al were analyzed FVIII gene for possibility detection of prenatal and hemophilia carrier through gene cloning method (Antonarakis et al., 1985), the problems and limitations of these methods were evaluated by other researchers (Graham et al., 1985). The PCR RFLP method was used for prenatal diagnosis and hemophilia A carrier for the first time in 1990s (Rudzki et al., 1996; Herrmann et al., 1988; Kogan et al., 1987;). Missense and nonsense point mutations in FVIII gene of hemophilia A patient, prenatal and carrier hemophilia A were detected using DGGE method (Gitschier, 1989). Ball and colleagues were used oral cells, urine and hair follicles samples to identify prenatal and carrier hemophilia A (Ball et al., 1990). Various polymorphism and mutations have been detected in FVIII gene of hemophilia A patients (Wacey et al., 1996; Antonarakis et al, 1995; Naylor et al., 1991; Baranov et al., 1990; Gécz et al., 1990; Jedlicka et al., 1990; Sadler et al., 1990; Surin et al., 1990; Wehnert et al., 1990a; Wehnert et al., 1990b). Establishment the PCR technique in diagnostic laboratories was a large change in DNA analysis of FVIII gene for detecting carriers and individuals with hemophilia A (Song et al., 1993; Feng, 1991; Wadelius et al., 1991; Wu, 1991). Detection of unknown mutations is performed by universal mutation detection system methods such as SSCP (Arruda et al., 1995; Pieneman et al., 1995; David et al., 1994). Hemophilia diagnosis with PGD method was used by Michaelides (2006) and colleagues For the first time in 2006; they were diagnosed two point mutations in FVIII gene of donor (IVF) blastomere. Acquired hemophilia due to FVIII autoantibody is a rare disease and occurs one in a million, yearly; its mortality is 20 percent (Shetty et al., 2010).

### 5.1 Treatment of hemophilia A

Hemophilia treatment doing by replacing the natural (Brackmann & Gormsen, 1977) or recombinant FVIII (Kaufman, 1991) via intravenous injection. The half life of transfused FVIII in normal individuals or patients with hemophilia is 8 to 12 hours (White & Shoemaker, 1989). Using recombinant serum proteins in the treatment of hemophilia began in 1990 (Liras, 2008), but Homate P / humate-P is a derived pasteurized human plasma which was approved in Germany in 1981 and used administered intravenous injection for

25 years to control bleeding in patients with hemophilia A and von Willebrand disease (Berntorp, 2009; Carter & Scott, 2007; Czapek et al, 1988). The main presentation following hemophilia treatment is creating inhibitory antibodies against the FVIII which observed 5% in patients with hemophilia B and 40 -20 percent in patients with severe hemophilia A (Hong & Stachnik, 2010; Kempton et al., 2010; Eckhardt et al., 2009; Ghosh & Shetty, 2009; von Auer et al., 2005; Sharathkumar et al., 2003; Scharrer, 1999; de Biasi et al., 1994). Complication inhibitory antibodies seem to produce with plasma-derived FVII severe than with recombinant one (Lusher, 2002; Lusher, 2000), also with FVIII (Qadura et al., 2009; Delignat et al., 2007; Goudemand et al., 2006; Yoshioka et al., 2003; Fijnvandraat et al., 1997). The B cell epitopes mutated, produced FVIII inhibitory antibody is reduced, and some one proposed that this phenomenon is safe vaccine for people with hemophilia (Parker et al, 2004). Antibody production against FVIII has been studied in hemophilia patients and indicates that most nonsense mutations and large deletions in FVIII gene and chromosomal recombination lead to produce FVIII inhibitory antibody (Schwaab et al., 1995). Treatment of hemophilia by FVIII overdose administration is effective for producer antibodies hemophilia patients (Scandella et al., 2000).

The OBI (BDD- rpFVIII) was introduced by Ipsen and Inspiration Biopharmaceuticals Inc Company and passed clinical trial phases 1 and 2. It shows porcine FVIII biochemical properties and procoagulant activity and less immunogenicity than plasma derived pFVIII (Toschi, 2010).

In 1960 Los Angeles Red Cross Blood Center was treated hemophilia patients using anti hemophilic globin (Rapaport et al., 1960). 1-Deamino-8-d-arginine vasopressin (DDAVP) (a FVII autologous) have been used instead of plasma derived factors for treatment of hemophilia A and B (Mannucci et al., 1977). Hultin and colleagues were used cyclophosphamide as immunosuppression drug for antibody producer hemophilia patients (Hultin et al., 1976). Lian et al. were treated hemophilia using cyclophosphamide, vincristine and prednisone (CVP) (Lian et al., 1989). Blatt et al were removed FVIII inhibitory antibody with prothrombin complex concentrates (PCC) (Blatt et al., 1977). Paleyanda and colleagues were transferred FVIII cDNA into pig lactate system; the pig was produced FVIII more than 10 times as normal plasma (Paleyanda et al., 1997). Specific thrombin anticoagulant Bivalirudin (Krolick, 2005) and monoclonal antibody Retoximab (Franchini, 2007; Wiestner et al., 2002) are also used for hemophilia treatment and patients with FVIII autoantibody, respectively. Idiotype vaccines will neutralize anti human FVIII antibody in hemophilia A patients (Lacroix-Desmazes et al., 2002).

Production and characterization of recombinant FVIII for the treatment of hemophilia was conducted in 1984 for the first time (Toole et al., 1984; Wood et al., 1984). Use of recombinant proteins to replace clotting factors and treatment of hemophilia opened a new arena in treatment of disease. Circulating blood factors are the first generation recombinant proteins and second generation drugs made by recombinant DNA and protein engineering technology cause changes in proteins for specific applications, like FVIIa (Levy & Levi, 2009; Pipe, 2008). FVII alone or in combination with its analogues have been used to reduce bleeding (Lauritzen et al., 2008a; Lauritzen et al., 2008b; Allen et al., 2007). Use of recombinant FVIIa for hemophilia patients with FVIII antibody (Oberfell et al., 2010; Margaritis, 2010; Margaritis et al., 2010), also people who have bleeding into the joint or prevent bleeding in surgery is economically efficient (Stephens et al., 2007).

## 5.2 Gene therapy of hemophilia A

For the first time in 1996, Connelly et al were administrated intravenous hemophilia dog by adenovirus containing hFVIII gene in which related protein was detectable in plasma for two weeks (Connelly et al., 1996a). Connelly and colleagues were injected adenovirus containing BDD- hFVIII gene through the tail artery into mice; hFVIII was detected in plasma by ELISA method (Connelly et al., 1999; Connelly et al. 1995). Dwarki and colleagues were transfected fibroblast by retrovirus containing BDD- FVIII gene, transfected fibroblasts were transferred into mice, human FVIII was observed in plasma after one week (Dwarki et al., 1995). Connelly et al were transferred adenovirus containing hFVIII gene into mice, hFVIII was stable in mouse for five months. (Connelly et al., 1996b). Ill et al were prepared suitable plasmid with necessary elements for FVIII expression in liver cells (Ill et al., 1997). Zhang et al were prepared a mini-adenovirus containing FVIII -equipped human albumin gene promoter for hemophilia A gene therapy. This structure was transferred to cell line; the hFVIII was consistently produced in mouse transferred ell line (Zhang et al., 1999). Gene therapy of hemophilia were done by liver cell transfected by adeno associated viruses or lentiviral viruses containing FVIII and FIX. Also use non-viral vector is also considered. Antibody production in gene therapy of hemophilia with FVIII and FIX can be depended on vector serotype (viral), expression rate (a long time, especially in the liver), the promoter used, method of gene delivery and transduced cell types (Margaritis et al., 2009; Ohmori et al., 2008; VandenDriessche et al., 2003; Chuah et al., 2001).

To overcome adenovirus toxicity phenomena, Andrews and colleagues were used adenovirus defected early genes E1, E2a, E3, E4 (four-generation defected vector), and transferred albumin promoter -controlled FVIII gene into mice, but was not suitable for use in vivo (Andrews et al., 2001). Chuah et al were inhibited bleeding in hemophilia A SCID mice using intravenous injection of adenovirus carrying BDD -FVIII gene (Chuah et al., 2003). Shi et al believed that platelet/ megakaryocyte is a target for hemophilia A gene therapy, they were transferred equipped specific platelet glycoprotein IIb promoter BDD-hFVIII to Domi cells, hFVIII was biosynthesised (Shi et al., 2003). Sarkar and colleagues were transferred AAV carrying hFVIII to deficient FVIII mice through portal, intravenous and spleen injections, they observed secreted hFVIII in transgenic animals but no in neonatal animal (Sarkar et al., 2003). Scallan et al were transferred FVIII gene into mice by AAV2 vector. The construct was equipped with liver cell specific promoter (Scallan et al., 2003). Kang and colleagues were used liver specific promoter equipped FIV retrovirus containing BDD- hFVIII gen for intravenous injection in hemophilia mice, hFVIII was secreted in mice for months without anti FVIII antibody production (Kang et al., 2005). Kumaran et al were treated hemophilia mice by cell therapy, a mixture of hepatocytes, liver endothelial sinusoids and liver kupffer cells was injected into mice peritoneum, FVIII was observed in mouse blood (Kumaran et al., 2005). Jiang et al were transferred FVIII in to hemophilia dog by AAV types 2, 5, 6 and 8, their report indicated that the performance of virus types 2 and 5 for gene therapy is more than viruses type 6 and 8 (Jiang et al., 2006). Sarkar et al believed that gene therapy duration in the dog with AAV8 containing FVIII have prolonged up to two years (Sarkar et al., 2006). Durable gene therapy based on AAV containing FVIII have also been reported by McCormack and colleagues (McCormack et al., 2006). Shi and colleagues findings suggest that targeted FVIII gene expression by platelets specific promoter is effective in the treatment of hemophilia A (Shi et al., 2006). Shi and colleagues were suggested that ectopic expression of FVIII in platelets with lentiviral virus via bone

marrow gene therapy is effective for human hemophilia treatment They were transferred lentiviral vector containing FVIII - Induced glycoprotein IIb platelet specific promoter into null mice bone marrow, the permanent secretion of FVIII in platelets lysates mice was observed. (Shi et al., 2007). Liu and colleagues were targeted rDNA of HL7702 hepatocytes by non-viral vector pHrneo containing FVIII gene for treatment of hemophilia (Liu et al., 2007). Doering has been transferred swine FVIII gene into mouse bone marrow mesenchymal cells for hemophilia treatment (Doering, 2008). Ishiwata and colleagues have been treated hemophilia mice using AAV8 vector containing canine BDD -FVIII gene (Ishiwata et al., 2009). Sabatino and colleagues report indicated that canine BDD- FVIII dogs is stable than human BDD- FVIII, it can be considered in the hemophilia treatment (Sabatino et al., 2009). Doering et al were transferred hFVIII - sFVIII hybrid in to hematopoietic stem cells with lentiviral vector; cells expressed FVIII more than 100-8 times of cells transfected with hFVIII only (Doering et al., 2009). Zatloukal and colleagues report suggested that expressed FVIII would be observed if the adenovirus containing FVIII -transfected fibroblasts or myoblasts move into liver or spleen cells, but do not observe in the transfected muscle cells (Zatloukal et al., 1995). Because there are no acceptable phenotypic correction of hemophilia mice, Liars was used induced pluri potent stem cell therapy technology, these cells suggested converting into all cells and can be transfected by recombinant AAV or lentiviral vectors (Liars, 2011). Studies conducted so far suggest that blood factors gene therapy with AAV in animal muscle (dogs and mice) was healthy for FIX, but did not sufficiently much success for FVIII (Haurigot et al., 2010; Wang & Herzog, 2005). It is believed that the clinical correction of hemophilia B depends on the dose of vector transfer into muscular hosts (mice and dogs) (Hagstrom et al., 2000; Kay et al., 2000).

### 5.3 Hemophilia gene therapy with factor VII

Activated FVII is used as recombinant factor VII (rFVII) can go around process dependent coagulation FVIII and FIX (Mackman et al., 2007), it helps blood coagulation through extrinsic pathway. It is an ideal choice in the treatment of patients with FVIII producing antibodies and hemophilia patients to be considered (Johannessen et al., 2000; Lauritzen et al., 2008a). FVII is used in patient's surgery with hemophilia A (Lauritzen et al., 2008a) also effective in term of homostatic process (Hedner et al., 2000; Kenet et al., 1999). The VIIa (Novoseven; rhFVIIa) has been achieved great success in treating patients with hemophilia. On the FVIII or FIX defects or presence of inhibitory antibodies, FVIIa - tissue factor complex will activate coagulation FX. FVIIa can activate coagulation cascade which cause clot formation and bleeding is inhibited (Hong & Stachnik, 2010; Levy & Levi, 2009). The main drug problems are short half-life (3-6 hours) and highly price (Ramanarayana et al., 2011; Puetz, 2010; Agersø et al., 2011; ), more than one full dose of medication, especially for homeostasis regulation during surgeries are required. Hence, research groups around the world are trying different methods of gene transfer to express stable FVIIa in cells without the need to drug re administration (Ramanarayana et al., 2011). After biosynthesis of rhFVII as zymogen, it will be cut by proteases and biologically active through purification process (Huntington, 2009). The produced protein will breaks down at Arg152 and Ile153 to FVIIa. It is proposed that FVII gene transfer eliminates short half-life of rFVII and FVIII inhibitory antibody production (Ramanarayana et al., 2011). Emamgholipour et al (2009) and Margaritis (2010) established furin enzyme digestion site between Arg152 and Ile153 to generate FVIIa from FVII zymogene break down inside the targeted cell during FVII gene therapy strategy (Figure 6). Margaritis and colleagues were successfully corrected canine hemophilia B with this method



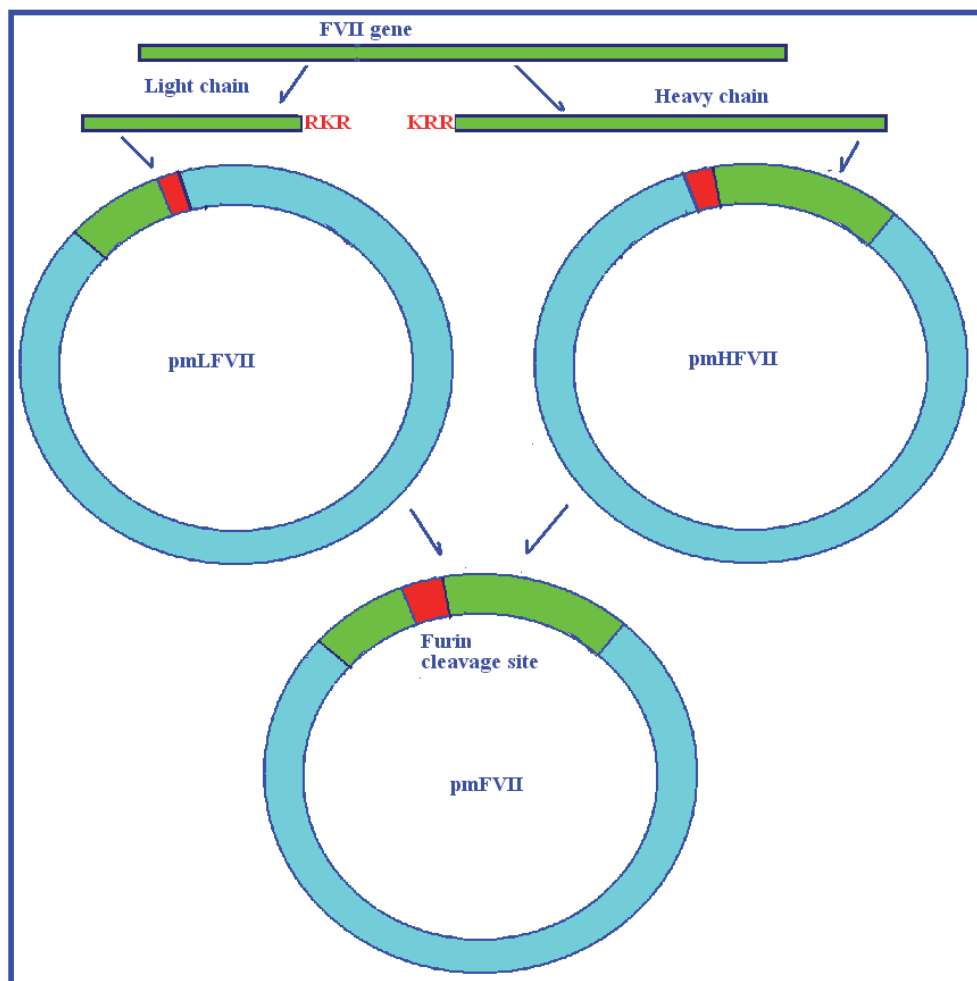


Fig. 6. Mutagenesis method to create furin digestion site on the FVII protein (Proposed based on Emamgholipour et al., 2009; Margaritis, 2010)

(Margaritis et al., 2004). Margaritis and colleagues were injected the mice through gene therapy by AAV contained FVII gene, FVII was produced in host cells (Margaritis et al., 2004). Miller et al were injected mice muscle myoblasts with plasmids coding FVIII and FVII cDNA (muscle specific elements and poly A were placed on both sides of genes); they were observed FVII and related antibody after 4-5 days. They believed that post translation modification process was occurred in the muscle cells (Miller et al., 1995). Tomokiyo and colleagues showed that the composition of plasma FVIIa and FXa in the treatment of monkeys hemophilia B more effective than FVIIa alone (Tomokiyo et al., 2003). Ohmori et al have been used ectopic expression FVIIa in platelets to hemophilia A treatment, they were transferred SIV containing platelet glycoprotein Ib alpha specific promoter into bone marrow cells, was lead to FVII expression on the platelets surface. This construct was

corrected mouse hemophilia A phenotype (Ohmori et al., 2008). Margaritis have been treated canine hemophilia by AAV containing FVII through the portal vein (Margaritis et al., 2009). To overcome the repeated injection problem, Obergfell and colleagues were studied hemophilia treatment and suggested permanent expression of FVII in canine hemophilia model through gene therapy method (Obergfell et al., 2010).

## 6. Conclusion

There are some reported of FVIII and FIX gene transfer by viral vectors in animal models, but no evidence so far reported successful treatment of human hemophilia gene therapy by this method. Researchers have used FVII to overcome antibody production in treatment of FVIII deficiency. Despite several reports of curing hemophilia A with FVII in animals, there is no yet successful reported in human.

Methods proposed for the future: reviewing the history of hemophilia gene therapy by viral vectors, identified several reasons that cannot be sure of the viruses used for gene transfer:

1) Application of viruses is associated with inappropriate chromosomal insertion and makes the undesirable point mutations (Nakai et al., 2003; Miller et al., 2002). 2) Viruses are carcinogens (Check, 2003). 3) The viruses will cause the host immune response (Lefesvre et al., 2003) which is temporarily being transgene. 4) Because viruses genome are great than non-viral vectors the sequences of the viruses cannot be controlled by reseaechers. 5) Preparation of this vector requires a lot of time and money. Although non-viral gene transfer is less efficient than virus vector but have been told no above disadvantages and their use for gene transfer in human can be safer than viruses. Vectors are suggested to be prepared as non-viral vector for targeting the rDNA locus of human genome by homologous recombination method, and as ex vivo gene transfer in humans to be done with them.

## 7. Acknowledgment

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## 8. References

- Agersø H, Brophy DF, Pelzer H, Martin J, Carr M, Hedner U, Ezban M (2011). Recombinant human factor VIIa (rFVIIa) cleared principally by antithrombin following intravenous administration in hemophilia patients. *J Thromb Haemost*; 9(2): 333-338. ISSN: 1538-7933
- Allen GA, Persson E, Campbell RA, Ezban M, Hedner U, Wolberg AS (2007). A variant of recombinant factor VIIa with enhanced procoagulant and antifibrinolytic activities in an in vitro model of hemophilia. *Arterioscler Thromb Vasc Biol* ;27(3):683-689. ISSN 1049-8834
- Andrews JL, Kadan MJ, Gorziglia MI, Kaleko M, Connelly S (2001). Generation and characterization of E1/E2a/E3/E4-deficient adenoviral vectors encoding human factor VIII. *Mol Ther*; 3 (3):329- 336. ISSN: 1525-0016

- Antonarakis SE, Waber PG, Kittur SD, Patel AS, Kazazian HH Jr, Mellis MA, Counts RB, Stamatoyannopoulos G, Bowie EJ, Fass DN, et al. (1985). Hemophilia A. Detection of molecular defects and of carriers by DNA analysis. *N Engl J Med*; 313(14): 842-848. ISSN 0028-4793
- Antonarakis SE, Kazazian HH, Tuddenham EG (1995). Molecular etiology of factor VIII deficiency in hemophilia A. *Hum Mutat*; 5(1): 1-22. ISSN: 1059-7794
- Arruda VR, Pieneman WC, Reitsma PH, Deutz-Terlouw PP, Annichino-Bizzacchi JM, Briët E, Costa FF (1995). Eleven novel mutations in the factor VIII gene from Brazilian hemophilia A patients. *Blood*; 86(8): 3015-3020. ISSN 0006-4971
- Ball J, Warnock LJ, Preston FE (1990). Rapid assessment of haemophilia A carrier state by non-invasive techniques using the polymerase chain reaction. *J Clin Pathol*; 43(6): 505-507. ISSN:0021-9746
- Baranov VS, Aseev MV, Gorbunova VN, Ivashchenko TE, Mikhaïlov AV, Gornostaeva NI, Surin VL (1990). Use of molecular and genetic approaches in prenatal diagnosis and prevention of hemophilia A and Duchenne muscular dystrophy. *Akush Ginekol (Mosk)*; (11): 26-28. ISSN: 0002-3906
- Bartlett A, Dormandy KM, Hawkey CM, Stableforth P, Voller A (1976). Factor-VIII-related antigen: measurement by enzyme immunoassay. *Br Med J*; 1(6016): 994-996. ISSN: 09598138
- Berntorp E.(2009). Haemate P/Humate-P: a systematic review. *Thromb Res*;124 Suppl 1: S11-4. ISSN:0049-3848
- Blatt PM, White GC 2nd, McMillan CW, Roberts HR (1977). Treatment of anti-factor VIII antibodies. *Thromb Haemost*; 38(2): 514-523. ISSN:0340-6245
- Brackmann HH, Gormsen J (1977): Massive factor-VIII infusion in haemophilic with factor-VIII inhibitor, high responder. *Lancet*; 2: 933. ISSN: 0140-6736
- Cahill MR, Colvin BT (1997). Haemophilia. *Postgrad Med J*; 73: 201-206. ISSN: 0022-3859
- Carter NJ, Scott LJ (2007). Human Plasma von Willebrand Factor/Factor VIII Complex (Haemate(R) P/Humate-P(R)): In von Willebrand Disease and Haemophilia A. *Drugs*; 67 (10): 1513-1519. ISSN 0012-6667
- Check E (2003). Cancer risk prompts US to curb gene therapy. *Nature*. Mar 6;422(6927):7. ISSN : 0028-0836
- Chuah MK, Collen D, VandenDriessche T (2001). Gene therapy for hemophilia. *J Gene Med*; 3(1): 3-20. ISSN: 1099-498X
- Chuah MK, Schiedner G, Thorrez L, Brown B, Johnston M, Gillijns V, Hertel S, Van Rooijen N, Lillicrap D, Collen D, VandenDriessche T, Kochanek S (2003). Therapeutic factor VIII levels and negligible toxicity in mouse and dog models of hemophilia A following gene therapy with high-capacity adenoviral vectors. *Blood*;101(5):1734-1743. ISSN: 0006-4971
- Connelly S, Mount J, Mauser A, Gardner JM, Kaleko M, McClelland A, Lothrop CD Jr (1996a). Complete short-term correction of canine hemophilia A by in vivo gene therapy. *Blood*; 88(10): 3846-3853. ISSN: 0006-4971
- Connelly S, Smith TA, Dhir G, Gardner JM, Mehaffey MG, Zaret KS, McClelland A, Kaleko M (1995). In vivo gene delivery and expression of physiological levels of functional human factor VIII in mice. *Hum Gene Ther*; 6(2): 185-193. ISSN: 1043-0342 ISSN: 1043-0342

- Connelly S, Gardner JM, Lyons RM, McClelland A, Kaleko M (1996b). Sustained expression of therapeutic levels of human factor VIII in mice. *Blood* ; 87(11): 4671-4677. ISSN: 0006-4971
- Connelly S, Andrews JL, Gallo-Penn AM, Tagliavacca L, Kaufman RJ, Kaleko M (1999). Evaluation of an adenoviral vector encoding full-length human factor VIII in hemophiliac mice. *Thromb Haemost*; 81(2): 234-239. ISSN:0340-6245
- Czapek EE, Gadarowski JJ Jr, Ontiveros JD, Pedraza JL (1988). Humate-P for treatment of von Willebrand disease [letter]. *Blood*; 72: 1100. ISSN: 0006-4971
- David D, Moreira I, Lalloz MR, Rosa HA, Schwaab R, Morais S, Diniz MJ, de Deus G, Campos M, Lavinha J, et al (1994). Analysis of the essential sequences of the factor VIII gene in twelve haemophilia A patients by single-stranded conformation polymorphism. *Blood Coagul Fibrinolysis*; 5(2): 257-264. ISSN: 1473-5733
- de Biasi R, Rocino A, Papa ML, Salerno E, Mastrullo L, De Blasi D (1994). Incidence of factor VIII inhibitor development in hemophilia A patients treated with less pure plasma derived concentrates. *Thromb Haemost*; 71(5): 544-7. ISSN:0340-6245
- Delignat S, Dasgupta S, André S, Navarrete AM, Kaveri SV, Bayry J, André MH, Chtourou S, Tellier Z, Lacroix-Desmazes S (2007). Comparison of the immunogenicity of different therapeutic preparations of human factor VIII in the murine model of hemophilia A. *Haematologica* ;92(10): 1423-1426. ISSN is 0390-6078
- Denson KW, Biggs R, Haddon ME, Borrett R, Cobb K (1969). Two types of haemophilia (A+ and A-): a study of 48 cases. *Br J Haematol*; 17(2): 163-171. ISSN 0007-1048
- Deutz-Terlouw PP, Losekoot M, Olmer R, Pieneman WC, de Vries-v d Weerd S, Briët E, Bakker E (1995). Inversion in the factor VIII gene: improvement of carrier detection and prenatal diagnosis in Dutch haemophilia A families. *J Med Genet*; 32(4): 296-300. ISSN: 0148-7299
- Dimitrios P Agaliotis, Robert A Zaiden, Saduman Ozturk (2009). Hemophilia, Overview. <http://emedicine.medscape.com/article/210104-overview>
- Doering CB, Denning G, Dooriss K, Gangadharan B, Johnston JM, Kerstann KW, McCarty DA, Spencer HT (2009). Directed engineering of a high-expression chimeric transgene as a strategy for gene therapy of hemophilia A. *Mol Ther*; 17(7): 1145-1154. ISSN: 1525-0016
- Doering CB (2008). Retroviral modification of mesenchymal stem cells for gene therapy of hemophilia. *Methods Mol Biol*; 433: 203-212. ISSN: 1064-3745
- Dwarki VJ, Belloni P, Nijjar T, Smith J, Couto L, Rabier M, Clift S, Berns A, Cohen LK. (1995). Gene therapy for hemophilia A: production of therapeutic levels of human factor VIII in vivo in mice. *Proc Natl Acad Sci U S A*; 92(4): 1023-1027. 0027-8424. ISSN
- Eaton DL, Wood WI, Eaton D, Hass PE, Hollingshead P, Wion K, Mather J, Lawn RM, Vehar GA & Gorman C (1986). Construction and characterization of an active factor VIII variant lacking the central one-third of the molecule. *Biochemistry*; 25(26): 8343-8327. ISSN:1742-464X
- Eckhardt CL, Menke LA, van Ommen CH, van der Lee JH, Geskus RB, Kamphuisen PW, Peters M, Fijnvandraat K (2009). Intensive peri-operative use of factor VIII and the Arg593-->Cys mutation are risk factors for inhibitor development in mild/moderate hemophilia A. *J Thromb Haemost*; 7(6): 930-937. ISSN: 1538-7933
- Ellison N (1977). Diagnostic and management of Bleeding disorders. *Anesthesiology*; 47(2): 171-180. ISSN: 0003-3022

- Emamgholipour S, Bandehpour M, Shabani P, Maghen L, Yaghmaee B, Kazemi B (2009). Mutagenesis in sequence encoding of human factor VII for gene therapy of hemophilia. *DARU*; 17(4): 294-298. ISSN: 1560-8115
- Essien EM, Ingram GI (1967). Diagnosis of haemophilia: use of an artificial factor-VIII-deficient human plasma system. *J Clin Pathol*; 20(4): 620-623. ISSN:0021-9746
- Feng J (1991). Gene diagnosis of hemophilia A by PCR. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao*; 13(5): 384-388. ISSN: 0376-2491
- Franchini M (2007). Rituximab in the treatment of adult acquired hemophilia A: a systematic review. *Crit Rev Oncol Hematol*; 63(1):47-52. ISSN: 1040-8428
- Fijnvandraat K, Turenhout EA, van den Brink EN, ten Cate JW, van Mourik JA, Peters M, Voorberg J (1997). The missense mutation Arg593 --> Cys is related to antibody formation in a patient with mild hemophilia A. *Blood*; 89(12): 4371-2377. ISSN: 0006-4971
- Firshein SI, Hoyer LW, Lazarchick J, Forget BG, Hobbins JC, Clyne LP, Pitlick FA, Muir WA, Merkatz IR, Mahoney MJ (1979). Prenatal diagnosis of classic hemophilia. *N Engl J Med*; 300(17): 937-941. ISSN: 0028-4793
- Forbes CD, King J, Prentice CR, McNicol GP (1972). Serum enzyme changes after intramuscular bleeding in patients with haemophilia and Christmas disease. *J Clin Pathol*; 25(12): 1034-1037. ISSN:0021-9746
- Furie B, Limentani SA, Rosenfield CG (1994). A Practical Guide to the Evaluation and Treatment of Hemophilia. *Blood*; 84 (1 ): 3-9. ISSN: 0006-4971
- Géczy J, Kádasi L, Poláková H, Ferák V (1990). Use of DNA analysis in the diagnosis and prevention of hemophilia A. *Bratisl Lek Listy*; 91(3): 219-224. ISSN: 0006-9248
- Ghosh K, Shetty S (2009). Immune response to FVIII in hemophilia A: an overview of risk factors. *Clin Rev Allergy Immunol*; 37(2): 58-66. ISSN: 1080-0549
- Gitschier J (1989). Molecular genetics of hemophilia A. *Schweiz Med Wochenschr*; 119(39): 1329-1331. ISSN, 0036-7672
- Gitschier J, Wood WI, Tuddenham EG, Shuman MA, Goralka TM, Chen EY, Lawn RM (1985). Detection and sequence of mutations in the factor VIII gene of haemophiliacs. *Nature*; 315(6018):427-430. ISSN : 0028-0836
- Goldenberg, NA, Hathaway WE, Jacobson L, et al. (2006). "Influence of Factor VIII on Overall Coagulability and Fibrinolytic Potential of Haemophilic Plasma as Measured by Global Assay: Monitoring in Hemophilia A." *Haemophilia*.; 12: 605-614. ISSN 1351-8216
- Goodeve A, Preston FE, Peake IR (1994). Factor VIII gene rearrangements in patients with severe haemophilia A. *Lancet*; 343: 329-330. ISSN: 0140-6736
- Goudemand J, Rothschild C, Demiguel V, Vinciguerrat C, Lambert T, Chambost H, Borel-Derlon A, Claeysens S, Laurian Y, Calvez T (2006). Influence of the type of factor VIII concentrate on the incidence of factor VIII inhibitors in previously untreated patients with severe hemophilia A. *Blood*; 107(1): 46-51. ISSN: 0006-4971
- Graham JB, Green PP, McGraw RA, Davis LM (1985). Application of molecular genetics to prenatal diagnosis and carrier detection in the hemophilias: some limitations. *Blood*; 66(4): 759-764. ISSN: 0006-4971
- Hagstrom JN, Couto LB, Scallan C, Burton M, McClelland ML, Fields PA, Arruda VR, Herzog RW, High KA (2000). Improved muscle-derived expression of human coagulation factor IX from a skeletal actin/CMV hybrid enhancer/promoter. *Blood*; 95(8): 2536-42. ISSN: 0006-4971

- Haurigot V, Mingozzi F, Buchlis G, Hui D, Chen Y, Tschakarjan EB, Arruda V, Radu A, Franck HG, Wright JF, Zhou S, Stedman HH, Bellinger DA, Nichols5 TC & High KA (2010). Safety of AAV Factor IX Peripheral Transvenular Gene Delivery to Muscle in Hemophilia B Dogs. *Mol Ther*; 18 (7): 1318-1329. ISSN: 1525-0016
- Hedner U (2000). NovoSeven as a universal haemostatic agent. *Blood Coagul Fibrinolysis*; 11 Suppl 1: S107-111. ISSN: 0957-5235
- Hellings JA, van Leeuwen FR, Over J, van Mourik JA (1982). 1712. Immunoradiometric assay of VIII:Cag, a potential tool to detect human anti-VIII:C antibodies. *Thromb Res* 15; 26(4):297-302. ISSN:0049-3848
- Herrmann FH, Kruse T, Wehnert M, Vogel G, Wulff K (1988). First experiences in application of RFLP analysis for carrier detection in preparation of prenatal diagnosis of hemophilia A in the GDR. *Folia Haematol Int Mag Klin Morphol Blutforsch*; 115(4): 489-93. ISSN : 1087-0156
- Higuchi M, Kochhan L, Schwaab R, Egli H, Brackmann HH, Horst J, Olek K (1989). Molecular defects in hemophilia A: identification and characterization of mutations in the factor VIII gene and family analysis. *Blood*; 74(3): 1045-1051. ISSN: 0006-4971
- History of hemophilia. <http://www.hemophilia-information.com/history-of-hemophilia.html>
- History of Hemophilia Disease. <http://www.buzzle.com/articles/history-of-hemophilia-disease.html>
- Hong I, Stachnik J (2010). Unlabeled uses of factor VIIa (recombinant) in pediatric patients. *Am J Health Syst Pharm*; 67(22):1909-19. ISSN: 0815-9319
- Hoyer LW, Breckenridge RT (1968). Immunologic studies of antihemophilic factor (AHF, factor VIII): cross-reacting material in a genetic variant of hemophilia A. *Blood*; 32(6): 962-971. ISSN: 0006-4971
- Hoyer LW, Carta CA, Golbus MS, Hobbins JC, Mahoney MJ (1985). Prenatal diagnosis of classic hemophilia (hemophilia A) by immunoradiometric assays. *Blood*; 65(6):1312-1317. ISSN: 0006-4971
- Hultin MB, Shapiro SS, Bowman HS, Gill FM, Andrews AT, Martinez J, Eyster EM, Sherwood WC (1976). Immunosuppressive therapy of Factor VIII inhibitors. *Blood*; 48(1): 95-108. ISSN: 0006-4971
- Huntington JA. (2009). Slow thrombin is zymogen-like. *J Thromb Haemost*; Suppl 1: 159-64. ISSN: 1538-7933
- Ill CR, Yang CQ, Bidlingmaier SM, Gonzales JN, Burns DS, Bartholomew RM, Scuderi P (1997). Cloning of the human factor VIII complementary DNA expression plasmid for gene therapy of hemophilia A. *Blood Coagul Fibrinolysis*; Suppl 2:S23-30. ISSN: 0957-5235
- Ishiwata A, Mimuro J, Mizukami H, Kashiwakura Y, Takano K, Ohmori T, Madoiwa S, Ozawa K, Sakata Y (2009). Liver-restricted expression of the canine factor VIII gene facilitates prevention of inhibitor formation in factor VIII-deficient mice. *J Gene Med* ; 11(11): 1020-1029. ISSN: 1099-498X
- Jedlicka P, Greer S, Millar DS, Grundy CB, Jenkins E, Mitchell M, Mibashan RS, Kakkar VV, Cooper DN (1990). Improved carrier detection of haemophilia A using novel RFLPs at the DXS115 (767) locus. *Hum Gene*; 85(3): 315-318. ISSN: 0340-6717
- Jenkins PV, Collins PW, Goldman E, McCraw A, Riddell A, Lee CA, Pasi KJ (1994). Analysis of intron 22 inversions of the factor VIII gene in severe hemophilia A: implications for genetic counseling. *Blood*; 84(7): 2197-2201. ISSN: 0006-4971

- Jiang H, Lillicrap D, Patarroyo-White S, Liu T, Qian X, Scallan CD, Powell S, Keller T, McMurray M, Labelle A, Nagy D, Vargas JA, Zhou S, Couto LB, Pierce GF (2006). Multiyear therapeutic benefit of AAV serotypes 2, 6, and 8 delivering factor VIII to hemophilia A mice and dogs. *Blood*; 108(1): 107-115. ISSN: 0006-4971
- Johannessen M, Andreassen RB, Nordfang O (2000). Decline of factor VIII and factor IX inhibitors during long-term treatment with NovoSeven. *Blood Coagul Fibrinolysis*; 11(3): 239-242. ISSN: 0957-5235
- Kang Y, Xie L, Tran DT, Stein CS, Hickey M, Davidson BL, McCray PB Jr (2005). Persistent expression of factor VIII in vivo following nonprimate lentiviral gene transfer. *Blood*; 106(5): 1552-1558. ISSN: 0006-4971
- Kaufman RJ (1991). Developing rDNA products for treatment of hemophilia A. *Trends Biotechnol*; 9(10): 353-359. ISSN: 0167-7799
- Kay MA, Manno CS, Ragni MV, Larson PJ, Couto LB, McClelland A, Glader B, Chew AJ, Tai SJ, Herzog RW, Arruda V, Johnson F, Scallan C, Skarsgard E, Flake AW, High KA (2000). Evidence for gene transfer and expression of factor IX in haemophilia B patients treated with an AAV vector. *Nat Genet*; 24(3): 257-261. ISSN:1061-4036
- Kempton CL, Soucie JM, Miller CH, Hooper C, Escobar MA, Cohen AJ, Key NS, Thompson AR, Abshire TC (2010). Non-severe hemophilia A the risk of inhibitor after intensive factor treatment is greater in older patients: a case-control study. *J Thromb Haemost*; 8(10): 2224-2231. ISSN: 1538-7933
- Kenet G, Walden R, Eldad A, Martinowitz U (1999). Treatment of traumatic bleeding with recombinant factor VIIa. *Lancet*; 354(9193): 1879. ISSN: 0140-6736
- Knights SF, Ingram GIC (1967). Partial thromboplastin time test with kaolin: diagnosis of haemophilia and Christmas disease without natural reference plasmas. *J clin. Path*; 20: 616-619. ISSN:0021-9746
- Kochhan L, Lalloz MR, Oldenburg J, McVey JH, Olek K, Brackmann HH, Tuddenham EG, Schwaab R (1994). Haemophilia A diagnosis by automated fluorescent DNA detection of ten factor VIII intron 13 dinucleotide repeat alleles. *Blood Coagul Fibrinolysis*; 5(4): 497-501. ISSN: 0957-5235
- Kogan SC, Doherty M, Gitschier J (1987). An improved method for prenatal diagnosis of genetic diseases by analysis of amplified DNA sequences. Application to hemophilia A. *N Engl J Med*; 317(16):985-990. ISSN: 0028-4793
- Krolick MA (2005). Successful percutaneous coronary intervention in a patient with severe haemophilia A using bivalirudin as the sole procedural anticoagulant. *Haemophilia*; 11(4): 415-417. ISSN 1351-8216
- Kumaran V, Bente D, Follenzi A, Joseph B, Sarkar R, Gupta S (2005). Transplantation of endothelial cells corrects the phenotype in hemophilia A mice. *J Thromb Haemost*; 3(9): 2022-2031. ISSN: 1538-7933
- Lacroix-Desmazes S, Bayry J, Misra N, Kaveri SV, Kazatchkine MD (2002). The concept of idiotypic vaccination against factor VIII inhibitors in haemophilia A. *Haemophilia*;8 Suppl 2:55-59. ISSN 1351-8216
- Lauritzen B, Tranholm M, Ezban M (2008a). rFVIIa and a new enhanced rFVIIa-analogue, NN1731, reduce bleeding in clopidogrel-treated and in thrombocytopenic rats. *J Thromb Haems*; 7(4): 651- 657. ISSN: 1538-7933
- Lauritzen B, Hedner U, Johansen PB, Tranholm M, Ezban M (2008b). Recombinant human factor VIIa and a factor VIIa-analogue reduces heparin and low molecular weight

- heparin (LMWH)-induced bleeding in rats. *J Thromb Haemost*; 6(5): 804-811. ISSN: 1538-7933
- Lefesvre P, Attema J, Lemckert A, Havenga M, van Bekkum D (2003). Genetic heterogeneity in response to adenovirus gene therapy. *BMC Mol Biol*. 5; 4: 4. ISSN 1471-2199
- Levy JH, Levi M (2009). A modified recombinant factor VIIa: can we make it work Harder, Better, Faster, Stronger?. *J Thromb Haemost*; 7(9): 1514- 1516. ISSN: 1538-7933
- Lian EC, Larcada AF, Chiu AY (1989). Combination immunosuppressive therapy after factor VIII infusion for acquired factor VIII inhibitor. *Ann Intern Med*; 110(10): 774-778. ISSN: 0003-4819
- Liars A (2011). Induced human pluripotent stem cells and advanced therapies Future perspectives for the treatment of haemophilia? *Thromb Res*; 2011 Mar 9. [Epub ahead of print] ISSN:0049-3848
- Liras A (2008). Recombinant proteins in therapeutics: haemophilia treatment as an example. *Int Arch Med*, 1:4 ISSN 1755-7682
- Liu X, Liu M, Xue Z, Pan Q, Wu L, Long Z, Xia K, Liang D, Xia J (2007). Non-viral ex vivo transduction of human hepatocyte cells to express factor VIII using a human ribosomal DNA-targeting vector. *J Thromb Haemost*; 5(2): 347-351. ISSN: 1538-7933
- Ljung R, Holmberg L (1982). Immunoradiometric assay of inhibitors of antihemophilic factor A. *Acta Paediatr Scand*; 71(6): 1019-1023. ISSN: 0001-656X
- Lusher JM (2000). Hemophilia treatment. Factor VIII inhibitors with recombinant products: prospective clinical trials. *Haematologica*; 85(10 Suppl): 2-5; discussion 5-6. ISSN: 0390-6078
- Lusher JM (2002). First and second generation recombinant factor VIII concentrates in previously untreated patients: recovery, safety, efficacy, and inhibitor development. *Semin Thromb Hemost*; 28(3): 273-276. ISSN: 0094-6176
- Mackman N, Tilley RE, Key NS (2007). Role of the extrinsic pathway of blood coagulation in hemostasis and thrombosis. *Arterioscler Thromb Vasc Biol*; 27(8): 1687-1693. ISSN 1049-8834
- Mannucci PM, Ruggeri ZM, Pareti FI, Capitanio A (1977). 1-Deamino-8-d-arginine vasopressin: a new pharmacological approach to the management of haemophilia and von Willebrand's diseases. *Lancet*; 1(8017): 869-872. ISSN: 0140-6736
- Margaritis P, Arruda VR, Aljamali M, Camire RM, Schlachterman A, High KA (2004). Novel therapeutic approach for hemophilia using gene delivery of an engineered secreted activated Factor VII. *Clin Invest*; 113(7): 1025-1031. ISSN: 0021-9738
- Margaritis P (2010). Long-term expression of canine FVIIa in hemophilic dogs. *Thromb Res*; 125 Suppl 1:S60-62. ISSN:0049-3848
- Margaritis P, Roy E, Aljamali MN, Downey HD, Giger U, Zhou S, Merricks E, Dillow A, Ezban M, Nichols TC, High KA (2009). Successful treatment of canine hemophilia by continuous expression of canine FVIIa. *Blood*; 113(16): 3682-3689. ISSN 0006-4971
- McCormack WM Jr, Seiler MP, Bertin TK, Ubhayakar K, Palmer DJ, Ng P, Nichols TC, Lee B (2006). Helper-dependent adenoviral gene therapy mediates long-term correction of the clotting defect in the canine hemophilia A model. *J Thromb Haemost*; 4(6): 1218-1225. ISSN: 1538-7933
- Michaelides K, Tuddenham EG, Turner C, Lavender B, Lavery SA (2006). Live birth following the first mutation specific pre-implantation genetic diagnosis for haemophilia A. *Thromb Haemost*; 95(2): 373-379. ISSN: 1538-7933



- Miller G, Steinbrecher RA, Murdock PJ, Tuddenham EG, Lee CA, Pasi KJ, Goldspink G (1995). Expression of factor VII by muscle cells in vitro and in vivo following direct gene transfer: modelling gene therapy for haemophilia. *Gene Ther*; 2(10): 736-742. ISSN : 0969-7128
- Miller DG, Rutledge EA, Russell DW (2002). Chromosomal effects of adeno-associated virus vector integration. *Nat Genet*; 30(2): 147-8. ISSN: 1061-4036
- Nakai H, Montini E, Fuess S, Storm TA, Grompe M, Kay MA (2003). AAV serotype 2 vectors preferentially integrate into active genes in mice. *Nat Genet*; 34(3): 297-302. ISSN: 1061-4036
- Naylor J, Brlinke A, Hassock S, M.Green P, Giannelli F (1993). Characteristic mRNA abnormality found in half the patients with severe haemophilia A is due to large DNA inversions. *Human Molecular Genetics*; 2(11): 1773-1778. ISSN: 0964-6906
- Naylor JA, Green PM, Montandon AJ, Rizza CR, Giannelli F (1991). Detection of three novel mutations in two haemophilia A patients by rapid screening of whole essential region of factor VIII gene. *Lancet*; 337(8742): 635-639. ISSN: 0140-6736
- Naylor JA, Green PM, Rizza CR, Giannelli F (1992). Factor VIII gene explains all cases of haemophilia A. *Lancet*; 340(8827):1066-1067. ISSN: 0140-6736
- Nowotny C, Niessner H, Thaler E, Lechner K (1976). Sonography: a method for localization of hematomas in hemophiliacs. *Haemostasis*; 5(3): 129-135. ISSN:0301-0147
- Obergfell A, Nichols T, Ezban M (2010). Animal models of FVIIa gene expression: their role in the future development of haemophilia treatment. *Haemophilia*; 16 Suppl 2: 24-27. ISSN 1351-8216
- Ohmori T, Ishiwata A, Kashiwakura Y, Madoiwa S, Mitomo K, Suzuki H, Hasegawa M, Mimuro J, Sakata Y (2008). Phenotypic correction of hemophilia A by ectopic expression of activated factor VII in platelets. *Mol Ther*; 16(8): 1359-1365. ISSN: 1525-0016
- Okamoto Y, Kojima T, Katsumi A, Yamazaki T, Hamaguchi M, Nishida M, Suzumori K, Saito H (1995). Carrier detection and prenatal diagnosis for hemophilia A using the inversion analysis of the factor VIII gene. *Rinsho Ketsueki*; 36(11): 1252-1256. ISSN: 0485-1439
- Paleyanda RK, Velandar WH, Lee TK, Scandella DH, Gwazdauskas FC, Knight JW, Hoyer LW, Drohan WN, Lubon H (1997). Transgenic pigs produce functional human factor VIII in milk. *Nat Biotechnol*;15(10):971-997. ISSN : 1087-0156
- Parker ET, Healey JF, Barrow RT, Craddock HN, Lollar P (2004). Reduction of the inhibitory antibody response to human factor VIII in hemophilia A mice by mutagenesis of the A2 domain B-cell epitope. *Blood*; 104(3):704-710. ISSN 0006-4971
- Petkova R, Chakarov S, Kremensky I (2004). Genetic analysis of haemophilia A in Bulgaria. *BMC Blood Disord*; 4(1): 2. ISSN 1471-2326
- Puetz J (2010). Optimal use of recombinant factor VIIa in the control of bleeding episodes in hemophilic patients. *Drug Des Devel Ther*; 4: 127-337. ISSN: 1177-8881
- Pieneman WC, Deutz-Terlouw PP, Reitsma PH, Briët E (1995). Screening for mutations in haemophilia A patients by multiplex PCR-SSCP, Southern blotting and RNA analysis: the detection of a genetic abnormality in the factor VIII gene in 30 out of 35 patients. *Br J Haematol*; 90(2): 442-449. ISSN: 0007-1048
- Pipe SW (2008). Recombinant clotting factors. *Thromb Haemost* ; 99: 840-850.
- Ramanarayana J, Krishnan GS, Hernandez-Ilizaliturri. Factor VII.  
<http://emedicine.medscape.com/article/209585-overview>

- Qadura M, Waters B, Burnett E, Chegeni R, Bradshaw S, Hough C, Othman M, Lillicrap D (2009). Recombinant and plasma-derived factor VIII products induce distinct splenic cytokine microenvironments in hemophilia A mice. *Blood* ; 114(4): 871-880. ISSN 0006-4971
- Rapaport SI, Patch MJ, Casey JE (1960). The antihemophilic globulin in plasma; content of freshly frozen single-donor plasma units prepared by the Los Angeles Red Cross Blood Center. *Calif Med*; 93:208-210.. ISSN:0008-1264
- Rudzki Z, Rodgers SE, Sheffield LJ, Lloyd JV (1996). Detection of carriers of haemophilia A: use of bioassays and restriction fragment length polymorphisms (RFLP). *Aust N Z J Med*; 26(2): 195-205. ISSN: 0004-8291
- Sabatino DE, Freguia CF, Toso R, Santos A, Merricks EP, Kazazian HH Jr, Nichols TC, Camire RM, Arruda VR (2009). Recombinant canine B-domain-deleted FVIII exhibits high specific activity and is safe in the canine hemophilia A model. *Blood*; 114(20): 4562-4565. ISSN 0006-4971
- Sadler JE (1990). Recombinant DNA methods in hemophilia A: carrier detection and prenatal diagnosis. *Semin Thromb Hemost* ; 16(4): 341-347. ISSN:0094-6176
- Salviato R, Belvini D, Are A, Radossi P, Tagariello G (2002). Large FVIII gene deletion confers very high risk of inhibitor development in three related severe haemophiliacs. *Haemophilia*; 8(1): 17-21. ISSN 1351-8216
- Sarkar R, Mucci M, Addya S, Tetreault R, Bellinger DA, Nichols TC, Kazazian HH Jr (2006). Long-term efficacy of adeno-associated virus serotypes 8 and 9 in hemophilia a dogs and mice. *Hum Gene Ther*; 17(4): 427-439. ISSN: 1043-0342
- Sarkar R, Xiao W, Kazazian HH Jr (2003). A single adeno-associated virus (AAV)-murine factor VIII vector partially corrects the hemophilia A phenotype. *J Thromb Haemost*; 1(2): 220-226. ISSN:1538-7933
- Scallan CD, Liu T, Parker AE, Patarroyo-White SL, Chen H, Jiang H, Vargas J, Nagy D, Powell SK, Wright JF, Sarkar R, Kazazian HH, McClelland A, Couto LB (2003). Phenotypic correction of a mouse model of hemophilia A using AAV2 vectors encoding the heavy and light chains of FVIII. *Blood*; 102(12):3919- 3926. ISSN 0006-4971
- Scandella D, Reyes H, Felch M, Sakurai Y (2000). Characterization of antibodies to factor VIII in hemophilia A patients treated by immune tolerance therapy. *Haematologica*; 85(10 Suppl): 86-88. ISSN: 0390-6078
- Scharrer I (1999). Recombinant factor VIIa for patients with inhibitors to factor VIII or IX or factor VII deficiency. *Haemophilia*; 5(4): 253-259. ISSN 1351-8216
- Schwaab R, Brackmann HH, Meyer C, Seehafer J, Kirchgesser M, Haack A, Olek K, Tuddenham EG, Oldenburg J (1995). Haemophilia A: mutation type determines risk of inhibitor formation. *Thromb Haemost* ; 74(6): 1402-1406. ISSN:0340-6245
- Schwartz RA, Klujszo E, McKenna Ri. FVIII.  
<http://emedicine.medscape.com/article/201319-overview>
- Sharathkumar A, Lillicrap D, Blanchette VS, Kern M, Leggo J, Stain AM, Brooker L, Carcao MD (2003). Intensive exposure to factor VIII is a risk factor for inhibitor development in mild hemophilia A. *J Thromb Haemost*; 1(6): 1228-1236. ISSN: 1538-7933
- Shetty S, Bhawe M, Ghosh K (2010). Acquired hemophilia A: Diagnosis, aetiology, clinical spectrum and treatment options. *Autoimmun Rev*. 2010 Nov 27. [Epub ahead of print]. ISSN: 1568-9972

- Shi Q, Wilcox DA, Fahs SA, Weiler H, Wells CW, Cooley BC, Desai D, Morateck PA, Gorski J, Montgomery RR (2006). Factor VIII ectopically targeted to platelets is therapeutic in hemophilia A with high-titer inhibitory antibodies. *J Clin Invest*; 116(7): 1974-1982. ISSN: 0021-9738
- Shi Q, Wilcox DA, Fahs SA, Fang J, Johnson BD, DU LM, Desai D, Montgomery RR (2007). Lentivirus-mediated platelet-derived factor VIII gene therapy in murine haemophilia A. *J Thromb Haemost*; 5(2): 352-361. ISSN: 1538-7933
- Shi Q, Wilcox DA, Fahs SA, Kroner PA, Montgomery RR (2003). Expression of human factor VIII under control of the platelet-specific alphaIIb promoter in megakaryocytic cell line as well as storage together with VWF. *Mol Genet Metab*; 79(1): 25-33. ISSN: 1096-7192
- Song KS, Lee CH, Chung CS, Lee K, Yang YH, Kim KY (1993). The prevalence study on restriction fragment length polymorphism analysis for the detection of hemophilia A carrier. *Yonsei Med J*; 34(3): 239-242. ISSN: 0513-5796
- Stephens JM, Joshi AV, Sumner M, Botteman MF (2007). Health economic review of recombinant activated factor VII for treatment of bleeding episodes in hemophilia patients with inhibitors. *Expert Opin Pharmacother*; 8(8): 1127-1136. ISSN: 1465-6566
- Stites DP, Hershgold EJ, Perlman JD, Fudenberg HH (1971). Factor 8 detection by hemagglutination inhibition: hemophilia A and von Willebrand's disease. *Science*; 171(967): 196-197. ISSN 0036-8075
- Surin VL, Zhukova EL, Krutov AA, Solov'ev GI, Likhacheva EA, Pliushch OP, Grineva NI (1990). [Detection of hemophilia A carriers by testing polymorphic Bcl I and HINDIII sites using the PCR method with internal splitting control]. *Gematol Transfuziol*; 35(3): 3-6. ISSN: 0234-5730
- Thompson AR (1986). Structure, function, and molecular defects of factor IX. *Blood*; 67: 565-572. ISSN 0006-4971
- Tomokiyo K, Nakatomi Y, Araki T, Teshima K, Nakano H, Nakagaki T, Miyamoto S, Funatsu A, Iwanaga S (2003). A novel therapeutic approach combining human plasma-derived Factors VIIa and X for haemophiliacs with inhibitors: evidence of a higher thrombin generation rate in vitro and more sustained haemostatic activity in vivo than obtained with Factor VIIa alone. *Vox Sang*; 85(4): 290-299. ISSN 0042-9007
- Toole JJ, Knopf JL, Wozney JM, Sultzman LA, Buecker JL, Pittman DD, Kaufman RJ, Brown E, Shoemaker C, Orr EC, et al. (1984). Molecular cloning of a cDNA encoding human antihemophilic factor. *Nature*; 312(5992): 342-347. ISSN : 0028-0836
- Toschi V (2010). OBI-1, porcine recombinant Factor VIII for the potential treatment of patients with congenital hemophilia A and alloantibodies against human Factor VIII. *Curr Opin Mol Ther*; 12(5): 617-625. ISSN: 1464-8431
- Toy L, Young EA, Longenecker JB (1983). Ascorbic acid, vitamin A, folic acid, and amino acids in blood of patients with hemophilia. *Blood*; 62(3): 532-537. ISSN 0006-4971
- Van de Water NS, Williams R, Nelson J, Browett PJ (1995). Factor VIII gene inversions in severe hemophilia A patients. *Pathology*; 27(1): 83-85. ISSN:0031-3025
- VandenDriessche T, Collen D, Chuah MK (2003). . Gene therapy for the hemophilias. *J Thromb Haemost*; 1(7): 1550-1558. ISSN: 1538-7933
- von Auer Ch, Oldenburg J, von Depka M, Escuriola-Ettinghausen C, Kurnik K, Lenk H, Scharer I (2005). Inhibitor development in patients with hemophilia A after continuous infusion of FVIII concentrates. *Ann N Y Acad Sci*; 1051: 498-505. ISSN: 0077-8923

- Wacey AI, Kemball-Cook G, Kazazian HH, Antonarakis SE, Schwaab R, Lindley P, Tuddenham EG (1996) The haemophilia A mutation search test and resource site, home page of the factor VIII mutation database: HAMSTeRS. *Nucleic Acids Res*; 24(1): 100-102. ISSN: 0305-1048
- Wadelius C, Blombäck M, Goonewardena P, Anvret M, Lindstedt M, Gustavson KH, Pettersson U (1991). Evaluation of DNA-based diagnosis for haemophilia A. *Scand J Clin Lab Invest* ; 51(7): 625-633. ISSN: 0036-5513
- Wang L, Herzog RW (2005). AAV-mediated gene transfer for treatment of hemophilia. *Curr Gene Ther*. 2005 Jun; 5(3): 349-360. ISSN: 1566-5232
- Wehnert M, Shukova EL, Surin VL, Schröder W, Solovjev GYa, Herrmann FH (1990a). Prenatal diagnosis of haemophilia A by the polymerase chain reaction using the intragenic hind III polymorphism. *Prenat Diagn*; 10(8): 529-532. ISSN: 0197-3851
- Wehnert M, Shukova EL, Surin VL, Schröder W, Solovjev GYa, Grinjeva NI, Herrmann FH (1990b). Genomic carrier detection and prenatal diagnosis of haemophilia A in families at risk using the polymerase chain reaction (PCR). *Folia Haematol Int Mag Klin Morphol Blutforsch*; 117(4): 617-622. ISSN: 0323-4347
- White GC, Shoemaker CB. (1989). Factor VIII Gene and Hemophilia A. *Blood*; 73(10): 1-12. ISSN 0006-4971
- Wiestner A, Cho HJ, Asch AS, Michelis MA, Zeller JA, Peerschke EI, Weksler BB, Schechter GP (2002). Rituximab in the treatment of acquired factor VIII inhibitors. *Blood*; 100(9): 3426-3428. ISSN 0006-4971
- Wood WI, Capon DJ, Simonsen CC, Eaton DL, Gitschier J, Keyt B, Seeburg PH, Smith DH, Hollingshead P, Wion KL, et al. (1984). Expression of active human factor VIII from recombinant DNA clones. *Nature*; 312(5992): 330-337. ISSN : 0028-0836
- Wu G (1991). Prenatal diagnosis of hemophilia A by DNA analysis. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao*; 13(6): 428-434. ISSN: 0376-2491
- Yoshioka A, Fukutake K, Takamatsu J, Shirahata A; Kogenate Post-Marketing Surveillance Study Group (2003). Clinical evaluation of a recombinant factor VIII preparation (Kogenate) in previously untreated patients with hemophilia A. *Int J Hematol*; 78(5): 467-474. ISSN: 0925-5710
- Yousoufian H, Antonarakis SE, Aronis S, Tsiftis G, Phillips DG, Kazazian HH Jr (1987). Characterization of five partial deletions of the factor VIII gene. *Proc Natl Acad Sci U S A*; 84(11): 3772-3776. ISSN: 0027-8424.
- Zatloukal K, Cotten M, Berger M, Schmidt W, Wagner E, Birnstiel ML (1994). In vivo production of human factor VII in mice after intrasplenic implantation of primary fibroblasts transfected by receptor-mediated, adenovirus-augmented gene delivery. *Proc Natl Acad Sci U S A*; 91(11): 5148-5152. ISSN: 0027-8424
- Zhang WW, Josephs SF, Zhou J, Fang X, Alemany R, Balagué C, Dai Y, Ayares D, Prokopenko E, Lou YC, Sethi E, Hubert-Leslie D, Kennedy M, Ruiz L, Rockow-Magnone S (1999). Development and application of a minimal-adenoviral vector system for gene therapy of hemophilia A. *Thromb Haemost*; 82(2): 562-571. ISSN: 1538-7933



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