The Malmö International Brother Study (MIBS). Genetic defects and inhibitor development in siblings with severe hemophilia A

Jan Astermark
Johannes Oldenburg
Miguel Escobar
Gilbert C White II
Erik Berntorp
and the MIBS study group

Background and Objectives. The strongest risk factor identified for inhibitor development in people with severe hemophilia A is the type of factor VIII gene mutation. The objective of this study was to evaluate the mutation type dependent concordance rate of inhibitor formation in siblings.

Design and Methods. The gene defect, treatment and inhibitor history were evaluated in 113 families in which two or more siblings had severe hemophilia A.

Results. Seventy-nine of the families (69.9%) were concordant in that either all or none of the siblings had a history of inhibitors. The concordance in 59 families with inhibitors was 42.4%. The corresponding figures for the 74 families with intron 22 inversion were 63.5% and 40.0%, respectively, and the overall concordance within 14 families with nonsense mutations was 78.6%. The siblings in two families with large gene deletions had no inhibitor history. A small proportion of the families with missense mutations, small deletions/insertions and splice site mutations developed inhibitors, but in four of the families two or more siblings developed high-responding inhibitors. In 18 of the 25 concordant families (72.0%) with inhibitors, the inhibitor was also of the same type (high-responding).

Interpretations and Conclusions. This is the first study of the association between inhibitor formation and the causative factor VIII gene mutation in siblings. The data show that the type of mutation provides, to some extent, the basis for this relationship, but the mutation itself is not enough to predict the risk for therapy-induced inhibitor formation.

Key words: factor VIII gene mutation, hemophilia, inhibitors, MIBS, siblings.

Haematologica 2005; 90:924-930

©2005 Ferrata Storti Foundation

From the Department for Coagulation Disorders, University Hospital, Malmö, Sweden (JA, EB); Institute of Transfusion Medicine and Immunohaematology, University Clinic, Frankfurt, Germany (JO) Gulf States Hemophilia and Thrombophilia Center, Houston, Texas, US (ME); Center for Thrombosis and Hemostasis, Chapel Hill, NC, USA (GCW 2nd).

Correspondence: Jan Astermark, MD, PhD, Associate Professor, Department for Coagulation Disorders University Hospital SE-205 02 Malmö, Sweden. E-mail: Jan.astermark@medforsk.mas.lu.se

The formation of inhibitory allo-antibodies against the deficient factor is a serious complication of replacement therapy in patients with hemophilia.[1,2] In the case of hemophilia A, the antibodies inhibit the function of factor VIII and develop in approximately 30% of all subjects suffering from the severe form of the disease. In many cases these inhibitors become a long-standing problem that seriously affects health and quality of life and requires costly medical intervention.[3] Several genetic and environmental risk factors for the development of inhibitors have been evaluated, but in most cases without significant associations being found.[4-5] The strongest relationship that has been found is with the causative factor VIII gene mutation.[6-7] Patients with large gene deletions, nonsense mutations and intrachromosomal aberrations appear to have a relatively high risk of inhibitor development, whereas those with missense mutations, small deletions/insertions and splice site mutations experience this side-effect less frequently. A weak correlation has also been found with the major histocompatibility complex (MHC) class I/II genotypes, in that A3, B7, C7, DQA0102, DQB0602 and DR15 have been associated with relative risks from 1.9-4.0 for inhibitor development.[8,9] However, several patients with *high-risk* mutations and unfavorable genotypes do not develop inhibitors and the reason for this is unknown. The aim of the Malmö International Brother Study (MIBS) is to identify risk factors for inhibitor development. The study cohort consists of families containing two or more siblings with hemophilia with or without a history of inhibitors. In an analysis of 249 families with severe hemophilia A, we found an overall concordance between siblings of 78.3% and a relative risk of 3.2 for the development of an inhibitor for a patient whose older brother had previously been diagnosed with an inhibitor.[10] The study of factors affecting the immune response to

replacement therapy in siblings offers many benefits. The factor VIII gene mutation and the amount of circulating endogenous factor VIII antigen will be similar in all affected family members, and the variation and impact of socio-economic and environmental factors can be minimized. Previous family studies have not addressed the relationship between inhibitor development and the type of factor VIII mutation.[10,11] Since the type of mutation will be important when interpreting concordance within families, we evaluated the factor VIII gene defect and inhibitor history in 113 MIBS families with severe hemophilia A. We also compared our findings with the incidence figures and data available from the two largest registries of unrelated patients with hemophilia i.e. the one at the Bonn center and the database of the Haemophilia A Mutation, Structure, Test and Resource Site (HAMSTeRS).[12,13]

Design and Methods

Subjects

Centers participating in the Malmö International Brother Study (MIBS) were given a standardized questionnaire to accrue data from twins and non-twin brothers with severe hemophilia A (factor VIII:C <1%). Date of birth, ethnicity, type of hemophilia, treatment history, inhibitor history including peak titer and current titer in Bethesda units (BU/mL), type of causative factor VIII gene mutation and techniques used for analysis were recorded. In the case of twins, data concerning zygosity were requested. All families in which the factor VIII gene mutation was fully characterized were enrolled in the study. A high-responding inhibitor was defined as a historical peak titer >5 BU/mL and a low-responding inhibitor as one with a peak titer of ≤5 BU/mL.[14] The study was approved by the independent review board (IRB) and ethics committee.

Methods

Standard methods for genetic analyses were used including Southern blot and long range polymerase chain reaction (PCR) for inversion analysis, PCR and mutation screening methods, e.g. chemical mismatch cleavage (CMC), single stranded conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), denaturing high performance liquid chromatography (DHPLC), and direct DNA sequencing.[15] Splice site scores were calculated according to the website available at URL *http://cgsigma.cshl.org/new_alt_exon_db2/HTML/score.html*. Inhibitory antibodies were quantified according to the original Bethesda method and the Nijmegen modified assay.[16,17]

Statistical methods

The number of expected concordant families with intron 22 inversion and inhibitors in both siblings was calculated using the formula (i): [Expected inhibitor incidence in % i.e. either 21.0% (based on the Bonn data for unrelated patients) or 34.4% (based on the HAMSTeRS data for unrelated patients)/100]$_{\text{sibling 1}}$ × [Inhibitor incidence in %/100]$_{\text{sibling 2}}$ ×74 (i.e. the total number of families with this type of mutation). The expected number of concordant families without inhibitors in both siblings was calculated using the formula (ii): [100 – Expected inhibitor incidence in % / 100]$_{\text{sibling 1}}$ × [100 - Inhibitor incidence in % / 100]$_{\text{sibling 2}}$ × 74. The number of discordant families was calculated using the formula (iii): [Expected inhibitor incidence in % / 100]$_{\text{sibling 1}}$ × [100 - Inhibitor incidence in % / 100]$_{\text{sibling 2}}$ × 2 × 74. χ^2 analysis was used for evaluation of the observed versus expected number of concordant and discordant families. A p value less than 0.05 was considered to indicate statistical significance.

Results

Study cohort characteristics

The types of FVIII gene mutations identified in the 113 families containing 231 members with severe hemophilia A were intron 22 inversion in 74 families (150 subjects), nonsense mutations in 14 families (28 subjects), large gene deletions in 4 families (8 subjects), missense mutations in 9 families (20 subjects), small deletions/insertions in 9 families (19 subjects) and splice site mutations in 3 families (6 subjects). The median age was 31 years and all family members were above the age of 4 years. Two monozygotic twins were included. All families were of Caucasian ethnicity, except for 7 African-American families, and all subjects had been extensively exposed to factor VIII concentrates, far above the number of exposure days normally regarded as the highest risk period for inhibitor development i.e. <50-75 days. The inhibitor characteristics for each subgroup of mutation are shown in Table 1. The siblings in 79 of the 113 families (69.9%) were concordant in that either all siblings developed inhibitors or none of them did. The siblings in the remaining 34 families were discordant. All siblings had a history of inhibitor development in 25 out of the 59 inhibitor families (42.4%) and in 18 of these families (72.0%) the inhibitor was of the same type (high-responding). Altogether 88 of the 231 patients in the cohort (38.1%) had a history of inhibitors and in 70 of these cases (79.6%), the inhibitor was of the high-responding type.

©Ferrata Storti Foundation

Table 1. Number of concordant and discordant families divided into subgroups according to factor VIII gene mutation. The families with more than two siblings are included in the total number of families, but described in the footnote. Low- and high-responding inhibitors are defined as a historical peak titer ≤ 5 and >5 BU/mL, respectively (No: no history of inhibitors).

Type of mutation	Total no. of families	Inhibitor history in sibling1/sibling2				
		High/ High	High/ Low	High/ No	Low/ No	No/ No
Intron 22 inv	74*	12	5	17	9	29
Nonsense mutation	14	2	1	3		8#
Missense mutation	9°				2	6
Large deletion	4	1		1		2
Small deletion/ insertion	9§	2				6
Splice site mutation	3					3
Total	113	17	6	21	11	54

*Including 1 family with 3 siblings; two with high-responding inhibitors and one without a history of inhibitor and 1 family with 3 siblings of whom two were monozygotic twins and all had high-responding inhibitors; #including one pair of monozygotic twins; °including family with 4 siblings; two with high-responding inhibitors and two without inhibitors; §including 1 family with 3 siblings; two with high- and one with low-responding inhibitors.

Intron 22 inversion

Inhibitors were described in 45 of the 74 families with intron 22 inversion (60.8%) and in 65 of the total 150 patients with this mutation (43.3%). This is higher than the incidence of inhibitors for this mutation reported in unrelated patients from Bonn (21.0%) or in the HAMSTeRS database (34.4%). According to the figures in the databases, the expected number of concordant siblings with an inhibitor history in the 74 families in our series would have been 3.3 and 8.8 depending on which figure is used (Bonn vs. HAMSTeRS). However, the number of families with more than one sibling having a history of inhibitor in the study was 19, including an inhibitor discordant family with three siblings, two of whom had an inhibitor and one who did not. The corresponding expected numbers of discordant families with an inhibitor in only one of the siblings are 24.6 and 33.4, and the observed number of families was 26. The number of observed concordant families without an inhibitor history was 29 compared with the expected 31.8 and 46.2. The overall number of completely concordant families within the intron 22 inversion subgroup was 63.5% (47 out of 74 families). Among the 45 families with inhibitors, 18 (40.0%) were concordant in that all siblings had developed an inhibitor. The peak titers in these 18 concordant families ranged from 2.2 to >1,000 BU/mL (median titer 100 BU/mL). In the families with one sibling having a high- and one having a low-responding inhibitor, the peak titers for the high-responding patients were between 6.6 and >1,000 BU/mL. In the 26 discordant families, with an inhibitor identified in only one of the siblings, the peak titers varied from 1.0 to >1,000 BU/mL (median titer 17 BU/mL). The number of families with concordant siblings was significantly higher than the expected number in unrelated subjects (p<0.001).

Non-intron 22 inversion mutations

The non-intron 22 inversion mutations associated with inhibitors in our series are described in Table 2 together with the inhibitor response and peak titer in each sibling. The mutations without associated inhibitors are summarized in Table 3.

Nonsense mutations

As shown in Table 1, six of the 14 families and nine of all 28 siblings with nonsense mutations had developed inhibitors. This proportion of inhibitors is consistent with an inhibitor incidence of 31.0-38.4% observed in the databases for this subgroup of factor VIII mutations. The locations of the four mutations found in the six inhibitor families are shown in Table 2. Most of these mutations are located in the C1/C2 domains and have been relatively frequently reported to the databases.[6,18-22] The only mutation not previously associated with inhibitors is the *S2058X* mutation in exon 21 found in a discordant brother-pair with a high-titer inhibitor. Among the seven nonsense mutations in our series without inhibitors, four were novel and three had been previously described[23,24] (Table 3). The overall concordance within the subgroup of nonsense mutations was 78.6%.

Large deletions

The number of families with large deletions was small and no major conclusions can be made. However, according to database reports, the occurrence of inhibitor development associated with this type of mutation is high (35.7-41.0%) and it is interesting to note that a brother-pair with an exon 7 deletion was discordant and none of the siblings with the exon 26 and exon 1-6 deletions has developed inhibitory antibodies (Tables 1 and 2). The only concordant family with high-responding inhibitors in both siblings was that with a deletion of exons 11 to 25. This mutation, as well as the exon 1-6 deletion, has previously been associated with inhibitors, whereas no consistent associations with inhibitors have been reported for the other two mutations.[25,26]

Missense mutations

Inhibitors were described in three of nine families and four out of 20 siblings with missense mutations (Tables 1 and 2). According to the databases, the incidence of inhibitors in this subgroup is low (4.3-5.0%). However, in the family with the *G686R* mutation,

©Ferrata Storti Foundation

Table 2. Mutation type and peak titers found in the siblings of 14 non-intron 22 inversion families with a history of inhibitors. Low- and high-responding inhibitors are defined as described in Table 1.

Type of mutation	*Type of family*	*Mutation/ Amino acid change*	*Type of inhibitor response in each sibling*	*Peak titer (BU/mL)*	*Novel mutation (Yes/No)*	*Associated with inhibitors in the databases (Yes/No)*
Nonsense	Concordant	R1966X	High/High	934 (B1) 34 (B2)	No	Yes
	Discordant	S2058X	No/High	150 (B2)	No	No
	Discordant	R2147X	No/High	81 (B2)	No	Yes
	Concordant	R2209X	High/High	>1,000 (B1) 18 (B2)	No	Yes
	Concordant	R2209X	High/Low	563 (B1) 0.8 (B2)	No	Yes
	Discordant	R2209X	High/No	909 (B1)	No	Yes
Large deletion	Discordant	Exon 7 del	High/No	110 (B1)	No	Not known*
	Concordant	Exon 11-25 del	High/High	>1,000 (B1) >1,000 (B2)	No	Yes
Missense	Discordant	S534P	No/Low	4 (B2)	No	No
	Discordant	N684D	Low/No	1 (B1)	No	No
	Discordant	G686R	No/No/High/High	55 (B3) 12 (B4)	Yes	–
Small deletion	Concordant	nt 118 C del	High/High	8 (B1) 15 (B2)	No	Yes
	Concordant	nt 3091-3094 AAGA del	High/High	20 (B1) 566 (B2)	No	Yes
	Concordant	nt 4838 A del	High/High/Low	7 (B1) 5.6 (B2) 3 (B3)	Yes	–

*Discordant: inhibitors in only one or two of the siblings; concordant: inhibitors in all siblings; no: no history of inhibitors; B1: the oldest brother in the family; B2-4: the younger siblings in consecutive order to B1; *not known: missing data in the databases.*

©Ferrata Storti Foundation

two of the four brothers developed high-responding inhibitors (Table 2). This mutation is located in the A2-domain and has not been reported to the HAMSTeRS database. The mutation affects the donor splice site of exon 13, since the first nucleotide of the glycine codon GGT is the last nucleotide of exon 13. The donor splice score is reduced from 8.1 to 4.2, thus almost abolishing the splice site and causing a severe hemophilia A phenotype, similar to that caused by a serine substitution at the same location reported in the HAMSTeRS database. The *S534P* and *N684D* mutations have previously been identified, but not associated with inhibitors.[20,23] Among the remaining missense mutations found in the MIBS families without inhibitors, two mutations were novel, and only *R2304C* has previously been associated with inhibitors[27-29] (Table 3).

Small deletions/insertions

Inhibitors were found in three out of nine families and seven out of 19 subjects with small deletions or insertions (<50 bp). Interestingly, all three families were concordant in that all siblings developed inhibitors (Table 2). The nt118C deletion and the deletion in codons 1012-1013 (nt3091-3094AAGA del) cause a frameshift and were associated with high-responding inhibitors. The nt4838Adel mutation generates a subsequent stop codon and is not

Table 3. Mutation type found in the 25 non-intron 22 inversion families without any family history of inhibitors.

Type of mutation	*Mutation/ Amino acid change*	*No. of families (siblings)*	*Novel mutation (Yes/No)*	*Associated with inhibitors in the databases (Yes/No)*
Nonsense	W1535X	1 (2)	Yes	–
	Y1748X	1 (2)	Yes	–
	W2062X	1 (2)	Yes	–
	L2178X	1 (2)	Yes	–
	R336X	1 (2)	No	Yes
	R795X	2 (4)	No	No
	Q1686X	1 (2)	No	No
Large deletion	Exon 1-6 del	1 (2)	No	Yes
	Exon 26 del	1 (2)	No	Yes
Missense	S1849L	1 (2)	Yes	–
	C2169Y	1 (2)	Yes	–
	T118I	1 (2)	No	No
	N1922S	1 (2)	No	No
	R2304C	2 (4)	No	Yes
Small deletion/ insertion	nt2057-2060CACTdel	2 (4)	Yes	–
	nt2062Ains	2 (4)	No	No
	nt4321-4324AGAAdel	1 (2)	No	No
	nt4820-4825Ains	1 (2)	No	Yes
Splice site	IVS4 + 5G→A	1 (2)	Yes	–
	IVS6 + 3A→G	1 (2)	No	No
	IVS11 + 1G→A	1 (2)	Yes	–

found in the HAMSTeRS database. The families without inhibitors had a deletion or insertion in exon 13 involving codons 667-669 or in exon 14 (Table 3). Insertion at codon 669 has also been reported to the database without any associated inhibitor. The HAMSTeRS and Bonn data suggest an average inhibitor incidence of 7.4-16.0% for patients with small deletions/insertions, but the figures vary depending on the location of the mutation.

Splice site mutations

Splice site mutations have been associated with an inhibitor incidence of approximately 3% and, consistent with this low-risk profile, no inhibitor history was reported in the small group of three families with this type of mutation in our series (Table 3).

Discussion

Both genetic and environmental factors must be considered as determinants in the formation of inhibitory antibodies. One attractive way to address the issue of etiology is to study related subjects, since patients within a family will carry the same causative gene mutation, a similar amount of circulating antigen and are more homologous with respect to other immunological genes than unrelated subjects. In addition, the impact of environmental and epigenetic factors will be minimized. Therefore, family studies of the rate of inhibitor concordance should lead to more accurate findings than studies of unrelated subjects. The present study is the first to evaluate how different factor VIII gene mutations relate to therapy-induced inhibitor formation in siblings. Except for families with the intron 22 inversion, the number of families in each subgroup was low. However, the study was not designed to evaluate the risk of inhibitors in each subgroup of mutation, but focuses on the concordance rate between siblings having the same genetic basis for the disease in order to extend the knowledge about the influence of genetic and environmental factors. A large proportion of the families had the intron 22 inversion (65.5%). This may be explained by the fact that this mutation is easily tested for in most laboratories, whereas the identification of other mutations requires more effort and is not always performed. Although the questionnaire asked for siblings with a phenotype of severe hemophilia A and a FVIII:C level <1%, three of the missense mutations, i.e. T118I, N1922S and R2304C, have also been associated with a more moderate phenotype in the HAMSTeRS database.[27-29] This reflects the variation in classification that can be seen in international multicenter studies and the reason why a definition of <2% for severe hemophilia is used in most studies. However, in our study, the FVIII:C level is less crucial, since the concordance rate in the families is related to the causative mutation. In agreement with previous data, we found that the type of factor VIII gene mutation appears to play an important role in that large rearrangements of the gene and other protein-truncating mutations were relatively frequently associated with inhibitors. However, even though the concordance within families was high, it is obvious that the mutation itself does not supply sufficient information to predict whether therapy-induced inhibitory antibodies will develop or not. The overall figure of 69.9% concordance includes both inhibitor-negative and inhibitor-positive families and suggests that other unidentified genetic factors are as important as the factor VIII gene mutation itself. This is even more obvious when analyzing concordance in the subgroup of families with an inhibitor history in at least one of the siblings. In this subgroup, the figure was approximately 40% both overall and in the largest subgroup of patients with intron 22 inversion. Interestingly, although perhaps only of anecdotal value, the monozygotic twins with intron 22 inversion and those with nonsense mutation were both concordant. The mutations belong to the high-risk group, but only the twins with the inversion had developed inhibitors. The type of inhibitor response was the same in 18 of the 25 concordant families with inhibitors in all siblings. The factors that determine the type of inhibitor are not known, but it is tempting to believe that mutations and polymorphisms in genes for co-factors and cytokines involved in the B- and T-cell interaction may either stimulate or suppress the native immune response. It is well known that both hemophiliacs and normal subjects form antibodies to factor VIII,[30-32] but it has not yet been established whether the immune response associated with the formation of non-inhibitory antibodies in patients with hemophilia is different from that causing inhibitory antibodies. It would be important to evaluate the presence of non-inhibitory as well as anti-idiotypic antibodies in the discordant siblings, since this may add additional information about how siblings within the same family react against replacement therapy.

Some of the observed mutations require additional comments. The nonsense mutation *R2209X* was found in three unrelated families, two of which were concordant for inhibitors and one discordant with a high-responding inhibitor in only one of the siblings. This mutation, which is located in the C2 domain, has previously been associated with inhibitors and patients with this mutation seem to be at high risk of

©Ferrata Storti Foundation

forming inhibitory antibodies. The reason why one of these siblings did not develop inhibitors is unknown.

The missense mutation *G686R* in the A2-domain has not previously been described, but was found in a family with four siblings. The mutation affects the donor splice site of exon 13 to an extent similar to that observed with the serine substitution at the same location and reported to the HAMSTeRS database. Both mutations are associated with a severe phenotype. Two of the siblings with the G686R mutation had developed inhibitors while two had not. They are all between 6 and 9 years of age. The two siblings without inhibitors have been on primary prophylaxis for several years and have had well above the critical level of exposure to replacement therapy. While the mechanism of inhibitor formation can be explained for the G686R mutation, it is unknown for the other two missense mutations, *S534P* and *N684D*. Both positions are completely conserved in the gene sequence for human, porcine, murine and canine factor VIII in the HAMSTeRS database, indicating a crucial role for protein function. Moreover, in the *S534P* variant, proline represents a helix-breaking amino acid likely to change the three dimensional structure of the factor VIII protein, while *N684D* represents a non-conservative amino acid exchange with respect to acidity and polarity. In the HAMSTeRS mutation database the *S534P* variant has been reported in two patients without inhibitor formation.

The small deletions of a nucleotide in exon 1 (C118) and four nucleotides in exon 14 (AAGA 3091-3094) were found in families in which both brothers had developed high-responding inhibitors. The mutations create a frameshift and have, according to the HAMSTeRS database, been associated with inhibitors in other cases.

A weakness of all retrospective analyses of inhibitors is that some transient and low-responding inhibitors may be overlooked. In addition, no data are available to characterize the presence of non-inhibitory antibodies. In our cohort of families with predominantly clinically significant, high-responding inhibitors (79.6% of all inhibitors in our series), the data indicate a genetic predisposition to inhibitor development. The fundamental capacity of patients to develop therapy-induced inhibitory antibodies probably relates to the type of causative gene defect, but several other mutations and/or polymorphisms will affect the final outcome. In addition, even in family studies, non-genetic factors may differ and the impact of these parameters remains to be settled. To fully appreciate the nature of all significant genetic factors involved, including the role of the MHC class I/II system and different cytokines, genome screening of large cohorts of patients and their relatives is warranted.

Appendix

The MIBS study group consists of the following centers and investigators - Malmö, Sweden (J Astermark/E Berntorp); Amsterdam, The Netherlands (K Fijn van Draat); Bonn, Germany (H Brackmann/J Oldenburg); Bratislava, Slovakia (A Batorova); Cardiff, UK (P Collins); Chapel Hill, USA (GC White/M Escobar); Duarte, USA (NP Ewing); Denver, USA (M Manco-Johnson); Frankfurt, Germany (C Escuriola-Ettinghausen); Gothenburg, Sweden (L Tengborn); Helsinki, Finland (F Ebeling); La Coruna, Spain (J Batlle); Lille, France (J Goudemand); Lyon, France (C Négrier); Madrid, Spain (A Villar); New York, USA (D DiMichele); Stockholm, Sweden (P Petrini/S Schulman); Toronto, Canada (M Carcao); Utrecht, The Netherlands (M van den Berg/E Mauser-Bunschoten); Wabern, Switzerland (R Kobelt).

JA: primary responsibility for the paper and design of the study, analysis and interpretation of data, creation of Tables 1-3 and writing the final version of the manuscript; JO: analysis and interpretation of factor VIII mutation data, provision of study materials and data, contribution to the discussion and writing of the final version of the manuscript; ME, GCW2nd: provision of study materials and data, interpretation of data and contribution to the discussion and writing of the manuscript; EB: administrative and logistic support, interpretation of data and contribution to the discussion and writing of the final version of the manuscript. The authors declare that they have no potential conflicts of interests.

This study was supported by grants from Wyeth and the Research Fund at Malmö University Hospital. Sharyne M. Donfield is gratefully acknowledged for her kind assistance and comments on the manuscript.

Manuscript received January 28, 2005. Accepted May 25, 2005.

©Ferrata Storti Foundation

References

1. Wight J, Paisley S. The epidemiology of inhibitors in haemophilia A: a systematic review. Haemophilia 2003;9:418-35.
2. Kreuz W, Ettingshausen CE, Auerswald G, Saguer IM, Becker S, Funk M, et al. GTH PUP Study Group. Epidemiology of inhibitors and current treatment strategies. Haematologica 2003; 88: E-Rep04.
3. Astermark J. Treatment of the bleeding inhibitor patient. Semin Thromb Hemostas 2003;29: 77-86.
4. Vermylen J. How do some haemophiliacs develop inhibitors? Haemophilia 1998;4:538-42.
5. Oldenburg J, Brackmann HH, Schwaab R. Risk factors for inhibitor development in hemophilia A. Haematologica 2000; 85 Suppl 10:7-13.
6. Schwaab R, Brackman HH, Meyer C, Seehafer J, Kirchgesser M, Haack A, et al. Haemophilia A: mutation type determines risk of inhibitor formation. Thromb Haemost 1995;74:1402-6.
7. Tuddenhamn EGD, Mcvey JH. Genetic basis of inhibitor development in haemophilia A. Haemophilia 1998; 4: 543-5.
8. Oldenburg J, Picard JK, Schwaab R, Brackmann HH, Tuddenham EGD, Simpson E. HLA genotype of patients with severe haemophilia A due to intron 22 inversion with and without inhibitors of factor VIII. Thromb Haemost 1997;77:238-42.
9. Oldenburg J, El-Maarri O, Schwaab R. Inhibitor development in correlation to factor VIII genotypes. Haemophilia

2002;8 Suppl 2:23-9.
10. Astermark J, Berntorp E, White GC, Kroner BL. The Malmö International Brother Study (MIBS): further support for genetic predisposition to inhibitor development in hemophilia patients. The MIBS Study Group. Haemophilia 2001;7:267-72.
11. Gill JC. The role of genetics in inhibitor formation. Thromb Haemost 1999;82: 500-4.
12. Goodeve A. Genetic determinants of inhibitor formation in patients with hemophilia. Haematologica 2003; 88 Suppl 12:2-3
13. Oldenburg J, Schröder J, Brackmann HH, Muller-Reible C, Schwaab R and Tuddenham E. Environmental and genetic factors influencing inhibitor development. Semin Hematol 2004;41 Suppl 1:82-8.
14. White GC II, Rosendaal F, Aledort LM, Lusher JM, Rothschild C, Ingerslev J. Recommendation of the Scientific Subcommittee on Factor VIII and Factor IX of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. On behalf of the Factor VIII and Factor IX Subcommittee. Definitions in hemophilia. Thromb Haemost 2001;85:560.
15. Oldenburg J. Mutation profiling in haemophilia A. Thromb Haemost 2001;85:577-9.
16. Kasper CK, Aledort LM, Counts RB, Edson JR, Frantatoni J, Green D, et al. A more uniform measurement of factor VIII inhibitors. Thromb Diath Haemorrh 1975;34:869-72.
17. Verbruggen B, Novakova I, Wessels H, Boezeman J, van den Berg M, Mauser-Bunschoten E. The Nijmegen modification of the Bethesda assay for factor VIII:C inhibitors: improved specificity and reliability. Thromb Haemost 1995; 73:247-51.
18. Lin S-W, Lin SR, Shen MC. Characterization of the genetic defects of hemophilia A in patients of Chinese origin. Genomics 1993;18:496-504.
19. Schwaab R, Oldenburg J, Tuddenham EGD, Brackmann HH, Olek K. Mutations in haemophilia A. Br J Haematol 1993;83:450-8.
20. Waseem NH, Bagnall R, Green PM, Gianelli F. Start of UK confidential haemophilia A database: analysis of 142 patients by solid phase fluorescent chemical cleavage of mismatch. Thromb Haemost 1999;81:900-5.
21. Liu ML, Nakaya S, Thompson AR. Non-inversion factor VIII mutations in 80 hemophilia A families including 24 with alloimmune responses. Thromb Haemost 2002;87:273-6.
22. Goodeve AC, Williams I, Bray GL, Peake IR. Relationship between factor VIII mutation type and inhibitor development in a cohort of previously untreated patients treated with recombinant factor VIII (Recombinate). Recombinate PUP Study Group. Thromb Haemost 2000;83:844-8.
23. Liu M, Murphy ME, Thompson AR. A domain mutations in 65 haemophilia A families and molecular modelling of dysfunctional factor VIII proteins. Br J Haematol 1998;103:1051-60.
24. Higuchi M, Kochhan L, Schwaab R, Egli H, Brackmann HH, Horst J, et al. Molecular defects in hemophilia A: identification and characterization of mutations in the factor VIII gene and family analysis. Blood 1989;74:1045-51.
25. Millar DS, Steinbrecher RA, Wieland K, Grundy CB, Martinowitz U, Krawczak M, et al. The molecular genetic analysis of haemophilia A; characterization of partial deletions in the factor VIII gene. Hum Genet 1990;86:219-27.
26. Citron M, Godm ilow L, Ganguly T, Ganguly A. High throughput mutation screening of the factor VIII gene (F8C) in hemophilia A: 37 novel mutations and genotype-phenotype correlation. Human Mutation 2002;20:267-74.
27. Schwaab R, Oldenburg J, Schwaab U, Johnson DJD, Schmidt W, Olek K, et al. Characterization of mutations within the factor VIII gene of 73 unrelated mild and moderate haemophiliacs. Br J Haematol 1995;91:458-64.
28. Higuchi M, Kazazian HH, Kasch L, Warren TC, McGinniss MJ, Phillips JA, et al. Molecular characterization of severe hemophilia A suggests that about half the mutations are not within the coding regions and splice junctions of the factor VIII gene. Proc Natl Acad Sci USA 1991;88:7405-9.
29. Liu ML, Shen BW, Nakaya S, Pratt KP, Fujikawa K, Davie EW, et al. Hemophilic factor VIII C1- and C2-domain missense mutations and their modeling to the 1.5-angstrom human C2-domain crystal structure. Blood 2000;96:979-87.
30. Algiman M, Dietrich G, Nydegger U, Boieldieu D, Sultan Y, Kazatchkine MD. Natural antibodies to factor VIII (anti-hemophilic factor) in healthy individuals. Proc Natl Acad Sci USA 1992;89:3795-9.
31. Gilles JG, Saint-Remy JM. Healthy subjects produce both anti-factor VIII and specific anti-idiotypic antibodies. J Clin Invest 1994;94:1496-505.
32. Moreau A, Lacroix-Desmazes S, Stieltjes N, Saenko E, Kaveri SV, D'Oiron R, et al. Antibodies to the FVIII light chain that neutralize FVIII procoagulant activity are present in plasma of nonresponder patients with severe hemophilia A and in normal polyclonal human IgG. Blood 2000;95: 3435-41.

©Ferrata Storti Foundation