Polymorphisms in the *IL10* but not in the *IL1beta* and *IL4* genes are associated with inhibitor development in patients with hemophilia A

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The aim of the Malmö International Brother Study (MIBS) is to evaluate host genetic factors associated with the development of inhibitory antibodies in patients with hemophilia. Factor VIII gene mutations and genetic polymorphisms of the *IL1beta*, *IL4*, and *IL10* genes, known to influence antibody production in autoimmune diseases, were analyzed in 164 patients (124 with severe, 26 with moderate, and 14 with mild disease) in 78 unrelated families with hemophilia A. Seventy-seven (47%) patients in 54 families had a history of inhibitors (57 high responding, 20 low responding). Inversions were found in 36 families (75 patients). There was no association between the development of inhibitor and the *IL1beta TaqI* RFLP alleles in exon 5 or the −590 C/T single nucleotide polymorphism (SNP) in the promoter region of *IL4*. There was, however, a strong association between an allele with 134 bp in one of the CA repeat microsatellites, *IL10G*, located in the promoter region of the *IL10* gene, and the development of inhibitor (odds ratio [OR], 4.4; 95% confidence interval [95% CI], 2.1-9.5; $P < .001$). The association was consistent in the subgroup of families with severe hemophilia and inversions. *IL10* is the first gene located outside the causative factor VIII gene mutation to be associated with inhibitor development. (Blood. 2006;107:3167-3172)

Introduction

Inhibitory antibodies to factor VIII develop in 10% to 15% of all patients with hemophilia A and in 25% to 30% of patients with the severe form of the disease after exposure to factor VIII concentrates.[1,2] Often the inhibitors become a longstanding problem that seriously affects quality of life and requires costly medical intervention.[3,4] Genetic and environmental risk factors for inhibitor development have been evaluated, usually without consistent results.[5,6] Except for some local outbreaks of inhibitors caused by neoantigenicity of a modified factor VIII molecule, no environmental risk factors have been unequivocally associated with inhibitor development. On the other hand, studies of related subjects have provided evidence for the major importance of genetic determinants.[7,8] We have previously reported an overall concordance of 78.3% between siblings in 249 families with severe hemophilia A and a higher frequency of inhibitors in African Americans compared with white persons.[8] Among the genetic factors, an association between large rearrangements of the factor VIII and IX genes and a higher risk for inhibitors has been described.[9-12] However, most patients with null mutations, including the intron 22 inversion, do not develop inhibitory antibodies. For example, the concordance rate for inhibitor development between siblings in families with intron 22 inversion was found to be only 40%.[13] It is obvious that other genetic markers influencing the immune response to replacement therapy in patients with hemophilia remain to be identified, and the aim of Malmö International Brother Study (MIBS) is to characterize these factors.[8,13]

Cytokines are more or less directly involved in the antibody-mediated immune response and therefore have the potential to be determinants for the immune response. IL-1 is a multifunctional proinflammatory cytokine affecting most immunomodulatory cells.[14,15] Two agonists (IL-1α and IL-1β) and one receptor antagonist (IL-1Rα) belong to this family. In addition to its proinflammatory actions, IL-1 also acts as an adjuvant during antibody production.[16] Associations with autoimmune diseases and high antibody production have been described for polymorphisms in the *IL1beta* gene, the most important of which is the *Taq*I restriction fragment length polymorphism (RFLP) 13.4-kb allele in exon 5, referred to as allele 2 (A2).[17,18] This A2 allele is associated with high spontaneous IL-1β production. IL-4 is essential for the generation of T_H2 cells and for total immunoglobulin and IgE production. Polymorphisms in the *IL4* gene, including a single nucleotide polymorphism (SNP) at position −590 in the promoter region, seem to be primarily associated with atopy and allergic disorders but are also associated with certain autoimmune and inflammatory conditions.[19] IL-10 is an important anti-inflammatory cytokine, and it exerts a broad spectrum of activities. IL-10 also enhances the in vitro production of all types of immunoglobulins

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A complete list of the members of the Malmö International Brother Study Group appears in "Appendix."

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Table 1. Characteristics of the study cohort with respect to inhibitor status

Type of hemophilia A	Distribution by severity of hemophilia and inhibitor status, no. (%)			Total, no.
	HR	LR	No inhibitor	
Severe	52 (41.9)	11 (8.9)	61 (49.2)	124
Moderate	2 (7.7)	5 (19.2)	19 (73.1)	26
Mild	3 (21.4)	4 (28.6)	7 (50.0)	14
Total	57 (34.8)	20 (12.2)	87 (53.0)	164

by peripheral blood mononuclear cells (PBMCs) in patients with systemic lupus erythematosus (SLE), and the serum concentration of IL-10 is correlated with disease activity in these patients.[20,21] In addition, a 134-bp–long variant of a CA repeat microsatellite in the promoter region of the *IL10* gene is associated with autoantibody concentrations in SLE, myasthenia gravis, and Wegener granulomatosis and with the concentration of the monoclonal immunoglobulin in multiple myeloma.[22-24] In the present study, we evaluated whether polymorphisms of these 3 cytokine genes may also confer susceptibility to inhibitor development in patients with hemophilia.

Patients, materials, and methods

Subjects

Centers participating in MIBS were given a standardized questionnaire to accrue data from siblings with severe (factor VIII:C, less than 1%), moderate (factor VIII:C, 1%-5%), and mild (factor VIII:C, greater than 5%-40%) hemophilia A. Patient date of birth, ethnicity, disease severity, treatment history, and inhibitor history, including peak titer and current titer in Bethesda units (BU/mL), were collected. A high-responding inhibitor was defined as a historical peak titer greater than 5 BU/mL, and a low-responding inhibitor was defined as one with a peak titer of 5 BU/mL or less.[25] Approval was obtained from the Lund University institutional review board for these studies. Informed consent was provided according to the Declaration of Helsinki.

Methods

Standard methods for the analyses of the factor VIII gene were used and included Southern blot and long-range polymerase chain reaction (PCR) for inversion analysis, PCR and mutation screening methods (eg, single-stranded conformation polymorphism [SSCP]), denaturing gradient gel electrophoresis (DGGE), denaturing high-performance liquid chromatography (DHPLC), and direct DNA sequencing.[26] Factor VIII and IX clotting activity were measured according to standard techniques. Inhibitory antibodies were quantified according to the original Bethesda method and the Nijmegen modified assay.[27,28]

DNA extraction and PCR reactions

Genomic DNA was extracted from EDTA-preserved blood using a kit (Qiagen genomic tip; Kebo Lab, Stockholm, Sweden). *IL1beta Taq*I RFLP, *IL4* −590 SNP, and *IL10G* microsatellites were essentially determined as previously described.[17,18,22,23,29,30] The oligonucleotides 5′GTTGTCATCAGACTTTGACC3′ and 5′TTCAGTTCATATGGACCAGA3′ were used to amplify the region containing the *Taq*I polymorphic site within exon 5 of the *IL1beta* gene.[17] Amplification was performed using a thermal cycler (PHC-3 Dri-Block; Techne, Cambridge, United Kingdom). *Taq*I digestion of the 249-bp PCR product resulted in 2 fragments of 135 and 114 bp (allele 1) or remained intact (allele 2).[18] The primers used to amplify the *IL4* gene were previously described by Noguchi et al.[29] A mismatch was introduced in the forward primer with a T at position 594 altered to G, creating a restriction site for the enzyme *Ava*II. The following primers were used: forward, 5′TAA ACT TGG GAG AAC ATG GT3′; reverse, 5′TGG GGA AAG ATA GAG TAA TA3′. The digestion of *IL4* PCR products was performed using 1.5 U *Ava*II and 10 μL PCR product in a 20-μL final volume reaction, incubated at 37°C overnight. Genotyping for the dinucleotide microsatellite *IL10G* was performed by the use of PCR and a fluorescence-based technique.[22,23] Primers for the highly polymorphic microsatellites *IL10G* were designed as 5′TetGTCCTTCCCCAGGTAGAGCAACACTCC3′ and 5′CTCCCAAAGAAGCCTTAGTAGTGTTG3′.[30] A fluorescent dye was introduced in the 5′ end of the forward primers. A mixture of PCR products and internal size standard (Gene Scan 350-TAMRA; Perkin Elmer, Norwalk, CT) was loaded onto 5% denaturing polyacrylamide gel (Long Ranger) in the ABI 377 sequencer. Data were analyzed with the GeneScan 672 (version 1.0) and the Genotyper software (version 2.1; Perkin Elmer). Alleles were named after the lengths of PCR products.[22,23]

Statistical analysis

Chi-square analysis was used for evaluation of the frequency of alleles in patients with and without a history of inhibitory antibodies. For observed numbers lower than 5, Fisher exact test was used instead. All *P* values were 2-sided; *P* less than .05 was considered to indicate statistical significance.

Results

The numbers of patients and families with no history of inhibitors and a history of high-responding (HR) and low-responding (LR) inhibitors are shown in Tables 1 and 2. Median age was 24 years (range, 3-79 years). Seventy-seven (47%) of the 164 subjects enrolled had a history of inhibitors. They were members of 54 unrelated inhibitor families. Thirty-four of these families were discordant and 20 were concordant with respect to inhibitor development. In 24 families, no inhibitor was reported in the siblings. Seven families (4 inhibitor concordant, 3 inhibitor discordant) included 3 or 4 siblings with hemophilia.

Factor VIII gene mutations

The type of causative factor VIII mutation was characterized in all families, and these data, together with the polymorphisms of the *IL1beta*, *IL4*, and *IL10* genes and the inhibitor history in each sibling, are summarized in Table 3. Seventy-one patients with

Table 2. Concordance and discordance in the 78 MIBS families with respect to inhibitor development

Type of hemophilia A	No. discordant families with 2 siblings		No. concordant families with 2 siblings				No. discordant families with more than 2 siblings			No. concordant families with more than 2 siblings			No. total
	HR/No	LR/No	HR/HR	LR/LR	HR/LR	No/No	HR/No/No	HR/LR/No	HR/No/No/No	HR/HR/HR	LR/LR/LR	No/No/No	
Severe	20	5	11	0	5	15	1	1	0	1	0	1	60
Moderate	2	2	0	0	0	6	0	0	0	0	1	1	12
Mild	1	1	0	1	1	1	0	0	1	0	0	0	6
Total	23	8	11	1	6	22	1	1	1	1	1	2	78

No = no inhibitor history.

Table 3. Characteristics of all 164 enrolled siblings in 78 unrelated MIBS families with severe hemophilia A

Family no.	Type of hemophilia A	Type of inhibitor*	Peak titer, BU/mL*	Factor VIII gene mutation	Novel mutation	*IL1beta* genotype*	*IL4* genotype*	*IL10G* 134 microsatellite*
1	Severe	High/Low	3000/4	intron 22 inv	No†	A1A1/A1A2	CC/CC	134/NA
2	Severe	No/No	NA	Ser170X	No	A2A2/A1A2	CC/TT	NA/134
3	Severe	No/No	NA	intron 22 inv	No	A1A2/A1A1	TT/CC	None with 134
4	Severe	High/Low	31/2	intron 22 inv	No	A2A2/A1A2	CC/CC	134/NA
5	Severe	High/No	565/NA	intron 22 inv	No	A2A2/A1A2	CT/CC	134/NA
6	Severe	No/High	NA/7	delA(3146-3149)	Yes	A1A1/A2A2	CT/CC	134/134
7	Severe	No/No	NA	intron 22 inv	No	A1A2/A1A2	CC/CT	None with 134
8	Severe	No/No	NA	intron 22 inv	No	A1A2/A1A2	CC/CC	None with 134
9	Severe	No/No	NA	IVS6 + 3A > G	No	A1A1/A1A2	CC/CC	None with 134
10	Severe	No/High	NA/150	Arg427X	No	A1A2/A1A2	TT/CT	None with 134
11	Severe	High/High	20/566	delAAGA3091-94	No†	A1A1/A1A1	CC/CC	134/134
12	Severe	Low/No	3/NA	intron 22 inv	No	A1A2/A1A2	TT/TT	None with 134
13	Severe	High/High	282/6	delT5574	No	A1A1/A1A1	CC/CT	134/134
14	Severe	No/High	NA/40	delA(3629-3637)	No	A2A2/A2A2	CC/CT	None with 134
15	Severe	Low/No	4/NA	insA(3864-3870)	No	A1A1/A1A1	CC/CC	134/NA
16	Severe	High/No	6/NA	Ser535Thr	Yes	A1A1/A1A2	CC/CC	134/NA
17	Severe	High/High/High	1100/1560/164	intron 22 inv	No	A1A1/A1A1/A1A1	CT/CT/CT	NA/NA/134
18	Severe	High/No	55/NA	intron 22 inv	No	A1A2/A1A2	TT/CC	None with 134
19	Severe	No/No	NA	intron 22 inv	No	A2A2/A2A2	CT/CC	None with 134
20	Severe	High/High	38/300	intron 22 inv	No	A1A2/A2A2	CT/TT	None with 134
21	Severe	High/Low	153/1	intron 22 inv	No	A1A2/A1A2	CC/CT	None with 134
22	Severe	No/No	NA	insA(3864-3870)	No	A1A2/A1A2	CC/CC	NA/134
23	Severe	No/No	NA	Arg2163Leu	Yes	A1A2/A1A2	CC/CC	None with 134
24	Severe	No/No	NA	Leu1856Arg	Yes	A1A1/A1A1	TT/CT	None with 134
25	Severe	No/No	NA	intron 22 inv	No	A1A2/A1A2	CC/CC	None with 134
26	Severe	No/High	NA/1024	intron 22 inv	No	A2A2/A2A2	CT/CC	None with 134
27	Severe	No/High	NA/22	intron 22 inv	No	A1A1/A1A1	CT/CC	None with 134
28	Severe	High/High	15/8	intron 22 inv	No	A1A2/A1A2	CC/CC	134/134
29	Severe	No/High	NA/90	intron 22 inv	No	A1A2/A1A2	CC/CC	None with 134
30	Severe	Low/High/No	1/70/NA	delT4159	Yes	A1A1/A2A2/A1A2	CC/CT/CC	134/NA/NA
31	Severe	No/High	NA/26	intron 22 inv	No	A2A2/A2A2	CC/TT	None with 134
32	Severe	High/No	560/NA	intron 1 inv	No†	A2A2/A1A1	CT/CT	134/134
33	Severe	No/No	NA	No mutation found	NA	A1A2/A1A1	CT/CT	134/NA
34	Severe	Low/No	3/NA	intron 22 inv	No	A1A1/A1A2	CC/CC	None with 134
35	Severe	No/No/No	NA	intron 22 inv	No	A1A1/A2A2/A1A1	All 3 sibs TT	None with 134
36	Severe	No/High	NA/43	intron 22 inv	No	A1A1/A1A2	CT/CT	None with 134
37	Severe	No/High/No	NA/146/NA	intron 22 inv	No	A1A1/A1A1/A1A1	CT/CT/CC	None with 134
38	Severe	Low/No	1/NA	intron 22 inv	No	A1A1/A1A1	CC/CC	None with 134
39	Severe	High/High	17/150	intron 22 inv	No	A2A2/A1A1	CC/CC	None with 134
40	Severe	High/High	11/12	intron 22 inv	No	A1A1/A1A2	CC/CC	134/NA
41	Severe	Low/High	1/6	Trp585Cys	No	A1A2/A1A1	CC/TT	134/NA
42	Severe	No/No	NA	No mutation found	NA	A2A2/A1A2	CC/CT	NA/134
43	Severe	Low/No	3/NA	intron 22 inv	No	A1A1/A1A1	CC/CT	None with 134
44	Severe	No/No	NA	intron 22 inv	No	A1A1/A1A1	CT/CT	None with 134
45	Severe	High/No	71/NA	Tyr629X	Yes	A1A1/A1A1	CC/CC	134/NA
46	Severe	High/Low	700/2	intron 22 inv	No	A1A2/A1A2	CC/CC	134/NA
47	Severe	No/No	NA	intron 22 inv	No	A1A1/A1A1	CT/CT	None with 134
48	Severe	High/High	840/72	del exon 1-6	No†	A1A2/A2A2	CC/CC	None with 134
49	Severe	High/High	6/6	intron 1 inv	No	A1A2/A1A2	TT/CT	134/134
50	Severe	High/High	6/6	intron 22 inv	No	A1A2/A1A2	CT/CT	None with 134
51	Severe	High/High	6/6	Trp1108X	Yes	A1A1/A1A1	CT/CT	None with 134
52	Severe	No/High	NA/11	intron 22 inv	No	A1A1/A1A1	CC/CC	None with 134
53	Severe	No/No	NA	insAC6321	No	A1A1/A1A1	CC/CC	None with 134
54	Severe	High/No	164/NA	intron 22 inv	No	A1A1/A1A2	CC/TT	134/134
55	Severe	No/High	NA/14	insA(5955-5961)	No†	A1A2/A1A2	CC/TT	None with 134
56	Severe	No/High	NA/19	intron 22 inv	No	A1A1/A1A1	CT/CC	NA/134
57	Severe	High/No	753/NA	intron 22 inv	No	A1A1/A1A1	CT/TT	134/NA
58	Severe	No/High	NA/68	insA(2940-2945)	No	A2A2/A2A2	CC/CT	None with 134
59	Severe	No/High	NA/10	delAT1631-1632	Yes	A1A2/A1A2	CC/CC	NA/134
60	Severe	High/High	6/6	Trp382X	Yes	A1A1/A1A1	CT/CC	134/NA
61	Moderate	No/No	NA	His209Arg	No	A1A1/A1A1	TT/TT	None with 134
62	Moderate	No/No	NA	Arg531Cys	No	A1A2/A1A2	CC/CC	None with 134
63	Moderate	Low/No	3/NA	His209Arg	No	A1A1/A1A1	CC/CC	NA/134
64	Moderate	No/No	NA	Arg1781Cys	No	A1A1/A1A1	CT/TT	None with 134

Table 3. Characteristics of all 164 enrolled siblings in 78 unrelated MIBS families with severe hemophilia A (continued)

Family no.	Type of hemophilia A	Type of inhibitor*	Peak titer, BU/mL*	Factor VIII gene mutation	Novel mutation	*IL1beta* genotype*	*IL4* genotype*	*IL10G* 134 microsatellite*
65	Moderate	No/No/No	NA	Arg2163His	No†	A1A1/A1A1/A1A2	CC/CC/CC	None with 134
66	Moderate	No/High	NA/19	Gly691Trp	No	A1A1/A1A2	CC/CC	None with 134
67	Moderate	No/No	NA	insA(3629-3637)	No	A2A2/A1A2	CC/CT	NA/134
68	Moderate	No/High	NA/9	Asp569Glu	Yes	A1A2/A1A2	CC/CC	NA/134
69	Moderate	Low/Low/Low	2/3/3	Leu184Pro	Yes	A1A1/A1A2/A1A1	CC/CC/TT	134/NA/NA
70	Moderate	No/No	NA	Val1733Leu	Yes	A2A2/A2A2	CT/CC	NA/134
71	Moderate	No/Low	NA/1	Pro1761Gln	Yes	A1A1/A1A2	CC/CC	NA/134
72	Moderate	No/No	NA	Val483Gly	Yes	A1A1/A1A2	CC/CC	None with 134
73	Mild	No/No/High/No	NA/NA/6/NA	Tyr2105Cys	No†	A1A1/A1A1/A1A1/A1A1	CC/CC/CC/CC	134/NA/134/NA
74	Mild	Low/Low	2/1	Phe2101Cys	No	A2A2/A2A2	CC/CC	134/134
75	Mild	High/No	7/NA	Pro1854Leu	No	A2A2/A1A1	CC/CT	NA/134
76	Mild	No/No	NA	Arg1696Gly	No	A1A1/A1A1	CC/CC	None with 134
77	Mild	Low/High	1/42	Arg593Cys	No†	A1A1/A1A1	TT/CT	None with 134
78	Mild	Low/No	2/NA	Arg593Cys	No†	A1A1/A1A2	CT/CC	None with 134

Numbering of the mutations was according to cDNA. Siblings are numbered in consecutive order, with sibling 1 the oldest brother.
*All values are for siblings 1/2/3/4.
†Mutation previously associated with inhibitors.

intron 22 inversion and 4 patients with intron 1 inversion were identified in 36 unrelated families. Forty (53.3%) of these patients belonging to 28 families had experienced inhibitors. Siblings were concordant with respect to inhibitors in 11 families and discordant in 17 families. In the 42 families without inversions, the following types of mutation were identified: nonsense mutations in 5 families, a large deletion in 1 family, missense mutations in 21 families, a splice site mutation in 1 family, and small deletions/insertions in 12 families (Table 3). In 2 families, no mutation was identified. Fourteen mutations were novel and not previously described in the HAMSTeRS database (Table 3). Among these novel mutations, 10 were associated with inhibitors; in 3 families, all siblings developed inhibitors (Leu184Pro, Trp382X, Trp1108X). Among the 6 mutations previously described and associated with inhibitors, only the Arg2163His mutation was not associated with inhibitors in our cohort.

IL1beta

Frequencies of *IL1beta Taq*I genotypes in patients with and without inhibitory antibodies are summarized in Table 4. Genotype A2/A2 was found in 28 (17.1%) of the patients, 22 of whom had severe hemophilia A. Among these 28 patients, 16 (57.1%) had experienced an inhibitor and 12 had not (42.9%; not significant [NS]). Corresponding figures for the A1/A2 genotype were 26 (44.1%) and 33 (55.9%; NS) of 59 patients. No significant association between the A2 allele and the development of inhibitors was seen. Inhibitory antibodies were described in 42 of 87 patients with allele A2 (48.3%) compared with 35 (45.5%) of 77 patients without this allele (NS). The prevalences of genotype A2/A2 and allele A2 were higher than previously described in a Swedish white population.[18]

IL4

IL4 genotypes identified in the cohort are summarized in Table 5. No significant associations between the genotypes or the individual C- and T-alleles were found. The most common genotype, CC, was found in 97 (59.1%) of the patients, of whom 45 (46.4%) had a history of inhibitors. The CT genotype was found in 45 (27.4%) patients, of whom 23 (51.1%; NS) had inhibitors. Corresponding figures for the TT genotype were 22 (13.4%) and 9 (40.9%; NS), respectively. Altogether, inhibitors were described in 32 (47.8%) of 67 with the T-allele to be compared with 45 (46.4%) of 97 patients without this allele (NS). The prevalence of genotypes and alleles were comparable to those described in a white population.[29]

IL10

The frequency of allele 134 in the microsatellite *IL10G* is shown in Table 6. The allele was identified in 44 of 164 (26.8%) patients with hemophilia A. Thirty-two of these 44 (72.7%) patients developed inhibitors (25 HR, 7 LR) compared with 45 (37.5%) of the 120 patients without the allele (32 HR, 13 LR). Among all 77 patients with a history of inhibitors, allele 134 was found in 32 (41.6%) patients compared with 12 (13.8%) of 87 inhibitor-negative patients ($P < .001$). This corresponds to an odds ratio (OR) of 4.4 with a 95% confidence interval (95% CI) of 2.1 to 9.5. A significant

Table 4. Frequencies of *IL1beta Taq*I genotypes and allele A2

IL1beta	No. with severe hemophilia A		No. with moderate Hemophilia A		No. with mild hemophilia A		Total, no. (%)
	Inhibitor positive	Inhibitor negative	Inhibitor positive	Inhibitor negative	Inhibitor positive	Inhibitor negative	
Genotype							
A1/A1	28	26	3	10	4	6	77 (47.0)
A1/A2	22	26	4	6	0	1	59 (36.0)
A2/A2	13	9	0	3	3	0	28 (17.1)
Allele							
A2 positive	35	35	4	9	3	1	87 (53.0)
A2 negative	28	26	3	10	4	6	77 (47.0)
Total	63	61	7	19	7	7	164 (100.0)

Table 5. Frequencies of *IL4* genotypes and allele T

	No. with severe hemophilia A		No. with moderate Hemophilia A		No. with mild hemophilia A		
IL4	Inhibitor positive	Inhibitor negative	Inhibitor positive	Inhibitor negative	Inhibitor positive	Inhibitor negative	Total (%)
Genotype							
CC	35	33	6	13	4	6	97 (59.1)
CT	21	18	0	3	2	1	45 (27.4)
TT	7	10	1	3	1	0	22 (13.4)
Allele							
T positive	28	28	1	6	3	1	67 (40.9)
T negative	35	33	6	13	4	6	97 (59.1)
Total	63	61	7	19	7	7	164 (100.0)

association between allele 134 and the development of inhibitors was also found in a subgroup analysis of patients with severe hemophilia A—that is, in 26 (41.3%) of 63 patients with inhibitors and in 7 (11.5%) of 61 patients without inhibitors, corresponding to an OR of 5.4 (95% CI, 2.1-13.7; $P < .001$). Fourteen (87.5%) of 16 patients with an inversion and allele 134 had inhibitors compared with 26 (44.1%) of 59 patients with an inversion but not the *IL10* allele (OR, 8.9; 95% CI, 1.9-42.6; $P = .002$). Among the patients with moderate and mild hemophilia, the allele was identified in 6 (42.9%) of 14 patients with inhibitors and in 5 (19.2%) of 26 patients without inhibitors, corresponding to an OR of 3.2 ($P = .147$). Similar to the polymorphisms of the *IL1beta* gene, the prevalence of allele 134 was higher than that described in a Swedish white cohort.[23]

Among the 34 inhibitor discordant families, only one family was identified in whom the subject without allele 134 had developed an inhibitor, whereas the brother who was allele 134 positive had not (Table 3). This is a family with moderate hemophilia, and the inhibitor developed in adulthood after more than 100 exposure days (EDs) during hepatitis C treatment with interferon and ribavirin. It is a low-responding inhibitor, and the patient achieved successful tolerization with immune tolerance induction therapy. Among the other families, 12 others were also discordant with regard to allele 134, but the allele was only found in the sibling who had developed an inhibitor. In the remaining inhibitor-discordant families, either both or none of the siblings were allele 134 positive.

Discussion

In this study, we found a highly significant association between an allele with 134 bp in the CA repeat microsatellite *IL10G* and the development of inhibitory antibodies against factor VIII, whereas no association was found with the polymorphisms in the genes coding for IL-1β and IL-4. The association with *IL10* was consistent in the subgroup of patients with severe hemophilia and an inversion of the factor VIII gene. In the entire series, only one subject without this allele developed inhibitor, and his brother with allele 134 did not. In all other families, there was a consistent relation between the presence of allele 134 and the development of inhibitor. *IL10G* is located in the promoter region of the *IL10* gene (that is, in a part of the gene containing several potential transcription factor binding sites). The relation of allele 134 to high antibody production has been established in autoimmune disorders and in multiple myelomas.[22-24] IL-10 promotes activated B lymphocytes to produce antibodies, and results from one study on patients with multiple myeloma indicate that the effect of allele 134 is to increase the secretion of IL-10.[24] Patients with the *IL10G* allele 134 might be "high-secretor" phenotypes who, on antigenic stimuli with the deficient coagulation factor, develop an expansion of B cell clones and ultimately inhibitor development. Whether the polymorphism in these patients is associated with a higher IL-10 secretion in vivo remains to be analyzed. Interestingly, Lozier et al[31] recently described that in addition to the MHC H-2 (class II) genes in mice, linkage was established between the antibody response and polymorphic markers near other candidate immunoregulatory genes, including *Il10*. In addition, mice deficient in IL-10 had low antibody response to human factor IX, suggesting that IL-10 promotes the formation of high antibody concentrations.

The prevalence of *IL1beta* allele A2 and of *IL10G* allele 134 were significantly higher than previously described in a Swedish population.[18,22,23] Whether this reflects differences in the ethnic composition of this study or a true genetic association in the hemophilia population remains to be determined.

The type of causative factor VIII and IX mutations is considered an important genetic determinant for inhibitor development, but the mutation itself will probably not serve as the most critical determinant for the outcome. The most common factor VIII mutation in patients with severe hemophilia A is the intron 22 inversion. This is a null mutation causing a complete rearrangement of the gene and is considered to be a high-risk mutation for the development of inhibitors. In our series of 124 patients with severe hemophilia A, an inversion was identified in 75 subjects in 36 unrelated families. Within these families, all siblings in 11 families had developed inhibitors, in 8 families none of the siblings had a history of inhibitors, and in 17 families the siblings were discordant. In agreement with this finding, we recently reported a 40% concordance for inhibitors in families with intron 22 inversion.[13] The findings strengthen

Table 6. Frequencies of allele 134 bp in the *IL10G* microsatellite

	No. with severe hemophilia A		No. with moderate Hemophilia A		No. with mild hemophilia A		
IL10G	Inhibitor positive	Inhibitor negative	Inhibitor positive	Inhibitor negative	Inhibitor positive	Inhibitor negative	Total (%)
Allele 134 positive	26	7	3	3	3	2	44 (26.8)
Allele 134 negative	37	54	4	16	4	5	120 (73.2)
Total	63*	61	7	19	7	7	164 (100)

*OR, 5.4 (95% CI, 2.1-13.7; $P < .001$) for the presence of allele 134 among patients with severe hemophilia A and inhibitors.

the concept of inhibitor formation to be a polygenic process. *IL10* is the first gene located outside the causative factor gene mutation to be associated with this side effect of treatment. Functional analyses of IL-10 and other cytokines should be performed in patients after antigenic challenge. In addition, further studies of genetic markers for inhibitor development are warranted because they will be a prerequisite for the development of new and less immunogenic therapeutic approaches in the future.

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Appendix

The MIBS study group consists of the following geographic locations and investigators: Malmö, Sweden (J. Astermark, E. Berntorp); Amsterdam, The Netherlands (K. Fijn van Draat, M. Peters); Bratislava, Slovakia (A. Batorova); Bucharest, Romania (V. Uscatescu); Budapest, Hungary (L. Nemes); Gothenburg, Sweden (L. Stigendahl); Helsinki, Finland (F. Ebeling); Izmir, Turkey (K. Kavakli, C. Balkan, D. Yilmaz); La Coruna, Spain (J. Batlle); London, United Kingdom (T. Yee, C. Lee); Madrid, Spain (A. Villar, M. Morado); Oslo, Norway (G. Tjønnfjord); Santander, Spain (C. Sedano); Stockholm, Sweden (P. Petrini, S. Schulman); Toronto, Canada (M. Carcao); Utrecht, The Netherlands (M. van den Berg, E. Mauser-Bunschoten); Wabern, Switzerland (R. Kobelt); Warsaw, Poland (J. Windyga).

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