HHS Public Access

Author manuscript

Am J Hematol. Author manuscript; available in PMC 2015 July 16.

Published in final edited form as:

Am J Hematol. 2014 April ; 89(4): 375–379. doi:10.1002/ajh.23645.

Mutation analysis of a cohort of US patients with hemophilia B

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Abstract

Hemophilia B (HB) is a disorder resulting from genetic mutations in the Factor 9 gene (*F9*). Genotyping of HB patients is important for genetic counseling and patient management. Here we report a study of mutations identified in a large sample of HB patients in the US. Patients were enrolled through an inhibitor surveillance study at 17 hemophilia treatment centers. A total of 87 unique mutations were identified from 225 of the 226 patients, including deletions, insertions, and point mutations. Point mutations were distributed throughout the *F9* gene and were found in 86% of the patients. Of these mutations, 24 were recurrent in the population, and 3 of them (c.316G>A, c.1025C>T, and c.1328T>A) accounted for 84 patients (37.1%). Haplotype analysis revealed that the high recurrence arose from a founder effect. The severity of HB was found to correlate with the type of mutation. Inhibitors developed only in severe cases with large deletions and nonsense mutations. None of the mild or moderate patients developed inhibitors. Our results provide a resource describing *F9* mutations in US HB patients and confirm previous findings that patients bearing large deletions and nonsense mutations are at high risk of developing inhibitors.

Introduction

Hemophilia B (HB) is caused by mutations in the Factor 9 gene (*F9*) that result in decreased or defective Factor IX protein (FIX). The disorder is characterized by recurrent and prolonged bleeding. Based on the activity level of FIX, the disease severity is classified as severe: <1% of the normal FIX activity, moderate: 1–5% of the normal FIX activity, and mild: >5% and <40% of the normal FIX activity [1]. *F9* is mapped at chromosome Xq27.1 [2]. It contains 8 exons that span 33.5 kb. The gene, mainly expressed in liver cells, encodes a 2.8 kb mRNA, 1.4 kb of which is translated to synthesize a protein composed of 461 amino acids [3]. The protein is a zymogen containing a signal peptide, a pro-peptide, and a protease. The signal peptide and the pro-peptide are removed from the zymogen, and the serine protease is secreted into plasma. The protease participates in the intrinsic coagulation pathway after activation by Factor VII (FVII) or Factor XI (FXI) [3].

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Additional Supporting Information may be found in the online version of this article.

Conflict of interest: Nothing to report.

The current therapy for HB is replacement with highly purified, plasma-derived products or recombinant FIX [4]. However, population based studies indicate that 1–3% of HB patients develop inhibitory antibodies to treatment products [5], and the risk of inhibitor could increase to greater than 20% in patients with complete deletions of the FIX gene [6,7]. When compared with Factor VIII inhibitors in hemophilia A (HA), the incidence of FIX inhibitors is low, but unlike HA, most inhibitors in HB are high titer and occur concurrently with allergic reactions or anaphylaxis [8]. Importantly, immune tolerance induction (ITI) therapy is not effective for most HB patients [7,9]. As a result, the development of an inhibitor severely complicates the disease management and significantly increases the cost of treatment. It has been shown that the incidence of inhibitor development is associated with certain mutation types [7,8]. The inhibitor risk is estimated to be 50% for HB patients with complete deletions of *F9* and 20% for patients with nonsense or frame shift mutations [7]. For those with missense mutations, the risk is estimated to be almost zero [9]. Therefore, the molecular genotyping for HB provides valuable information for inhibitor prediction and could result in treatment alterations to prevent inhibitors.

More than 1,000 distinct mutations causing HB have been identified worldwide. These mutations are documented in various online databases [10,11]. The majority (>70%) of the reported mutations are point mutations, 16% are deletions. The rest are insertions, duplications, and combinations of deletions and insertions [10,11]. A few large re-arrangements have also been described [12]. These mutations are distributed throughout the *F9* gene, including exons, introns, promoter, and untranslated regions. Large deletions, nonsense mutations, and frameshift mutations, resulting from insertions and small deletions, are frequently associated with severe phenotype; while missense mutations are associated with severe, moderate or mild HB. Some mutations have been reported repeatedly. Most of these involve a CG to TG or CA change at CpG dinucleotides, which are considered as mutation hot spots due to the spontaneous deamination of methylated cytosine at these locations [13]. Some of the mutations, particularly those causing mild disease such as c. 1025C>T, have unusually high frequency in certain populations. This is caused, at least partly, by founder effects [14]. It is estimated that founder effect mutations account for 20–30% of mild HB disease [14,15].

Molecular genotyping for HB allows effective genetic counseling and provides insight into the mechanism through which a mutation causes HB. Population-based studies of *F9* mutations have been performed in various ethnic groups [6,16–19]. As of 2010, the number of HB patients enrolled in US federally funded Hemophilia Treatment Centers (HTCs) was 4,209 [20]. However, the genotypes for most of these US HB patients have not been reported. As a part of the Centers for Disease Control and Prevention (CDC) Hemophilia Inhibitor Research Study (HIRS) [21], we recruited HB patients from 17 US HTCs and conducted genotyping. Preliminary data were reported in 2011 [21]. Since then, 76 more HB patients were enrolled, and currently a total of 229 patients have been genotyped. In this paper, we report the mutations identified in these US HB patients, analyze the frequencies, and assess the risk for inhibitor development.

Methods

Data collection

The subjects of this study were HB male patients enrolled at 17 federally funded HTCs in the Hemophilia Inhibitor Research Study (HIRS) [22]. Patient information such as demographics, inhibitor history, and other variables were collected as previously described [22]. Ethnicity and race were self-reported and classified into six categories: White non-Hispanic (White), Black non-Hispanic (Black), Hispanic White (Hispanic), Asian/Pacific Islander, Native American (Indian/Alaskan), and Other or Mixed. Disease severity was determined based on the baseline activity level of FIX provided by the enrolling HTC and was classified as severe (<1 unit per deciliter [U/dl]), moderate (≥1 and ≤5 U/dl) or mild (>5 and <40 U/dl) [1]. For those with a history of an inhibitor at enrollment, the historical peak inhibitor titer was recorded on the enrollment form by the enrolling sites. The protocol was approved by the Institutional Review Boards of the CDC and each participating HTC. Informed consent was obtained from participants or parents of minor patients.

Laboratory methods

The blood collection, handling, DNA preparation, and methods for sequencing of *F9* in HB patients enrolled in the CDC surveillance project were described in Miller et al. [21]. The sequencing covered all exons, 5′- and 3′-untranslated regions (UTR), all exon–intron junction regions, and part of the introns. Large deletions were identified by the failure of sequencing analysis and validated by multiplex ligation-dependent probe amplificaiton (MLPA) according to the manufacturor's protocol (SALSA MLPA P207 F9, MRC Holland, Amsterdam, The Netherlands).

The sequencing data were analyzed with SeqScape (Applied Biosystems). The cDNA (c.) and protein (p.) nomenclature of *F9* mutations were based on nucleotide reference sequence NM_000133.3 and protein reference sequence NP_000124.1 according to the recommendations of the Human Genome Variation Society (HGVS). *F9* mutation numbering in the human genome (version 19) and the Yoshitake annotation were included as well. The codon number was determined based on the notation defined in Yoshitake et al. [23], with the number 1 assigned to the first amino acid of the processed mature protein. Novel mutations were identified by comparing with the FIX mutation database (http://www.factorix.org/) [10]. Based on the frequencies of polymorphisms in our patient population, variations at five sites of *F9* were used to construct *F9* haplotypes: c.–1145A/G, c.–822A/G (rs411017), c.580A/G (rs6048), c.687G/A (rs440051), and c. *836_*837GT/GTGT.

All the mutations, together with phenotypic severity and inhibitor information, are listed in a Microsoft Excel file named CHBMP-US, which can be accessed at http://www.cdc.gov/hemophiliamutations/.

Results

Mutation frequencies in US hemophilia B patients

Between 2006 and 2012, blood samples of 232 HB patients were collected from 17 US HTCs. Six were excluded due either to the refusal for genetic testing by the patient or to sample problems. A total of 226 patients were genotyped by direct sequencing. The characteristics of the patient population are shown in Table I. The age at registration ranged from <1 to 84 years, with an average of 18.4 years. More than 80% of the patients were White and of non-Hispanic ethnicity. Severe and moderate patients were more prevalent than mild cases. A total of 87 unique mutations in *F9* were identified in 225 (99.6%) of the 226 patients. There was one case in which no mutation in *F9* was identified; however, additional specimens for repeat sequencing were not available. A full list of the mutations is shown in Supporting Information Table SI. Sixty of the mutations (70%) were found each in only one patient, while the rest (30%) were recurrent mutations. Nine mutations were not previously reported in the HB database (http://www.factorix.org/) or in the literature at the time of analysis. These novel mutations included one missense, two splice site, two nonsense and four deletions (Supporting Information Table SI).

Table II shows the frequencies of the mutation types and reported inhibitor development. All the common mutation types in *F9* that were previously reported were represented in our patients, including: missense, frameshift caused by deletions or insertions, nonsense, synonymous, large deletions (>50 bp), small in-frame deletions, mutations in the promoter, and mutations at splicing sites. The most common mutation type was missense (70.4%). Nonsense (11.1%), large deletions (6.2%), and frameshift (4.4%) mutations were seen frequently as well. Three patients had substitutions in the promoter (c.–35G>A). Fourteen patients had large deletions (>50 bp), identified by the failure of sequencing and confirmed by MLPA. Of these, 11 involved complete deletion of *F9* (Supporting Information Figure S1). However, the exact break points in 13 of the 14 patients were not determined and the mutations were named at exon level. Three patients had synonymous mutations, which were the only mutations seen in those patients. Two of these had mild, and one had moderate disease (Supporting Information Table SI). Nine HB patients each carried two distinct mutations. One patient had 3 *F9* mutations. In one patient, the identified two mutations appeared to be both causative: one was c.836_837delCA, which resulted in frameshift near the 3′-end of exon 7, and the other one was c.277G>A/p.Asp93Asn, which was reported to cause severe HB in the HB database (http://www.factorix.org/) [10]. A duplication (c.839-20dupA) in intron 7 was found in two patients who carried additional mutations (c.880C>T/p.Arg294stop and c.223C>T/p.Arg75stop, respectively). c.839-20dupA was reported to be present in 6 HB patients in the HB mutation database (http://www.factorix.org/). However, there were insufficient data to support a causative role of this mutation. In the remaining cases, a primary mutation appeared to contribute to the disease, while the second or third mutation seemed to be variants and play a minor role. Two of the variants seen in patients with multiple mutations were silent mutations in coding regions (c.51C>T/p.Ile17Ile, and c.192T>C/p.Cys64Cys); all others were located in non-coding regions: two in 5′-UTR (c.–682G>C and c.–929G>A) and three in 3′-UTR (c. *858-*859delAC, c. *828_*872del, and c. *799A>T). All these variants have not been reported in

literatures to date. It is not clear if they play a causative role in HB. Inhibitors had developed in 15 subjects: 7 of them had large deletions in *F9*; 8 had nonsense mutations (Table II).

The mutations identified in our patients were distributed throughout the exons, introns, the promoter, and the UTR regions of *F9* gene, with the majority of them located in the coding regions. Most of them fell into the protease domain (Supporting Information Figure SI). Fewer mutations were identified in the signal peptide, pro-peptide, and activation domains. The missense mutations identified in these domains were positioned in the junction regions to their adjacent domains (Supporting Information Figure). These mutations could cripple the normal cleavage process of FIX during its secretion or activation. No other mis-sense mutations were identified inside these domains, indicating that they are dispensable for normal function of FIX.

Twenty-six mutations occurred in more than one patient. Of these, 18 were missense mutations, 4 were nonsense mutations, 3 were point mutations in promoter, 2 were point mutations in intron 4, and 2 were small deletions (Table III). These recurrent mutations accounted for 154 patients (68.1%). Ten of the mutations occurred in more than three patients. Three of them, c.316G>A, c.1025C>T, and c.1328T>C, were present in 26, 38 and 20 patients, respectively. These three mutations accounted for 84 (54.5%) of the 154 patients (Table III). Of these 84 patients, 81 exhibited either mild or moderate phenotypes. Of the patients with either mild or moderate disease, 59.6% had one of these 3 mutations. Notably, 11 of the 24 recurrent point mutations were involved in changes from CG to TG or CA at the CpG dinucleotides and occurred in 98 (65.3%) of the 150 patients with point mutations (Supporting Information Figure S2). To examine if a founder effect contribute to the 24 recurrent mutations, we performed a haplotype analysis in these patients using the following variants: c.–1145A/G, c.–822A/G (rs411017), c.580A/G (rs6048), c.687G/A (rs440051), and c. *836_*837GT/GTGT. Eleven Haplotypes were identified in our populations and were named H1–H10, respectively (Supporting Information Table SII). As shown in Table III, different haplotypes were found in patients with 10 of the 26 mutations, suggesting that these recurrent mutations arose independently. The remaining 14 mutations had one haplotype associated with each mutation, including c.316G>A (26 patients), c.1025C>T (38 patients) and c.1328T>C (20 patients), suggesting a common ancestor for at least these three most prevalent mutations.

Severity and inhibitors in hemophilia B

As shown in Table IV, the HB severities were correlated to mutation types. Certain mutation types predominantly resulted in severe HB. These mutations included: frameshift (90.0%), nonsense (84.0%), large deletion (100%), and mutations affecting splicing (100%). However, missense mutations and small deletions resulted in a wide spectrum of severity (Table IV). Notably, two patients with large deletions were reported with FIX activity levels of 15 and 2%, respectively. Since we relied on the site's report for FIX level, we could not confirm these findings. It is unlikely that patients with large deletions of F9 would have measurable FIX activity when not infused. These levels probably do not reflect true baseline levels. As a result, these two samples were excluded. Three children each carried a point mutation in the promoter (c.–35G>A) in the region of mutations reported to cause the

Leyden phenotype [24]. Two of them were moderate and one was mild at the time of testing when they were 2, 5, and 10 years old, respectively. It is highly possible that these patients will have normal level of FIX activity when they reach puberty. Synonymous mutations were identified in three patients with mild or moderate HB (Table IV). Two patients in whom we could not identify a mutation showed mild and moderate HB phenotypes, respectively. The mechanisms underlying this are not known.

Table V lists the 15 HB patients with inhibitors. The peak inhibitor titer before ITI ranged from 2.8 to 92.5 BU/ml. The exposure days at the time of inhibitor development ranged from <20 days to >150 days. Inhibitors were reported to develop primarily in HB patients with severe disease carrying large deletions, frameshift, or nonsense mutations [6,7]. Consistent with this, all of the 15 patients with inhibitors showed severe disease. All of them carried either a large deletion or a nonsense mutation. None of the patients carrying frameshift mutations developed inhibitors. Among the 10 nonsense mutations identified in our population, three mutations (p.Arg75*, p.Ser220*, and p.Arg294*) were associated with inhibitors (Table V). Two of these nonsense mutations (p.Arg75* and p.Arg294*) have been previously reported to be associated with inhibitors in the HB database (http://www.factorix.org/). The other nonsense mutation (p. Ser220* [c.659C>G]) is a novel mutation identified in this study; 5 of the 15 patients with inhibitors underwent ITI therapy, 3 failed, 1 is ongoing, and 1 succeeded. Owing to the low success rate of ITI for HB inhibitor patients [7,9], most of these patients were treated with bypass agents such as recombinant Factor VIIa (NovoSeven) (Table V).

Discussion

Identification of causative mutations in HB patients is important for genetic counseling and prediction of inhibitor risk. The genotyping for HB in various countries performed over the past decades is documented in HB databases, available online at http://www.factorix.org/ and http://www.cdc.gov/hemophiliamutations [10,11]; however, mutation and inhibitor development data reflecting the US HB patients are not well represented. In this study, we identified 87 unique mutations among 226 patients enrolled in federally funded US HTCs. Of the identified mutations, 86.3% were point mutations and 11.1% were deletions, which are similar to the frequencies reported in the factor IX mutation databases [10,11]. We could not identify mutations in one of the patients. One reason for this is that mutations could exist in introns which our sequencing did not cover. Another possibility is that these patients possess somatic mosaicisms, and the blood cells from which DNA was extracted for genotyping were normal. Three patients who showed HB symptoms carried synonymous mutations: c.484C>A (p.Arg162Arg), c.87A>G (p.Thr29Thr), and c.723G>A (p.Gln241Gln). These three synonymous mutations were reported in the FIX mutation database (http://www.factorix.org/) with 5, 7 and 4 patients, respectively. The previously reported patients were from different countries and exhibited mild, moderate or severe HB. It has been reported that synonymous mutation could cause human disease by affecting the structure and stability of mRNA or the synthesis rate of proteins [25]. Although the mechanisms of synonymous mutations in causing HB are not yet established, the recurrence of these mutations suggests the mutations are possibly causative.

Among the nine novel mutations not reported previously, three were large deletions, two were nonsense that potentially resulted in premature stop of translation, one was a small deletion causing a frameshift at p.Ala279, and one was a missense mutation (p.Tyr312Asp). Missense mutations affecting p.Tyr312 have not been reported before. The amino acid altered as a result of the missense mutation is located in the protease domain, is conserved in porcine, mouse and canine FIX, and may play an important role in maintaining normal structure or function of the enzyme. The remaining two novel mutations were located in introns. One was a point mutation at a splice site (c.277+1G>C). Different point mutations at this site (c.277+1G>A and c.277+1G>T) have been previously reported [10,11]. Another novel intronic mutation identified was a deletion in intron 4 (c.392-22_392-21del). A point mutation at c.392-22 (C>G), which possibly interferes with splicing, was previously identified in a mild HB patients [10,11]. In this study, the patients with deletion of 2 base pairs at this site showed severe HB. It would be reasonable to assume that these defects are causative of HB.

Three mutations, c.316G>A, c.1025C>T, and c.1328T>C, were found in 26, 38, and 20 of our patients, respectively, accounting for 84 (37.2%) of the 226 patients. These three recurrent mutations were present in patients enrolled in 8, 12, and 7 HTC sites, respectively, and thus were not clustered by HTC and region. A previous study reported that these three mutations contributed to 25% of HB in the US [26]. One mechanism contributing to the high recurrence of these mutations is the founder effect [26,27]. Haplotype analysis was consistent with a common ancestor for these three mutations in our patient population. Of the remaining recurrent mutations with lower (most under 5) occurrences, 10 are present in patients with different haplotypes (Table III), suggesting that these mutations arose independently. CpG dinucleotides are considered to be hot spots for mutations and are responsible for the recurrent mutations in unrelated families due to the spontaneous deamination of 5-methylcytosine to thymidine [28]. In agreement with this, 6 of the 9 (66.7%) recurrent point mutations, which occurred independently, were involved in a C>T or G>A change at CpG sites.

About 87% of the moderate and 93% of mild patients carried missense mutations, while 43% of the severe patients had a missense mutation. Some mutation types, including frameshift, nonsense, splice, and large deletion, were predominately associated with severe phenotypes, while small deletions and promoter mutations were related to moderate or mild phenotype. Nonsense and frameshift mutations are expected to produce a prematurely stopped, unstable protein and to cause severe HB [12]. Consistent with this, 90% of the frameshift and 84% of the nonsense mutations were reported as having severe disease. Interestingly, one patient with a frameshift caused by c.401_407del and four patients carrying nonsense mutations (c.880C>T, c.892C>T, and c.223C>T) showed moderate disease. These mutations were also reported in the FIX mutation database as showing phenotype variations (http://www.factorix.org/). A limitation of the study is that data on baseline factor levels were collected from the enrolling sites and were not confirmed in the CDC laboratory. For example, two patients with large deletions were reported to have baseline factor levels of 2% and 15%, respectively. These levels probably do not reflect true baseline levels.

When compared with HA, the incidence of inhibitors in HB is low, but most occur at high titer with anaphylaxis. Importantly, ITI therapy is not effective [7,9]. Large gene deletions and nonsense mutations are high risk factors for inhibitor development [6,7]. The risk for inhibitor development is estimated to be 50% for patients with large deletions and 20% for those carrying nonsense or frameshift mutations [7]. For missense mutations, the risk is almost zero [9]. Our study confirmed these previous findings: only patients with large deletions and nonsense mutations were reported to have developed inhibitors. Surprisingly, none of the frameshift mutations was associated with inhibitors. No inhibitors were reported in patients with other mutation types.

Knowledge of genotype may promote changes in treatment plan to prevent inhibitor development. Our study provides a resource containing *F9* mutations in a large sample of US HB patients and describes their heterogeneous mutation profile. Furthermore, it confirms previous findings that large deletions and nonsense mutations are risk factors for inhibitor development. With this information, the resource would be helpful for genetic counseling and the prediction of inhibitor development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank all patients and who participated and the study administrator and coordinators. The findings and conclusions in this paper are those of authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Contract grant sponsor: CDC Foundation (through Pfizer Inc. and Baxter Healthcare).

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TABLE I

Characteristics of Study Population

	Hemophilia B patients (n = 226)	
	N	**(%)**
Age at enrollment (years)		
<1	17	7.5
1–1	35	15.5
3–5	34	15.0
6–10	35	15.5
11–18	30	13.3
>18	75	33.2
Race		
White non-Hispanic	184	81.4
Black non-Hispanic	17	7.5
White Hispanic	12	5.3
Asian	3	1.3
Native American	1	0.4
Other or mixed	9	4.0
Severity (U/dl)		
Severe <1	90	39.8
Moderate 1–5	89	39.4
Mild >5	47	20.8

U/dl, units per deciliter.

TABLE II

Frequencies of Mutation Type and Inhibitors in US Hemophilia B Patients

Mutation type	No. of patients (%)	No. of patients with inhibitors (%)
Missense	159 (70.4)	0
Nonsense	25 (11.1)	8 (32.0)
Frameshift	10 (4.4)	0
Splice site change	6 (2.7)	0
Large deletion	14 (6.2)	7 (50.0)
Small deletion	5 (2.2)	0
Synonymous	3 (1.3)	0
Promoter	3 (1.3)	0
No mutation	1 (0.4)	0
Total	226 (100)	15 (6.6)

TABLE III

Recurrent Mutations in US HB Patients

HGVS cDNA name	HGVS protein name	No. of Patients	CpG site	Haplotype (No. of patients
c.127C>T	p.Arg43Trp	2	Y	H1(2)
c.128G>A	p.Arg43Gln	2	Y	H1(2)
c.224G>A	p.Arg75Gln	2	Y	H8(2)
c.223C>T	p.Arg75Stop	9	Y	H1(8); H3(1)
c.268C>T	p.Gln90Stop	2	N	H1(2)
c.301C>A	p.Pro101Thr	2	N	H3(2)
c.301C>G	p.Pro101Ala	4	N	H4(4)
c.304T>C	p.Cys102Arg	4	N	H1(4)
c.316G>C	P.Gly106Arg	2	N	H1(2)
c.316G>A	p.Gly106Ser	26	Y	H1(26)
c.317G>A	p.Gly106Asp	3	N	H3(2); H5(1)
c.572G>A	p.Arg191His	4	Y	H1(2);H3(1);H2(1)
c.677G>A	p.Arg226Gln	3	Y	H1(1);H2(1);H7(1)
c.786T>G	p.Ile262Met	2	N	H3(2)
c.802T>C	p.Cys268Arg	2	N	H1(2)
c.880C>T	p.Arg294Stop	5	Y	H1(2);H3(1);H7(1);H8(1)
c.881G>A	p.Arg294Gln	4	Y	H1(3); H2(1)
c.881G>T	p.Arg294Leu	4	N	H9(3);H10(1)
c.1025C>T	p.Thr342Met	38	Y	H1(38)
c.1067G>A	p.Trp356Stop	3	N	H1(1);H2(1);H3(1)
c.1240C>A	p.Pro414Thr	2	N	H3(2)
c.1328T>C	p.Ile443Thr	20	N	H4(20)
c.545_546del	p.Ser182Cysfs*6	2		H1(1);H4(1)
c.627delC	p.Ile210Phefs*35	2		H1(2)
c.–35G>A	promoter	3	Y	H1(2);H6(1)
c.392-1G>C	Intron 4	2	N	H9(2)

TABLE IV

Associations Between Severity and Mutation Type

	Severe	Moderate	Mild
Mutation type	n (% of total)	n (% of total)	n (% of total)
Missense	39 (24.5)	77 (48.4)	43 (27.0)
Frameshift	9 (90.0)	1 (10.0)	0
Nonsense	21 (84.0)	4 (16.7)	0
Synonymous	0	1 (33.3)	2 (66.7)
Splice site	6 (100.0)	0	0
Large deletion	12 (100.0)	0	0
Small deletion/insertion	3 (60.0)	2 (40.0)	0
Promoter	0	2 (66.7)	1 (33.3)
None	0	1(100.0)	0
Total	90(40.2)	88 (39.3)	46 (20.5)

Severity was determined by the FIX levels provided by the HTCs.

TABLE V

HB Patients with a History of Inhibitors

ID	HGVS cDNA name	Protein change	RACE	Exposure days at inhibitor development	Peak inhibitor titer before ITI	ITI[a]	Bypass agent prescribed
HIS3061	c.223C>T	p.Arg75*	WH	<20	30.0	Not done	Yes
HIS1969	c.223C>T	p.Arg75*	WH	>150	8.0	Not done	Yes
HIS1990	c.223C>T	p.Arg75*	WNH	<20	25.8	Not done	Yes
HIS2022	c.223C>T	p.Arg75*	WNH	>150	92.5	Not done	No
HIS1922	c.223C>T	p.Arg75*	WH	>150	65.0	Success	Yes
HIS1943	c.223C>T	p.Arg75*	WH	>150	40.0	Failure	Yes
HIS0286	c.–29-?_1386+?del	del exon 1–8	WNH	<20	2.8	Not done	N/A
HIS1985	c.–29-?_1386+?del	del exon 1–8	WNH	101–150	N/A	Not done	Yes
HIS2011	c.–29-?_1386+?del	del exon 1–8	WNH	6	46.0	Not done	Yes
HIS1940	c.–29-?_1386+?del	del exon 1–8	OTH	<20	N/A	Not done	Yes
HIS1939	c.–29-?_1386+?del	del exon 1–8	OTH	<20	N/A	Failure	Yes
HIS0248	c.–29-?_520+?del	del exon 1–5	OTH	51–100	19.2	Not done	Yes
HIS1978	c.659C>G	p.Ser220*	WNH	<20	12.0	Ongoing	Yes
HIS1925	c.880C>T	p.Arg294*	BNH	N/A	10.0	Not done	Yes
HIS1891	c.89–?_723+?del	del Exon 2–6	WNH	>150	N/A	Failure	Yes

[a] ITI, immune tolerance induction.