Identification of the Intron 22 and Intron 1 Inversions of the Factor VIII Gene in Iraqi Kurdish Patients With Hemophilia A

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Abstract
Hemophilia A (HA) is a severe coagulation disorder affecting 1 in 5000 to 10 000 male births. In severe cases, the most deleterious large DNA rearrangements are inversions of intron 22 (Inv22) and intron 1 (Inv1) of the factor VIII (FVIII) gene. These account for 40% to 50% and 1% to 5% of all causative mutations, respectively. Nevertheless, no genetic analysis to identify the actual causative mutation of FVIII, particularly Inv22 and Inv1, among Iraqi Kurdish hemophiliacs has been performed. In this study, we aimed to genotype Inv22 and Inv1 of the FVIII gene in our patients with HA and reveal the genotype/phenotype correlation with the inversion mutations and their role as a risk factor for the development of inhibitors. Analyses of the Inv22 and Inv1 mutations in 80 Iraqi Kurdish patients with HA (60 severe, 18 moderate, and 2 mild) were performed using the inverse shifting–polymerase chain reaction (IS-PCR) method. In severe cases, 46.7% (28/60) had Inv22 and 3.3% (2/60) had Inv1. The genotype/phenotype relation of Inv22 and Inv1 illustrated a statistically significant association ($P = .012$) between disease severity and inversion mutations. Slightly more patients with Inv22 (39%) developed inhibitors than those without Inv22 (28%; odds ratio $= 1.65$, 95% confidence interval $= 0.56-4.87$, $P = .361$). Inv22 is a major cause of severe HA in Iraqi Kurdish patients, and IS-PCR is a rapid, robust, and effective method that can be applied for carrier detection and prenatal diagnosis of HA in developing countries.

Keywords
FVIII mutations, hemophilia A, intron 1 inversion, intron 22 inversion, Iraqi Kurds, IS-PCR

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Introduction
Hemophilia A (HA) is the most common hereditary bleeding disorder. It is an X-linked recessive disease arising from a defect in the factor VIII (FVIII) gene leading to a deficiency in FVIII clotting activity. Clinically, the disorder is miscellaneous and is classified according to the residual FVIII clotting activity (FVIII: C); FVIII: C <1% is severe, FVIII: C 1%-5% is moderate, and FVIII: C >5%-40% is mild.1 Hemophilia A appears among all ethnic groups with an incidence of approximately 1 in 5000 to 10 000 live male births.2 In Iraq, the prevalence is 3.6 per 100 000 males according to the World Federation of Hemophilia.3

The FVIII gene is located at chromosome Xq28 and spans around 186 kb; it consists of 26 exons.4 The mutations reported in the FVIII gene are very heterogeneous and are often gene rearrangements, point mutations, or large deletions or insertions (HAMSTeRS http://europium.mrc.rpms.ac.uk).5 Large DNA inversions interrupting intron 22 of the FVIII gene (Inv22) and splitting the FVIII gene into 2 opposite parts are the most frequent defect that leads to HA. Inv22 is considered to be the causative mutation in around 40% to 50% of patients with severe HA.6 These mutations occur as a result of

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intrachromosomal homologous recombinations between semi-
identically directed duplicons—predominantly during male gametogenesis.\textsuperscript{5}

Inv22 ensues when a 9.5-kb region of intron 22—designated int22h-1 (intron 22 homologous region1) and positioned within the FVIII gene locus—recombines with either of the 2 copies in this region. Examples are int22h-2 (leading to Inv22-type 2 or proximal type) and int22h-3 (leading to Inv22-type 1 or distal type). These lie approximately 400 kb upstream from the FVIII gene.\textsuperscript{7,8}

The intron 1 inversion (Inv1) is also a large molecular defect and is found in 2\% to 5\% of patients with severe HA.\textsuperscript{9-11} Intron 1 of the FVIII gene involves a region of 1041 bp (Int1h-1) that has one extragenic copy (Int1h-2; 140 kb telomERICally). An intrachromosomal recombination between Int1h-1 and its extragenic copy, Int1h-2, causes FVIII Inv1.\textsuperscript{9} The 2 inversion mutations impede the formation of full-length FVIII messenger RNA (mRNA) and lead to the absence of the FVIII protein thus causing severe HA.\textsuperscript{6,7,9} It has been reported that both Inv22 and Inv1 mutations may augment the risk of the formation of inhibitors that interfere with treatment.\textsuperscript{6,7,12-14}

Carrier identification and prenatal diagnosis (PND) based on genetic testing are fundamental tools required for the prevention of this high-cost disease in developing countries. To date, in Iraq, genetic testing for carrier detection and PND of HA has not yet commenced. Direct mutation identification is considered better than indirect linkage analysis; nonetheless, a broad range of difficulties exist in their application because of restricted resources in developing countries. No genetic testing has yet been performed to identify the actual causative mutation of FVIII, particularly Inv22 and Inv1, among Iraqi Kurdish hemophiliacs. Thus, our goal here was to genotype the inversion mutations of intron 22 and intron 1 of the FVIII gene using the inverse shifting–polymerase chain reaction (IS-PCR) method among Iraqi Kurdish patients with HA to reveal the genotype/phenotype correlation with the inversion mutations as well as their role as a risk factor in the development of inhibitors.

**Patients and Methods**

**Patients**

Our study involved 80 patients with HA from 64 unrelated families registered at the local hemophilia treatment centers (Iraqi Society of Hemophilia). Approval for the study was obtained from the local institutional ethics committee (approval number 55 dated September 7, 2017). Informed consent was collected from all the patients. A diagnosis of HA was determined according to each patient’s personal and family history of bleeding, a prolonged activated partial thromboplastin time (aPTT), and a reduced FVIII level. Plasma FVIII: C was measured using an aPTT-based 1-stage clotting assay via the Stago blood coagulation analyzer (STA Compact Max; Diagnostica Stago, Asnières sur seine, France) according to the manufacturer’s instructions. Based on their conformance to the corresponding FVIII level, 75\% (60/80) of the hemophiliacs were classified as having severe HA (FVIII: C <1\%), 22.5\% (18/80) had moderate HA (FVIII: C 1\%–5\%); and 2.5\% (2/80) had mild HA (FVIII: C >5\%–40\%). No previous family history of HA (indicated by sporadic disease) was noted in the 19 (23.8\%) patients; 61 (76.2\%) patients were familial cases. The aPTT mixing study was performed to detect the presence of inhibitors. Of the 80 patients, 22 (27.5\%) developed inhibitors: 20 patients had severe and only 2 patients had moderate disease. Inhibitor titrations were carried out using the Nijmegen modification of the Bethesda assay.\textsuperscript{15} Most patients exhibited high titers and 2 patients showed low titers. The patients exhibited a mean antibody titer of 99.65 BU/mL (range: 4-825 BU/mL). The relevant clinical data such as age, gender, ethnicity, family history of inhibitors, age at first exposure to FVIII (recombinant or other blood products), and number of exposure days (EDs) were obtained from the patients.

**Genotyping of the Inv22 and Inv1 Mutations by IS-PCR**

We used the IS-PCR protocol of Rossetti et al\textsuperscript{16} to detect Inv22 and Inv1 mutations of the FVIII gene with minor modifications. Briefly, IS-PCR involves genomic DNA restriction by the BcII enzyme followed by self-ligation to form BcII circles and PCR analysis. Two PCR tests were carried out: (1) an Inv22 diagnostic test to detect Inv22 mutations (types 1 and 2) and (2) an Inv1 diagnostic test to identify Inv1 mutation.

**Inverse Shifting-PCR Protocol**

High molecular weight genomic DNA was extracted from 5 to 10 mL peripheral blood samples using a salting-out procedure.\textsuperscript{17} The genomic DNA was quality- and quantity-controlled by ultraviolet (UV) spectrophotometry or agarose gel electrophoresis. Then, genomic DNA (20 \(\mu\)L of 100 ng/\(\mu\)L or a larger volume of a lower concentration) was digested with 20 U of the restriction enzyme BcII (Thermo Fisher Scientific Inc., Rockford, IL, US) and incubated at 55°C for 4 hours in a 50-\(\mu\)L reaction volume. The digested DNA was purified using phenol–chloroform and ethanol precipitation. Alternatively, heat inactivation of the BcII enzyme was achieved via incubation at 80°C for 30 minutes. DNA circles were induced with 3 U of T4 DNA ligase (Promega Madison, Wisconsin, United States) in a 400-\(\mu\)L reaction volume incubated at 4°C overnight. The DNA ligation reaction required this large volume (400 \(\mu\)L) to produce DNA circles because it favors the self-ligation of both staggered ends of each DNA restriction fragment. This leads to an increase in the rate of circular to linear DNA segments. The ligated DNA samples were then isolated using equal volumes of the phenol–chloroform mixture; the upper aqueous phase was thoroughly removed, and the ethanol-precipitated DNA was retrieved in 30-\(\mu\)L distilled water.

Amplification used 25-\(\mu\)L final volume containing 3 and 6 \(\mu\)L of circularized DNA for the analysis of Inv1 and Inv22, respectively, with the addition of 0.6 \(\mu\)M of each primer (Sinclon Company, Tehran, Iran), 0.5 U of Super Taq DNA
polymerase (Gen Fanavar Co, Tehran, Iran), 1 × PCR buffer, 0.2 mM of each dNTP (Gen Fanavar Co, Tehran, Iran), and 1.4 mM MgCl2 (Gen Fanavar Co, Tehran, Iran). The primer sequences used for the Inv22 and Inv1 diagnostic tests were described earlier by Rossetti et al16 and are shown in Table 1.

The PCR program was set to an initial denaturation of 94°C for 2 minutes followed by 30 cycles of amplification that included 30 seconds of denaturation at 94°C, 1 minute of annealing at 56°C, 90 seconds of elongation at 72°C, and a final 5 minutes for extension at 72°C. The IS-PCR products were resolved using SYBR safe-stained 1.5% agarose gel electrophoresis and photographed under UV illumination. Even though the Inv22 and Inv1 diagnostic tests by IS-PCR had already been verified,16 we set the results of 10 blindly selected patients with an inverse PCR-based approach side by side. The blood samples were transferred to the molecular diagnostic center of the Tehran University of Medical Science, Iran, where inverse PCR was performed according to the Rossetti et al.18

**Statistical Methods**

The statistical package SPSS (version 23) was used to analyze the data: The continuous data are presented as the medians, means, and ranges. The χ2 test was applied to compare the differences in the categorical data. P values <.05 were regarded as statistically significant. Inhibitor risk associations with a particular genotype were reported as odds ratios (ORs) with 95% confidence intervals (CIs).

**Results**

To patients characteristics investigate Inv22 and Inv1 mutations, a group of 80 patients with HA from 64 unrelated families were analyzed using an IS-PCR method. The median age of all the patients was 19 years (range: 5-38 years). Of the 80 patients, 29 (36.3%) were positive for Inv22 (23 had Inv22-1 and 6 had Inv22-2). Among the studied patients, only 2.5% (2/80) had Inv1 (Table 2). The relative percentage of Inv22-1 to Inv22-2 was 79.3% and 20.7%, respectively. An accurate concordance between the Inv22 and Inv1 results was achieved from both the IS-PCR test and the Inverse-PCR-based assay for the 10 patients (4 were Inv22 positive and 6 were Inv22 negative; all were Inv1 negative).

### Table 1. The Sequences of Oligonucleotide Primers of Inv1 and Inv22.

| Primer | Sequence 5’ to 3’ | NC-000023.9 |
|--------|-------------------|-------------|
| I-IU   | GCCGATTGCTTATTTATATC | 153899635-54 |
| I-ID   | TCTGCAACTGTTACCTCATC | 153886959-77 |
| I-ED   | GCCATTTCAATCCACACT | 154030453-71 |
| 1-ID   | TCTGCAACTGTTACCTCATC | 153886959-77 |
| 1-IIU  | GCCGATTGCTTATTTATATC | 153899635-54 |
| 1-ED   | GCCATTTCAATCCACACT | 154030453-71 |

Abbreviation: ED, exposure day.

### Table 2. Frequency of Inversion Mutations in the Studied Hemophilia A Patients.

| Genotype of Inversion Mutations | Count (n = 80) | Percentage |
|--------------------------------|---------------|------------|
| Wild-type allele (normal)      | 49            | 61.3       |
| Intron 22 inversion type 1 (Inv22-1) | 23      | 28.7       |
| Intron 22 inversion type 2 (Inv22-2) | 6      | 7.5        |
| Intron 1 inversion (Inv1)       | 2             | 2.5        |
| Total                           | 80            | 100        |

### Table 3. Genotype–Phenotype Relation of Inversion Mutations of FVIII Gene in the Studied Hemophilia A Patients.

| Disease Severity | Intron 22 and 1 Inversion Mutations |
|------------------|-------------------------------------|
|                  | Mild (n = 2) | Moderate (n = 18) | Severe (n = 60) | P Value |
| Inv22             | 0    | 1 | 5.5 | 28 | 46.7 | .012 |
| Inv1              | 0    | 0 | 0  | 2  | 3.3  |     |
| Wild-type         | 2    | 100 | 17 | 94.5 | 30 | 50 |
| Total             | 2    | 100 | 18 | 100 | 60 | 100 |

Abbreviation: FVIII, factor VIII.

The genotype/phenotype relation of the FVIII Inv22 and Inv1 mutations in the studied patients with HA demonstrated a statistically significant association (P = .012) between disease severity and the inversion mutations (Table 3). Among the hemophiliacs with severe disease, 46.7% (28/60) had Inv22 (22 patients had Inv22-1, 6 patients had Inv22-2), 3.3% (2/60) had Inv1, and only 5.5% (1/18) of patients with moderate HA had Inv22. The 2 patients with mild HA had neither Inv22 nor Inv1 mutations (Table 3). Twelve of the 31 (38.7%) inversion mutation-positive patients (29 Inv22 positive, 2 Inv1 positive) were sporadic HA cases. The remaining 19 (61.3%) patients were familial cases.

Table 4 details the presence of the Inv22 and Inv1 mutations and the formation of inhibitors. Among the 29 Inv22 positive patients, 38% (11/29) with severe HA developed inhibitors and 3.4% (1/29) with moderate HA developed inhibitors. The 2 carriers of the Inv1 mutation did not develop inhibitors.
**Table 4. Presence of Intron 1 and 22 Inversions and Development of FVIII Inhibitors.**

| Inversion Mutations | Mild, N | Moderate, N | Severe, N |
|---------------------|---------|-------------|-----------|
| Inv1                | 0       | 0           | 2         |
| Inv22-1             | 0       | 0           | 15        |
| Inv22-2             | 0       | 0           | 2         |
| Wild Type           | 2       | 16          | 21        |
| Total               | 2       | 16          | 40        |

| Inhibitor Positive (n = 58) | Inhibitor Negative (n = 60) |
|----------------------------|-----------------------------|
| Inhibitor Positive (n = 20) | Inhibitor Negative (n = 40) |
| Age in years:               |                              |
| Mean (±SD)                  | 15 (±8)                     |
| Range                       | 5-30                        |
| Age at first exposure to FVIII, N (%): | | |
| <6 months                   | 6 (30)                      |
| ≥6 months                   | 14 (70)                     |
| Mean in months (±SD)        | 18 (±15)                    |
| Exposure days, N (%):       |                              |
| <150                        | 20 (100)                    |
| ≥150                        | 0 (0)                       |
| Mean exposure days (±SD)    | 58 (±27)                    |
| Range                       | 9-120                       |
| Positive family history of inhibitors, N (%): | |
| Inhibitor Positive (n = 20) | Inhibitor Negative (n = 40) |
| Total                       | 20 (33.3)                   |

**Table 5. Characteristics of the Studied Patients With Severe HA With Respect to Age, Age at First Exposure to FVIII, Exposure Days, and Family History of Inhibitors.**

| Severe HA (n = 60) | Inhibitor | Inhibitor |
|--------------------|-----------|-----------|
| Positive (n = 20)  | Negative  |
| Age in years:      |           |           |
| Mean (±SD)         | 15 (±8)   | 22 (±8)   |
| Range              | 5-30      | 5-30      |
| Age at first exposure to FVIII, N (%): | | |
| <6 months          | 6 (30)    | 11 (27.5) |
| ≥6 months          | 14 (70)   | 29 (72.5) |
| Mean in months (±SD) | 18 (±15) | 35 (±47) |
| Exposure days, N (%): |       |       |
| <150               | 20 (100)  | 12 (30)  |
| ≥150               | 0 (0)     | 28 (70)  |
| Mean exposure days (±SD) | 58 (±27) | 188 (±82) |
| Range              | 9-120     | 35-450   |
| Positive family history of inhibitors, N (%): | |
| Inhibitor Positive (n = 20) | Inhibitor Negative (n = 40) |
| Total              | 20 (33.3) |

**Table 6. Correlation of Intron 22 Inversion With Development of FVIII Inhibitors in Patients With Severe Hemophilia A.**

| FVIII Inhibitors | Positive, N (%) | Negative, N (%) | Total | P Value | OR (95% CI) |
|------------------|-----------------|-----------------|-------|---------|-------------|
| Intron 22        | Positive        | Negative        |       |         |             |
| Inversions       | N (%)           | N (%)           |       |         |             |
| Positive         | 11 (39)         | 17 (61)         | 28 (100) | .361    | 1.65 (0.56-4.87) |
| Negative         | 9 (28)          | 23 (72)         | 32 (100) |         |             |
| Total            | 20 (33.3)       | 40 (66.7)       | 60 (100) |         |             |

Abbreviations: FVIII, factor VIII; HA, hemophilia A; SD, standard deviation.

Overall, 41.4% (12/29) of the patients with positive Inv22 had developed inhibitors. In addition, the characteristics of the severe patients with HA with regard to age, age at first exposure to FVIII, EDs, and family history of inhibitors are detailed in Table 5.

The value of Inv22 as a predisposing factor for the development of inhibitors was also evaluated in patients with severe HA (60 cases). The presence of the FVIII Inv22 mutation was not a major predisposing factor for the formation of inhibitors. Nonetheless, slightly more patients with the Inv22 mutation (11/28 [39%]) developed inhibitors than patients without Inv22 (9/32 [28%]) giving an OR of 1.65 (95% CI = 0.56-4.87, P = .361) as shown in Table 6.

**Discussion**

In this study, we analyzed FVIII Inv22 and Inv1 mutations among 80 Iraqi Kurdish patients with HA using IS-PCR. To the best of our knowledge, this report is the first such study on this population. Inv22 appeared to be a remarkable cause of HA: It was found in 36.3% (29/80) of the patients in our study.

Furthermore, this mutation was detected in 46.7% (28/60) of the severe hemophiliacs. The literature shows that Inv22 is the most frequent large structural rearrangement of FVIII arising during male meiosis due to the high extent of similarity and the opposite orientation of the extragenic copies (int22h-2, int22h-3) versus the intragenic homologous region (int22h-1). The higher rate of Inv22 in males might be explained by the unpaired X chromosome during spermatogenesis which promotes the flipping of the tip of its long arm. Missing a normal X chromosome as a template for gene repair also places male gametes at a disadvantage versus oocytes when facing recombinant errors during gene duplication. The frequency of Inv22 in patients with HA in different studies is somewhat variable.

The frequency of Inv22 in the severe hemophiliacs in our study is consistent with previous reports from some countries (40%-55%), these values are higher than reports from the United Kingdom, France, and Lebanon in which lower frequencies of Inv22 ranging from 17.6% to 31% were recorded. This is most probably due to the inclusion of patients with all categories of disease severity. A comprehensive review of the frequency of Inv22 in severe HA in different countries worldwide is illustrated in Table 7.

The majority of the Inv22 mutations observed here involved the distal homologous region (Int22h-3) leading to Inv22-1 (79.3%). This finding is consistent with Antonarakis et al who showed that type 1 (distal) Inv22 is 5 times more frequent than type 2 (proximal) inversion. Likewise, other studies have demonstrated that most (70%-96%) Inv22 includes the distal A gene (type 1).

The reported prevalence of Inv1 in the literature is around 2% to 5% in patients with severe HA, which agrees with our...
result of 3.3% among severe cases. Table 7 shows the prevalence of Inv1 in severe HA from various regions of the world.

This study proved the main contribution of Inv22 and Inv1 as causative mutations in severe HA. The genotype–phenotype correlation of the Inv22 and Inv1 mutations in the investigated patients with HA showed that, among the severe cases, 46.7% (28/60) had Inv22 and 3.3% (2/60) had Inv1. Both inversion mutations are recognized as large structural rearrangements in the FVIII gene that interfere with the production of full-length FVIII mRNA thus leading to severe disease. The high frequency of Inv22 observed among severe cases in our study agrees with reports from other countries such as the United Kingdom (45%), Germany (45%), Italy (42%), France (46%), Iran (40%), India (44%), Mexico (45%), Brazil (49%), and Argentina (45%). Nevertheless, the frequency is lower than that of Arabs (55%) and higher than earlier reports from China (37%), Japan (33.3%), and Albania (10.5%), as shown in Table 7.

In this study, only 1 (5.5%) of the 18 patients with moderate HA had Inv22; the 2 mild cases carried the wild-type allele. These findings agree with Polakova et al and Abou-Elew et al because they recorded the presence of Inv22 in 8% and 10% of the patients with the moderate phenotype, respectively, and in none with mild disease. Importantly, Margaglione et al contradicted the premise of Inv22 as a cause of mild or moderate HA. In our study, the patient with moderate HA who was found to carry the Inv22 mutation was a 6-year-old boy who had an FVIII:C of 1% (assayed twice), no family history of HA, and inhibitors (14.24 BU/mL) to infused recombinant FVIII at the age of 20 months after 10 EDs. He experienced a moderately severe phenotype with multiple joints and mucous membrane bleeding. A possible explanation is that the index case is a somatic mosaic and a common event in HA—particularly in sporadic cases. Furthermore, Goodwe and Peake noticed that many patients with HA have a disparity between FVIII:C measured by 1- or 2-stage assays and their genotype.

In this series, more than one-third (38.7%) of the inversion mutation-positive patients comprised sporadic HA cases consistent with the literature. An isolated case of HA may follow the transmission of the defective FVIII gene through an asymptomatic carrier female, from a de novo mutation in the mother (resulting in her being a carrier), or a new mutation in the hemophilic (true de novo mutation). The existence of somatic and germ line mosaicism has to be considered as well. Patients with HA having somatic mosaicism and mutations including point mutations, large deletions, small deletions, and distal Inv22 have been reported. According to Oldenburg et al, somatic mosaicism of Inv22 indicates that this mutation is not completely confined to meiotic cell divisions; rather, it may also occur during mitotic cell division at the beginning of embryogenesis either in germ cells or in somatic cells.

Our data revealed that 41.4% (12/29) of the patients with positive Inv22 had developed inhibitors. According to the literature, the proportion of patients with Inv22 who develop inhibitors is quite variable (5%-51%).

Variable multicenter and review studies have shown that the major genetic factor involved in inhibitor formation is the type of FVIII mutation. Inv22 is the main hotspot mutation of the FVIII gene and a common cause of severe HA; thus, it is a risk factor worth screening for in patients with inhibitors. Here, the presence of the FVIII Inv22 mutation was not a major risk factor for the development of inhibitors. However, slightly more patients with an Inv22 mutation developed inhibitors (11/28 [39%]) than those without Inv22 (9/32, [28%]) giving an OR of 1.65 (95% CI = 0.56-4.87, P = .361); this finding agrees with prior reports.

One limitation of this study was the inability to screen the inversion-negative patients for other mutations due to the large size and complexity of the FVIII gene (~186 kb length comprising 26 exons) as well as its highly mutational heterogeneity. This makes the screening of the mutations challenging in underresourced molecular diagnostic laboratories.

In conclusion, we show here that Inv22 and Inv1 accounted for 46.7% and 3.3% of patients with severe HA, respectively. Thus, Inv22 can be considered a major cause of severe HA in Iraqi Kurdish patients. Although direct mutation identification is a resource-intensive method, the FVIII Inv22 and Inv1 IS-PCR approach is a rapid, robust, and effective method that can be applied for carrier detection and PND of HA in developing countries. Moreover, although inversion mutations were found to be associated with the severe phenotype in our study, Inv22 was not a major risk factor for inhibitor development.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Table 7. Frequency of Inv22 and Inv1 in Patients With Severe HA From Different Region of the World.

| Country            | No. of Patients | Inv22 (%) | Inv1 (%) | Reference |
|--------------------|----------------|-----------|----------|-----------|
| Kurdistan of Iraq  | 80             | 46.7      | 3.3      | Current   |
| United Kingdom     | 209            | 45        | 5        | study     |
| United Kingdom     | 51             | 17.6      | 5.9      |           |
| Germany            | 753            | 45        | 2        |           |
| Italy              | 93             | 42        | 3        |           |
| France             | 241            | 46        | 1        |           |
| France             | 94             | 22.3      | NA       |           |
| Iran               | 124            | 40        | 2        |           |
| Iran               | 30             | 47        | 7        |           |
| India              | 80             | 44        | 4        |           |
| North India        | 110            | NA        | 3.6      |           |
| Mexico             | 31             | 45        | 0        |           |
| Brazil             | 49             | 49        | NA       |           |
| Argentina          | 80             | 45        | 1        |           |
| Saudi Arabia       | 22             | 50        | NA       |           |
| China              | 112            | 37        | NA       |           |
| Albania            | 19             | 10.5      | 0        |           |
| Serbia             | 50             | 48        | 6        |           |

Abbreviations: HA, hemophilia A; NA, not available.
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