Free research paper on genetic indicators and variable relationship

Science, Genetics

1. 0 Introduction

Hemophilia A (HA) is an inherited X-linked bleeding disorder that results from a wide range of mutations in the factor VIII (FVIII) gene which leads to the absence or quantitative and/or qualitative deficiency of the said blood clotting factor ((Jacquemin, et al., 2000; Guillet, et al., 2006; Venceslá, et al., 2008). FVIII gene is one of the most extensively investigated of all human genes and these extensive studies have led to the identification of a wide range of different types of mutations responsible for HA. Some of the mutation types identified include point mutations (resulting in missense, nonsense and splice site mutations), inversions, deletions, insertions and unidentified mutations. This wide range of mutations within the large FVIII gene makes molecular diagnosis of HA very challenging. This paper will focus on the FVIII gene, the wild type and the mutations responsible for HA, the gene products as while as the interactions of the gene and psychosocial, occupational and environmental factors.

Suffice to say that while the deleterious mutations in the FVIII gene result in a reduction of the circulating levels and/or activity of the factor, the associated disorder (HA) is categorized based on the plasma pro-coagulant levels of FVIII. Severe HA occurs where the pro-coagulant level of FVIII is > 0. 01 IU/ml and is associated with spontaneous bleeding; in moderate HA the pro-coagulant level of FVIII is 0. 01-0. 05 IU/ml while it is 0. 05-0. 4 IU/ml in mild forms of HA. The latter to categories of HA are associated with bleeding that only occurs after minor trauma or surgery. It is also vital to note that there is a clear correlation between the mentioned categories of HA and the mutations that have been identified. The mutations leading to severe HA

were studied more but the last three decades have seen extensive characterization and identification of over 200 mutations linked to the etiology of mild/moderate HA.

2. 0 Overview of the FVIII gene and the gene product

FVIII gene is large (~ 180 kb) and complex in structure with 26 extrons and 25 introns. The gene is located on the long arm of the X chromosome, towards the end at Xq28 (a vulnerable position during meiosis in a male). Within the large gene, there is a small intronless gene (less than 2kb), FVIIIA(gene within a gene) that is transcribed in the opposite direction to the larger FVIII gene). Towards the telomere and outside the larger FVIII gene, there other two similar small genes (FVIII A' and FVIIIA", but they are transcribed in the same direction as the large gene, (see figure 1 below) .

Figure 1: FVIII gene and the associated small genes adopted from Kasper & Buzin (2007).

The transcript (mRNA) for the FVIII gene is approximately 9010 bases long and has a short (150 bases) 5'untranslated section, an open reading frame and its stop codon (7056 bases) and a long (1806 bases) 3' untranslated section. The reading frame encoded a 19 amino acids signal peptide that directs the movement of FVIII through the cell as a 2332 amino acids mature inactive pro-cofactor. The activation of FVIII occurs through a thrombin-catalyzed proteolysis on C-terminal side of arginine residues 372 (Arg 372-Ser 373), 740 (Arg 740-Ser 741) and 1689 (Arg 1689-Ser 1690). This same sites are cleavage sites for activated FX during the coagulation cascade leading to fibrin formation and they flank the B domain that is released from FVIII on activation to leave a heterotrimer peptide made up of an N-terminal

heavy chain and a C-terminal light chain that are non-covalently held together by a calcium ion bridge. FVIII has 3 types of domains (3 A (A1-A3) domains homologous to ceruloplasmin, a unique B domain of variable lengths and 2 C domains homologous to the discoidin protein family. The heavy chain consist A1, A2 and B subunit of variable lengths while the light chain is made up of A3, C1 and C2 subunits (see figure 2 below). Suffice to say that in circulation FVIII is bound to von Willebrand factor (VWF) (a carrier protein) and is released from the latter thrombin mediated activation.

(A)

-19 1 336 719 1691 2025 2332

Arg 372 Arg 740 Arg 1689

(B)

Ca2+

Figure 2: (A) Newly synthesized FVIII molecule made of a pre-sequence of 19 amino acids and a mature 2332 amino acids FVIII peptide. (B) Activated factor VIII comprising a heterotrimer in which the dimeric N-terminal heavy chain is held together with the monomeric C-terminal light chain by a metal ion bridge (Ca2+). (Adopted from Bowen, 2002)

FVIII has no enzymatic activity per se but a cofactor for FIX which is a serine protease. Once activated in the presence of Ca2+ and phospholipid (provided by activated platelets), FVIII releases VWF protein and interacts with FIX to form a complex (intrinsic Xase/tenase complex) which in turn activates FX through cleavage of a single peptide bond. These forms an

essential part of the coagulation cascade (see figure 3 below) Intrinsic pathway

Kallikrein Pre-kallikrein

Factor XII Factor XIIa

Factor XI Factor Xia

Factor IX Factor IXa. Factor VIIIa Factor V

F X FXa. Factor Va extrinsic pathways

Tissue factor FVII

Factor VIIa

Factor II Factor IIa

(prothrombin) (thrombin)

Fibrogen Fibrin

Figure 3: schematic representation of coagulation cascade (adopted from Bowen (2002)

4. 0 The amino acid alterations in the mutant form

The limited scope of this paper cannot elaborate all the mutations that have been linked to HA but a few will be considered. Generally, in terms of effects and alterations, the mutations are classified into: those that impair FVIII folding into the quaternary structure or intracellular processing hence result in reduced secretion, those that slow the thrombin-dependent activation of FVIII, those alter the stability of the activated FVIII(mostly by interfering with the interaction with VWF) and those that interfere with FVIII's interaction with FIXa.

Some of the mutations responsible for impaired/reduced FVIII synthesis,

processing and/or secretion include mutations at residue Arg2307 in the C2 domain, deletion of Phe652 in the A2 domain, Arg593Cys (substitution) and Arg2150His (substitution) in the A2 and C1 domains respectively. Mutations on the Arg2150 and Arg 2307 result in misfolded FVIII. The binding of FVIII to VWF protects the form rapid clearance and/or clearance hence reduced binding essentially translates to low levels of FVIII. The mutations that reduce FVIII/VWF interaction include those that affect Tyr1680 (mostly substitution) in the A3 domain. These mutations inhibit sulfation which is vital for the binding of FVIII to VWF. Other mutations that reduce the affinity of FVIII to VWF include Ser2173Ile, Ala2201Pro, Val2232Ala, Trp2249Cys, Gln2246Arg, Pro2300Leu, Arg2304Cys/Gly, Arg2320Thr in the C2 domain. Substitutions Ile2098Ser, Ser2119Tyr, Asn2129Ser, Arg2150His , Pro2153Gln, Gln2087Glu, Arg2090Cys, Arg2150Cys, Arg2159Cys, Arg2163Cys and Met2164Arg in the C1 domain also reduce FVIII/VWF interactions .

Impaired thrombin activation is mainly due to mutations at the Arg residues at the thrombin cleavage sites (substitutions of Arg372 and Arg1689 have been identified). Tyr346Cys Glu321Lys mutations have also been found to interfere with thrombin activation. The stability of FVIII can be impaired due to accelerated dissociation of the A2 domain. This has been linked to mutations like the Gly1948Asp, Arg531His, Ala284Pro/Glu, His1954Leu, Ser289Leu, substitutions. Glu720Lys and Arg527Trp mutations have been found to alter the interaction of FVIII and FIXa.

While the mutations discussed above cause mild to moderate HA a substitution N1922S in the A3 domain often result in severe HA due to

reduced FVIII secretion resulting from abnormal folding in the A3 and C1 domains. 40-50% of the cases of severe HA has been linked to an inversions due to breakage in intron 22. Inversions resulting from breakages at site cause 2% of the severe HA cases. The deletion of nucleotide T in the A (8) TA(2) sequence of exon 14 has been linked to severe HA. In conclusion, single nucleotide substitutions are the most common mutation in HA and are found in 90% of all patients, deletions occur in 5-10% of HA patients while inversions and insertions are very rare except for those that occur in patients with severe HA.

5. 0 Relationship between the genetic indicators and other factors

Often the etiology, pathophysiology, prognosis and management of disease is associated with the interaction of both genetic and non-genetic factors. In the case of HA, the non-genetic factors do not influence etiology but they have a significant influence on the symptoms (pathophysiology) and the treatment/management/prognosis (mainly through the formation of inhibitors/outoantibodies) . This section will focus on the relationship between the genetic indicators, psychosocial, occupational and environmental factors.

5. 1 Psychosocial factors

Psychosocial factors often influence the adjustment and coping which are necessary in this day and age of comprehensive healthcare. Adjusting and coping ensures good prognosis and treatment outcomes. The main direct effects of psychosocial factors on hemophiliacs are the emotional effect of the disease on the growing child, the effects of the physical symptoms and the impact of the disease on the family. Physical symptoms and

complications such as severe joint involvement make it impossible for the child to participate in aggressive activities and thus the child feels isolated from the hallmarks of childhood. This isolation can have far-reaching effects on the child’s ability to cope and adapt and is recipe for depression. A hemophilia diagnosis is bound to arouse a wide range of dissenting emotions (such as fear, sadness, anger and guilt) in the family and caregivers. Effective resolution (acceptance) of these emotions goes a long way in helping the child to adapt and cope. In addition, family and social support is a key factor in helping the child to cope with the chronic illness as a well in taking the necessary cautionary measures. In some instances, overzealous family support (overprotection) is detrimental because the family members become distant. The pain, spontaneous bleeding and complications associated with the disease always distress the patient. The very knowledge that one has an incurable disease and will be under medication for life certainly causes immense negative emotions. Some studies have shown that teenagers with chronic diseases are predisposed to deviant sexual activities and other high-risk behaviors such as drug and substance abuse and participating in individual and gang crimes . In addition to the direct psychosocial influences, there is a major indirect influence that has been extensively investigated.

Another psychosocial influence worth mentioning has to do with the emerging popularity of prenatal diagnosis and the ethical dilemma and controversy surrounding the abortion of fetuses diagnosed t with hemophilia. In developed countries prenatal diagnosis (PD) and carrier-testing (CT) is a key component of comprehensive care for hemophilia. There is a controversy

on whether hemophilia is sufficiently serious to warrant abortion based on a positive PD. The controversy, procedure and the psychological impact of abortion puts families with hemophilia history under enormous psychological pressure.

5. 2 Environmental and Occupational factors

Very little attention has been given to the occupational factors in research. However, occupation has a direct influence on living with hemophilia. Given that hemophilia is a chronic disease associated with spontaneous (the severe form) bleeding, pain in the joints and other health complications such as progressive arthritis, it can render in capable of getting involved in certain occupations. People with severe hemophilia are often not able to work in jobs that require a lot of physical activities and that are linked to higher chances of occupational accidents. One study revealed that people with severe hemophilia are involved less in full-time paid work compared to the general population. This was attributed to higher incidences of occupational disability in the former population particularly those at the age between 31 and 64 years old. However, introduction of prophylactic therapy has reduced the number of hemophiliac with occupational disability. In addition, the complications and ailments of ageing may be more complicated among hemophiliacs and thus hemophiliacs often retire earlier.

The most investigated environmental factors are those associated with the formation of inhibitors (alloantibodies against FVIII replacement therapy). The formation inhibitors is a serious treat complication that occurs in 10-30% of all patients following FVIII replacement therapy and can preclude effective patient management. While a myriad of genetic factors have been

associated with development of inhibitors, patient-related environmental factors have been identified (Oldenburg & Pavlova, 2006; Goudemand, et al., 2006; Astermark, 2006; Ghosh & Shetty, 2009; Astermark, et al., 2010). However, there is a differing opinion on the extent to which the individual environmental factors influence inhibitor development. In fact, recent studies have faulted some findings of earlier studies of got very different findings. The major risk factors for the development of inhibitors that have been identified include:

- The intensity of the treatment and surgery increases the risk of inhibitor development (Astermark, 2006; Gouw, van der Bom, & van den Berg, 2007). One recent meta-analysis faulted this finding on the basis of insufficient evidence and recommended more research on this.
- The type and purity of treatment (recombinant-higher risk than plasma derived FVIII) (Astermark, 2006). Onather study concluded that the type of treatment has no effect. Very high purity FVIII concetrates increase the risk of inhibitor development because they are more antigenic than the older concetrate.
- Age at the first treatment and doses of the initial treatment-early exposure to high doses seem to increase risk of inhibitor development (Astermark, 2006; Gouw, van der Bom, & van den Berg, 2007). However, some investigators feel that delayed treatment does not prevent inhibitor development but just defers it and expose the children to hemophilia complications (Santagostino, et al., 2005; Astermark, 2006).
- Mode of administration (periodic bolus injection have higher risk than continuous infusions) .

- Early regular prophylaxis treatment seems to decrease the risk of inhibitor development (Astermark, 2006; Gouw, van der Bom, & van den Berg, 2007). Suffice to say that the genetic risks may not be changed but the modification of the non-genetic factors may improve treatment and hence the outcome (prognosis). As such, a better understanding of the interaction of the genetic and non-genetic factors will go a long way in the management of HA.

References

Agle, D. (2006). PSYCHOLOGICAL FACTORS IN HEMOPHILIA—THE CONCEPT OF SELF-CARE. Annals of the New York Academy of Sciences , 221–225.

Astermark, J. (2006). Why do inhibitors develop? Principles of and factors influencing the risk for inhibitor development in haemophilia. Haemophilia , 12 (3), 52–60.

Astermark, J., Altisent, C., Batorovial, A., Dinz, M. J., Gringeri, A., Holme, P. A., et al. (2010). Non-genetic risk factors and the development of inhibitors in haemophilia: a comprehensive review and consensus report. Haemophilia , 16 (5), 747–766.

Bowen, D. J. (2002). Haemophilia A and haemophilia B: molecular insights. Journal of Clinical Pathology: Molecular Pathology , 55, 127-144.

Bussing, R., & Burket, R. (1993). Anxiety and Intrafamilial Stress in Children with Hemophilia after the HIV Crisis. Journal of the American Academy of Child & Adolescent Psychiatry , 32 (3), 562–567.

CDC. (2013, May 7). Hemophilia. Retrieved October 22, 2013, from http://www. cdc. gov/ncbddd/hemophilia/data. html

Ghosh, K., & Shetty, S. (2009). Immune Response to FVIII in Hemophilia A: An

Overview of Risk Factors. Clinical Reviews in Allergy & Immunology , 37 (2), 58-66.

Goodeve, A. C., & Peake, I. R. (2003). The Molecular Basis of Hemophilia A: Genotype-Phenotype Relationships and Inhibitor Development. Seminars in Thrombosis and Hemostasis , 29 (1), 023-030.

Gouw, S. C. (2010). The etiology of inhibitor development in children with severe hemophilia A. S. C. Gouw, Utrecht: Utrecht University.

Guillet, B., Lambert, T., d'Oiron, R., Proulle, V., Plantier, J.-L., Rafowicz, A., et al. (2006). Detection of 95 novel mutations in coagulation factor VIII gene F8 responsible for hemophilia A: results from a single institution. Human Mutation , 27 (7), 676–685.

Jacquemin, M., De-Maeyer, M., D'Oiron, R., Lavend'Homme, R., Peerlinck, K., & Saint-Remy, J.-M. (2003). Molecular mechanisms of mild and moderate hemophilia A. Journal of Thrombosis and Haemostasi , 1 (3), 456–463.

Jacquemin, M., Lavend’homme, R., Benhida, A., Vanzieleghem, B., d’Oiron, R., Lavergne, J.-M., et al. (2000). A novel cause of mild/moderate hemophilia A: mutations scattered in the factor VIII C1 domain reduce factor VIII binding to von Willebrand factor. BLOOD , 96 (3), 958-965.

Kasper, C. K., & Buzin, C. H. (2007). GENETICS OF HEMOPHILIA A AND B . Los Angeles: THE CSL BEHRING FOUNDATION FOR RESEARCH AND ADVANCEMENT OF PATIENT HEALTH.

Kniffin, C. L., & McKusick, V. A. (2013, March 14). HEMOPHILIA A; HEMA. Retrieved November 2013, 2013, from http://omim. org/entry/306700

Lee, C. A., Kessler, C. M., Varon, D., Martinowitz, U., Heim, M., & Tedgard, U. (1998). Carrier testing and prenatal diagnosis of haemophilia — utilisation

and psychological consequences. Haemophilia , 4 (4), 365–369.

Oldenburg, J., & Pavlova, A. (2006). Genetic risk factors for inhibitors to factors VIII and IX. Haemophilia , 12 (6), 15–22.

Plug, I., Peters, M., Mauser-Bunschoten, E. P., Goede-Bolder, A. d., Heijnen, L., Smit, C., et al. (2007). Social participation of patients with hemophilia in the Netherlands . The American Society of Hematolog .

Rull, G. (2011, February 18). Haemophilia A (Factor VIII Deficiency). Retrieved October 22, 2013, from http://www. patient. co. uk/doctor/haemophilia-a-factor-viii-deficiency

Street, A., Hill, K., Sussex, B., Warner, M., & Scully, M. (2006). Haemophilia and ageing. Haemophilia , 12 (3), 8-12.

Summers, R. J., Meeks, S. L., Healey, J. F., Brown, H. C., Parker, E. T., Kempton, C. L., et al. (2010). Factor VIII A3 domain substitution N1922S results in hemophilia A due to domain-specific misfolding and hyposecretion of functional protein. Blood , 117 (11), 3190-3198.

Valencia, L. S., & Cromer, B. A. (2000). Sexual Activity and Other High-Risk Behaviors in Adolescents with Chronic Illness: A Review. Journal of Pediatric and Adolescent Gynecology , 13 (2), 53–64.

Venceslá, A., Corral-Rodríguez, M. Á., Baena, M., Cornet, M., Domènech, M., Baiget, M., et al. (2008). Identification of 31 novel mutations in the F8 gene in Spanish hemophilia A patients: structural analysis of 20 missense mutations suggests new intermolecular binding sites. BLOOD , 111 (7), 3468-3478.