

Minireview Article

Systematic review of genetic-related risk factor and inhibitor epidemiology in people with severe hemophilia A from Africa: A 2023 update

ABSTRACT

Background and Aims: Prevalence of factor VIII inhibitors in patients with hemophilia A varies from study to study, ranging from 15% to 30%. The important risk of inhibitor development is factor VIII mutation responsible for hemophilia A. Few studies have reported factor VIII mutations in Africa. The aim of this study was to review on factor VIII mutations of severe hemophilia A in Africa, and those associated with inhibitor development.

Study design and methodology: A systematic review was carried out using the electronic databases Pubmed, Science Direct, Index Medicus Global and African Journals online and the key words "hemophilia A", "inhibitor", "genetic" and "Africa". Studies written in French or English on the African continent and published between 2012 and 2023 were included. Publications relating to acquired hemophilia and duplicates were excluded. In the end, 17 articles were selected.

Results: The factor VIII mutations involved in severe hemophilia A in Africa are variable, consisting of intron 22 inversion, large or point deletions, nonsense and missense mutations and splicing abnormalities. Among the latter, numerous previously unrecorded mutations have been identified, and a single case of intron 1 inversion has been found in Algeria. Prevalence of factor VIII inhibitors in severe hemophilia A in Africa varies between 23 and 30%. Genetic abnormalities associated with inhibitors include intron 22 inversion, large deletions such as exon 1-13 deletion, nonsense mutations and c.1010-2A>G mutation.

Conclusion: A better knowledge of the factor VIII mutations involved in severe hemophilia A in Africa will help improve patient management.

Keywords: Hemophilia, Factor VIII, inhibitor, genetic mutations, Africa

1. INTRODUCTION

Hemophilia A is an X-linked bleeding disorder caused by a mutation in factor VIII gene and affecting at birth 24,6 cases for all severities of 100,00 males [1]. It is the most common of the inherited bleeding disorders. Hemophilia affects men and women are usually carriers. The amount of residual clotting factor VIII defines three clinical and biological forms: mild hemophilia A (6 IU/dL to < 40%), moderate hemophilia A (1- 5 IU/dL), and severe hemophilia A (< 1 IU/dL). [2]. Classically, patients with severe form prone to joint and muscle bleeds [3]. External bleeding after deep cutaneous lesions, mucous membrane bleeding (epistaxis, gingivorrhagia) or visceral bleeding (hematuria) may also be observed. Nowadays, the standard of care in patients with severe haemophilia A is primary prophylaxis. For many years, replacement therapy has been based on concentrated factor VIII injections, either on demand in the event of a bleeding episode or as prophylaxis [2]. The most challenging complication of this treatment is development of inhibitory alloantibodies which neutralize activity of FVIII, compromising the efficacy of the treatment [4]. Detection of these inhibitors is an integral part of the standard laboratory follow-up of hemophilia, and its prevalence varies from study to study in hemophilia A, ranging from 15% to 30% [5]. This prevalence is four times higher in severe hemophilia A [5], and one of the most important predictors of the risk of inhibitor development in severe hemophilia A is the F8 gene mutation type [6-8]. Few studies have reported F8 gene mutation type for hemophilia A in Africa. The aim of this study was therefore to review the literature on F8 gene mutation type in Africa and to study those associated with development of inhibitors. Our data were then compared with mutations reported in caucasian populations.

2. METHODOLOGY

We tried to identify all published studies that defined F8 gene mutation type in Africa and all studies on development of inhibitory alloantibodies against FVIII. We carried out a systematic literature review using the electronic databases Pubmed, Science Direct, Index Medicus Global and African Journals online and the key words "hemophilia A", "inhibitor", "genetic" and "Africa". A manual search was then carried out using the bibliographic references of the detected articles to identify other relevant publications. Full-text studies written in French or English, published from 2012 to 2023 and focusing on African patients with hemophilia A were included. Selected articles included information on the different variants of FVIII gene mutation type and/or genetic abnormalities associated with inhibitor development in one of the African countries. Articles describing the sociodemographic, clinical and biological characteristics of African hemophilia A patients who have developed inhibitors, as well as the prevalence of these inhibitors, were also retained.

On the other hand, articles relating to acquired hemophilia A or those dealing with constitutional hemophilia A but carried out outside Africa, as well as duplicates, weren't retained. The same applies to annotations or comments not reporting clinical cases. Of the 1956 articles identified, 17 were finally retained (Figure 1)

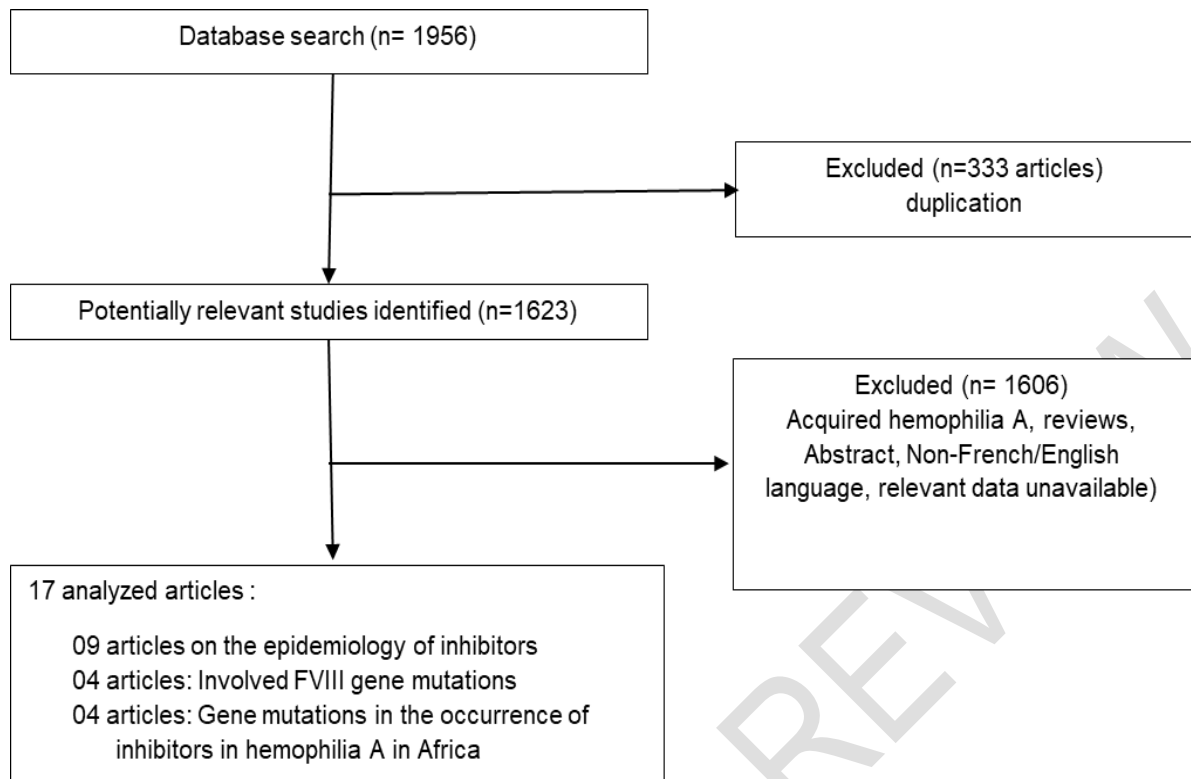


Figure 1: Flowchart of the inclusion of studies.

3. RESULTS

1- Study characteristics

Overall, we identified 17 references through the electronic and hand searches who met the inclusion criteria and were considered for this systematic review. Nine of which, studied the epidemiology of inhibitors in hemophilia A encountered in different African countries [9-17]. Four articles studied the factor VIII gene abnormalities involved in hemophilia A in Africa, and four others reported the FVIII gene variants associated with inhibitor development in hemophilia A [9, 18-20]. Studies were carried out in four Northern African countries (Morocco [12], Tunisia [16], Algeria [19] and Egypt [20]), two West African countries (Senegal [9, 10, 17] and Côte d'Ivoire [15, 18]), one Central African country (Cameroon [13]) and South Africa [11, 14].

2- F8 gene mutations profile in hemophilia A in Africa

The DNA extraction methods used varied from study to study. Some authors used organic solvents (phenol chloroform) [14, 23], while others used ion-exchange resin microcolumns [7, 16].

Molecular techniques used to identify FVIII gene mutation responsible for hemophilia A in Africa also varied from one publication to another: Detection of intron 22 inversion was carried out by Long Range PCR (Senegal, Côte d'Ivoire, Algeria, Tunisia) [7, 14, 16, 23] or by Inverse Shifting PCR (IS PCR) in Egypt [29]. Sometimes, research teams from the same country have used different molecular techniques. This is the case in Egypt, where Mosaad MR et al used IS PCR in Cairo [29], while Sherief LM et al used Long-Range PCR in Zagazig [18].

In the case of severe hemophilia, detection of intron 22 inversion was carried out as a first step, and in its absence, the search for intron 1 inversion was a second step. In the absence of intron 22 and intron 1 inversions, sequencing has been performed by all authors, most of them using the latest sequencing techniques [7, 16, 23, 29, 30].

At the end of these different strategies, various FVIII gene mutation type listed in databases such as HAMSTERS or EAHAD [28], like intron 22 inversion, intron 1 inversion, point mutations and large rearrangements, were found in Africa.

The frequency of intron 22 inversion of the factor VIII gene varies widely from one study to another and from one region of Africa to another. This mutation is reported in 36% [9], 38% [7] and 39.5% of severe PwH A [16] in South Africa, Senegal and Côte d'Ivoire respectively. However, in studies from Northern Africa such as Algeria and Morocco, intron 22 inversion appears to be more frequent, with frequencies of 89% [23] and 42.8% [10] respectively. Inversion of intron 1, on the other hand, is rare, with only one case reported in Algeria [17]. This mutation wasn't reported in any of the other studies, notably in Egypt, Tunisia, Côte d'Ivoire and Senegal.

Other mutations of the factor VIII gene were point mutations involving substitution, deletion, insertion, or inversion of one or more nucleotides. These mutations varied and found in all populations studied, resulting in nonsense mutations, missense mutations, or splicing alterations within the FVIII gene. In addition, frameshift mutations have been identified in six severe PwH A from Côte d'Ivoire [16], and two from Egypt [29]. The same applies to large FVIII gene deletions identified in Senegal and Côte d'Ivoire [7, 16].

However, mutations not reported in the HAMSTERS and EAHAD databases have also been identified in several African countries. In addition, no mutations were found in some PwH (Table 1).

Table 1 lists the number of patients with the various factor VIII gene variants involved in severe hemophilia A in Africa, as well as the new mutations identified.

Table 1. Distribution of FVIII gene variants and new mutations identified in severe hemophilia A in Africa

Countries,	StudyPo	Number of	Number of FVIII	New mutations in severe
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Years	pulation	severe HA	gene variants in case of severe HA	HA (HGVS notation)
Tunisia 2012 [21]	28	19	Inv22 : 7 Inv1 : 00 others : 12 Non-identified mutations : 00	5 new mutations : – Exons1-13del – c.592 T>C; p.C179(198)R – c.4844ins264pb – c.90-91insA; p.12(31)LfsX11 – c.2409 T>C; p.N784(803)N
Algeria 2014 [22]	24	22	Inv 22 : 13 Inv 1 : 01 Others : 06 Non-identified mutations : 02	02 new mutations – c.5219 p 1G > T – c.2189G > A
Côte d'ivoire 2020 [18]	54	43	Inv22 : 17 Inv1 : 00 Others : 24 Non-identified mutations : 02	5 new mutations : – c.266-?_*1788+?dup – c.1921T > G – c.[4738_4753del;4754_4771dup] – c.5322_5330del9 – c.6115 + 2T>A
Senegal [9]	22	21	Inv 22 : 8 Inv 1 : 00 Others : 12 Non-identified mutations : 01	05 new mutations : – c.3655_3659delAAGAA – c.4853dupT – c.3528_3530delinsGA – c.1010-2A>G – c.803A>G

HGVS : Human Genome Variation Society, HA : Hemophilia A

The involved genetic mutations study in severe hemophilia A is useful both for screening hemophilia carriers and for improving the management of PwH. In fact, the type of genetic abnormality involved in hemophilia is a factor associated with the appearance of anti-FVIII inhibitors. Large deletions and certain missense mutations are thus clearly associated with a high risk of developing an inhibitor [23]. As many authors claim that Africans develop inhibitors more frequently than caucasianPwH [24, 25], it was essential to study the epidemiology of inhibitors in severe hemophilia A in Africa, as well as the genetic mutations in the occurrence of these inhibitors.

3- FVIII inhibitors epidemiology in hemophilia A in Africa

3-1- Inhibitor prevalence in hemophilia A

Nine studies have determined the prevalence of inhibitors in hemophilia A. This prevalence varied from country to another, ranging from 7.8% in Côte d'Ivoire [15] to 29.2% in Algeria [19]. Among severe PwH A, the prevalence ranged from 4.6% in Côte d'Ivoire to 28% in Cameroon, depending on the series, and around half (45.5% in Morocco) or almost all (Senegal, Tunisia) of PwH who developed inhibitors were severe PwH (Table 2).

Table 2: Prevalence of inhibitors in hemophilia-A in Africa

Countries, Years of publication	Authors	Study population (N)	Number of severe HA	Prevalence of inhibitors	Prevalence of inhibitors in severe HA
Senegal, 2017	Seck M et al [9]	22	21	5 (22,7%)	5 (23,8%)
Ivory Coast, 2019	Lambert C et al [18]	50	-	6 (12%)	-
Ivory Coast, 2018	Lambert C et al [15]	64	43	5 (7,8%)	4 (4,6%)
Cameroon, 2014	Balôgôg PN et al [13]	38	23	7 (18,5%)	7 (30%)
South Africa, 2014	Lochan A et al [11]	216	-	29 (13%)	-
South Africa, 2022	Lethukuthula M et al [51]	36	-	18%	-
Algeria, 2014	Zemani-Fodil et al [19]	24	22	7 (29,2%)	6 (27,3%)
Egypt, 2020	Sherief LM et al [20]	120	-	21 (18%)	-
Marocco, 2022	Bouyadmar M et al [12]	95	-	21 (22%)	-
Tunisia, 2000	Ghali O et al [16]	46	23	7 (15,2%)	6 (26%)

However, prevalence studies do not always include PwH A with transient inhibitors, or patients whose inhibitors have disappeared after immune tolerance, hence the need for incidence studies.

3-2 Inhibitors incidence in hemophilia A patients in Africa

To our knowledge, one study has investigated the incidence of hemophilia in Africa. The study by Touré SA et al in Senegal determined incidence of inhibitors in severe PwH A treated with low-dose factor VIII concentrates for prophylaxis. Over a 3-year period, three of the 13 severe PwH A included had developed inhibitors or an incidence of 23% [10].

4- Factor VIII gene mutations type associated with risk of inhibitor development in PwH A from Africa

Studies on FVIII mutations and inhibitors are scarce in Africa, particularly in sub-Saharan Africa. Table 3 lists the FVIII gene mutations associated with inhibitors development in severe HA in Africa.

Among PwH A with intron 22 inversion, 12.5% and 37% respectively had developed inhibitors in Senegal [7] and Egypt [18]. In South Africa, PwH with intron 22 inversion were twice as likely to develop inhibitors ($p=0.05$) [9], whereas this risk was multiplied by three to four according to Sherief et al in Egypt ($p=0.03$) [18].

In addition to intron 22 inversion, the examined articles in this review reveal that large deletions were associated with the occurrence of inhibitors, as is the case for the del 1-13 deletion identified in Tunisia [30], as well as del exon 6+7 and del prom + exon 1 found in Senegal [7]. Similarly, the only large deletion identified in Côte d'Ivoire was also associated with inhibitor development [16]. When deletions are smaller, the consequences are variable and may or may not be associated with inhibitor development in hemophilia A [7, 16, 17]. Also, the c.322A>T.p.Lys108 mutation found in Algeria is associated with the occurrence of inhibitors [17].

Furthermore, several mutations implicated in severe hemophilia A and identified in the various African studies weren't listed in the Hamsters and EAHAD databases. Some of these were associated with the occurrence of inhibitors, such as mutations involving the c.1010-2A>G splice site [7] and the large deletion of exons 1 to 13 [30] identified in Senegal and Tunisia respectively.

Table 3: FVIII gene variants associated with the occurrence of inhibitors in severe hemophilia-A in different African countries

Countries	Mutation(HGVS notation)	Type	Location
Tunisia[26]	Exons1-13del	Large deletion	Exon 1- 13
Algeria [19]	inv 22	Inversion	Intron 22
	c.322A>T, p.Lys108	Nonsens	Exon 3
	c.3780G>C, p.Asp1260Glu	Polymorphism	Exon 14
Senegal[9]	Inv 22	Inversion	Intron 22
	c.(670 + 1_671-1)_(1009 + 1_1010-1)	Large deletion	Exon 6
	c.(?-1120)_(143 + 1_144-1)	Large deletion	Promotor + Exon 1
	c.6049delG	Small délétion	19
	c.1010-2A>G	Splice site	ivs7
Ivory Coast[18]	c.788+?-1009-?del	Large deletion	Exon 7
	Inv 22	Inversion	Intron 22

4. DISCUSSION

This literature review describes the different FVIII gene mutations in severe hemophilia A in Africa, and in the occurrence of inhibitors. The term "Africa" is an artificial geographical delimitation, covering socio-economic realities that vary tremendously from one country to another. However, there are many reasons for limiting our analysis to this scale. PwH are under-diagnosed in Africa, accounting for just 3% of PwH worldwide [27, 28]. This situation is probably due to the limited technical resources [27, 29] of medical analysis laboratories and the small number of hemophilia care centers in developing countries [29]. According to a study carried out by Mbanya in 2021, there were three HTC in Cameroon and five in Senegal [29]. Moreover, considering the limited socio-economic level of

patients or their relatives, and the great distance generally separating PwH' places of residence from hemophilia treatment centers, many PwH are treated on demand only in the event of bleeding episodes. And even when PwH manage to find the means to travel to hemophilia treatment centers, many of these centers lack the consumables and reagents needed [30] for factor VIII assay and systematic screening for inhibitors. This may explain very low number of publications on hemophilia in Africa.

However, this review is probably not exhaustive, due to the several studies carried out in Africa, which are difficult for the scientific community to access as they are not referenced in international databases of medical literature.

Prevalence of PwH who developed inhibitors at the review time in Africa ranged from 4.6% to 45.5%, depending on the country. This difference from one country to another is probably linked to the variability of the inclusion criteria used. Some authors included all hemophilia A patients in their cohort, while others included only unrelated hemophilia A patients. Similarly, while many of the PwH included in the various studies were on episodic treatment, the authors included varying proportions of patients on prophylaxis in their cohorts. Thus, these numerous limitations make it impossible to compare published results with each other or to generalize them to the African population. However, the prevalence of inhibitors in hemophilia A found in the various African studies remains similar to those found in the various studies in the literature, estimated at between 3.6% in Great Britain [31] and 26.8% in Greece [32]. In France, the prevalence of inhibitors in hemophilia A, whatever the degree of severity of the disease, was 7% [33]. After analysis of the numerous data from caucasian studies by Wight J et al, the overall prevalence of inhibitors, whatever the severity of the hemophilia, is 5 to 7%. This leads many authors to assert that the prevalence of inhibitors in Africans appears to be higher than in caucasians [8, 34].

All authors agree that mutation of the FVIII gene involved in hemophilia is one of the genetic factors determining the occurrence of inhibitors.

In severe hemophilia A, the search for intron 22 inversions was carried out in the majority of studies using the long-range PCR (LR PCR) technique, except in Egypt where inverted-shifting PCR (IS PCR) was used. The long-range PCR developed by Liu et al [35] is a rapid technique that uses a small quantity of DNA. However, it is technically difficult to standardize and requires a high-quality DNA extract. This has probably motivated the development of alternative methods such as reverse PCR used in Egypt [36, 37] or real-time PCR developed by Kloppers JF in South Africa in 2019 [38, 39].

The results of these different studies have revealed a prevalence of intron 22 inversion that varies from one country to another in Africa, ranging from 36 to 40% for sub-Saharan countries, while this prevalence seems slightly higher in Algeria (57.89%) [22] and Egypt (42.8%) [20]. These different prevalences of intron 22 inversion in the African population remain similar to the prevalence of this same mutation in the Colombian and Mexican populations, which were 42.4% [40] and 45% [41] respectively.

As for intron 1 inversion, it has been systematically investigated in the various studies carried out in Africa using various molecular techniques recommended by the United Kingdom HemophiliaCenter Doctors Organisation (UKHCDO) [37, 42]. But only one case of intron 1 inversion has been identified in western Algeria. This confirms the rarity of this mutation in severe hemophilia A in Africa. However, intron 1 inversion is just as rare in several other studies, such as in Iraq and Pakistan, where the prevalence was 3.3% [43] and 0.77% [44] respectively. This raises the question of the value of systematically searching for this inversion in the African population.

Furthermore, in the absence of introns 22 and 1 inversion in PwH with severe hemophilia A, the FVIII gene sequencing helped to identify several large deletions that were associated with the occurrence of inhibitors,

whereas missense mutations were not associated with these anti-FVIII Ac. In addition, our literature review revealed that the sole mutation of the FVIII gene involved in hemophilia A is not sufficient to explain the occurrence of an inhibitor in PwH because two PwH from the same family do not both necessarily develop inhibitors [22]. Other genetic factors such as Human Leucocyte Antigen (HLA) [45-47], haplotype [48, 49] and interleukin 10 [50] may be involved in the occurrence of inhibitors in hemophilia A.

Also, as described by many authors in the literature, no mutation could be identified in certain PwH during studies carried out in Algeria, Egypt, Senegal and Côte d'Ivoire. This could be related either to the performance of the tests or to as yet unidentified mechanisms involved in hemophilia

5. CONCLUSION

The genetic mutations spectrum causing hemophilia is similar in all countries. Intron 22 inversion is likely the most frequent mutation in Africa, and was often associated with the occurrence of inhibitors, but this can also occur without any statistically significant link. However, large deletions were almost always associated with the occurrence of inhibitors, unlike missense mutations, which were in no case associated with the occurrence of anti-FVIII inhibitors in the studies exploited. Nevertheless, it is necessary for all African countries to join their efforts to carry out a multicentric study including a larger study population with a more rigorous study method so as to obtain results that can be generalized to the African population.

ETHICS STATEMENT

Not applicable

CONSENT

Not applicable

REFERENCES

- 1lorio A, Stonebraker JS, Chambost H, Makris M, Coffin D, Herr C et al. Data and Demographics Committee of the World Federation of Hemophilia. Establishing the Prevalence and Prevalence at Birth of Hemophilia in Males: A Meta-analytic Approach Using National Registries. *Ann Intern Med.* 2019 Oct 15;171(8):540-546. doi: 10.7326/M19-1208.
2. Bolton-Maggs PH, Pasi KJ. Haemophilias A and B. *Lancet.* 2003;361(9371):1801-9. doi: 10.1016/S0140-6736(03)13405-8.
3. Alcalay M. Muscular complications of hemophilia. *Arch Pediatr.* 2009;16(2):196-200. French. doi: 10.1016/j.arcped.2008.11.007.
4. Cormier M, Batty P, Tarrant J, Lillicrap D. Advances in knowledge of inhibitor formation in severe haemophilia A. *Br J Haematol.* 2020;189(1):39-53. doi: 10.1111/bjh.16377.

5. Goudemand J. Les anticorps anti Facteur VIII chez l'hémophile. *Hématologie* 2001; 7: 170–183.
6. Calvez T, Chambost H, d'Oiron R, Dalibard V, Demiguel V, Doncarli A et al. Analyses of the FranceCoag cohort support differences in immunogenicity among one plasma-derived and two recombinant factor VIII brands in boys with severe hemophilia-A. *Haematologica* 2018; 103(1):179-189. doi: 10.3324/haematol.2017.174706.
7. Franchini M, Coppola A, Rocino A, Santagostino E, Tagliaferri A, Zanon E et al. Systematic review of the role of FVIII concentrates in inhibitor development in previously untreated patients with severe hemophilia-A: a 2013 update. *2013;39(7):752-66*. doi: 10.1055/s-0033-1356715.
8. Oldenburg J, Pavlova A. Genetic risk factors for inhibitors to factors VIII and IX. *Hemophilia* 2006; 12: 15–22.
9. Seck M, Costa C, Faye BF, Sy Bah D, Touré SA, Dieng N et al. Molecular diagnosis of hemophiliaA in patients from Senegal. *Haemophilia*. 2017;23(3):e225-e227. doi: 10.1111/hae.13207..
10. Touré SA, Seck M, Sy D, Senghor AB, Faye BF, Diop S. Clinical outcome and incidence of inhibitor development in severe hemophilia patients receiving low-dose prophylaxis: a 3-year follow-up study in Senegal, West Africa. *HematolTransfus Cell Ther*. 2023;45 Suppl 2:S95-S100. doi: 10.1016/j.htct.2022.04.004.
11. Lochan A, Macaulay S, Chen WC, Mahlangu JN, Krause A. Genetic factors influencing inhibitor development in a cohort of South African haemophilia A patients. *Haemophilia*. 2014;20(5):687-92. doi: 10.1111/hae.12436.
12. Bouyadmar M, Khorassani ME, Kababri ME, Kili A, Hessissen L. The prevalence of anti-factor VIII and anti-factor IX antibodies among patients with hemophilia in Rabat, Morocco: a single center experience. *Pan Afr Med J*. 2022;14(41):126. doi: 10.11604/pamj.2022.41.126.29571.
13. Balôgôgô PN, Tagny CT, Ndoumba A, Mbanya D. FVIII and FIX inhibitors in people living with hemophilia in Cameroon, Africa: a preliminary study. *Int J Lab Hematol*. 2014;36(5):566-70. doi: 10.1111/ijlh.12190.
14. Mafisa L, Dlova AN, Moodley V. The profile of patients with hemophiliamanaged at a hemophiliatreatmentcentre in Pretoria, Gauteng. *S Afr Fam Pract*. 2022;64(1):e1-e7. doi: 10.4102/safp.v64i1.5551.
15. Lambert C, Meité N, Sanogo I, Lobet S, Adjambri E, Eeckhoudt S et al. Hemophiliain Côte d'Ivoire (the Ivory Coast) in 2017: Extensive data collection as part of the World Federation of Hemophilia's twinning programme. *Haemophilia*. 2019;25(2):236-243. doi: 10.1111/hae.13682.
16. Ghali O, El Borgi W, El Mahmoudi H, Ben Lakhal F, Fekih Salem S, Achour Met al..Prévalence des inhibiteurs anti-FVIII chez les enfants hémophiles A: Experience d'un centred'hémophilie et revue de la littérature. *Jl M Sfax* 2020; 35: 45–49.
17. Touré SA, Seck M, Sy D, Bousso ES, Faye BF, Diop S.. Life-threatening bleeding in patients with hemophilia (PWH): a 10-year cohort study in Dakar, Senegal.pdf. *Hematology*. 2022 Dec;27(1):379-383. doi: 10.1080/16078454.2022.2047286.

18. Lambert C, Lannoy N, Meité N, Sanogo I, Eeckhoudt S, Hermans C. Inhibitor epidemiology and genetic-related risk factors in people with hemophilia from Côte d'Ivoire. *Haemophilia*. 2020 Jan;26(1):79-85. doi: 10.1111/hae.13902.
19. Zemani-Fodil F, Abdi M, Fodil M, Samia Aberkane M, Mesli N, Belazaar M et al. Factor 8 Gene Mutations and Risk of Inhibitor Development in Hemophilia-A Algerian Patients. *J Pharmacogenomics Pharmacoproteomics* 2014; 05: 1–4. DOI: 10.4172/2153-0645.1000124
20. Sherief LM, Gaber OA, Youssef HM, Sherbiny HS, Mokhtar WA, Ali AAA et al. Factor VIII inhibitor development in Egyptian hemophilia patients: Does intron 22 inversion mutation play a role? *Ital J Pediatr*. 2020;46(1):129. doi: 10.1186/s13052-020-00878-5.
21. Elmahmoudi H, Khodjet-el-khil H, Wigren E, Jlizi A, Zahra K, Pellechia D et al. First report of molecular diagnosis of Tunisian PwH A: Identification of 8 novel causative mutations. *DiagnPathol*. 2012 Aug 10;7:93. doi: 10.1186/1746-1596-7-93.
22. Abdi M, Zemani-Fodil F, Fodil M, Aberkane MS, Touhami H, Saidi-Mehtar N et al. First molecular analysis of F8 gene in Algeria: Identification of two novel mutations. *Clin Appl ThrombHemost*. 2014;20(7):741-8. doi: 10.1177/1076029613513321.
23. Bardi E, Astermark J. Genetic risk factors for inhibitors in hemophiliaA. *Eur J Haematol* 2015; 94: 7–10. doi: 10.1111/ejh.12495.
24. Howard TE, Viel KR, Karl M, Fernstrom B, Deshpande S, Ameri A et al. Allelically Mismatched Replacement Therapy Due to Common African-Restricted Haplotypes of the Factor (F)VIII Protein May Underlie the Increased Incidence of FVIII Inhibitors Observed in Hemophilia-A Patients of African-Descent. *Blood* 2006;108 (11): 765–765. doi.org/10.1182/blood.V108.11.765.765
25. Gunasekera D, Ettinger RA, Nakaya Fletcher S, James EA, Liu M, Barrett JC et al. Factor VIII gene variants and inhibitor risk in African American hemophilia-A patients. *Blood* 2015; 126(7):895-904. doi: 10.1182/blood-2014-09-599365.
26. Elmahmoudi H, Khodjet-el-khil H, Wigren E, Jlizi A, Zahra K, Pellechia D et al. First report of molecular diagnosis of Tunisian PwH A: Identification of 8 novel causative mutations. *DiagnPathol*. 2012 Aug 10;7:93. doi: 10.1186/1746-1596-7-93.
27. Diop S, Haffar A, Mahlangu J, Chami I, Kitchen S, Pierce G. Improving access to hemophilia care in sub-Saharan Africa by capacity building. *Blood Adv*. 2019;3(Suppl 1):1-4. doi: 10.1182/bloodadvances.
28. Mahony BO, Black C. Expanding Hemophilia Care in Developing Countries. *Semin ThrombHemost*. 2005 Nov;31(5):561-8. doi: 10.1055/s-2005-922228.
29. Mbanya DN, Diop S, NdoumbaMintya AN, El Kiaby M. Hemophilia care in Africa: Status and challenges. *Transfus Clin Biol*. 2021;28(2):158-162. doi: 10.1016/j.tracli.2021.01.008.

30. Davies J, Abimiku A, Aloba M, Mullan Z, Nugent R, Schneidman M et al. Sustainable clinical laboratory capacity for health in Africa. *Lancet Glob Health*. 2017 Mar;5(3):e248-e249. doi: 10.1016/S2214-109X(17)30024-4.
31. Yee TT, Pasi KJ, Lilley PA, Lee CA. Factor VIII inhibitors in PwH: a single-centre experience over 34 years. 1964 – 97. *Br J Haematol*. 1999 Mar;104(4):909-14. doi: 10.1046/j.1365-2141.1999.01268.x.
32. Aronis S, Platokouki H, Kapsimali Z, Adamtziki E, Kolokithas A, Mitsika A. Prevalence of inhibitor formation in a cohort of haemophilic children exposed to several products of various purities. *Haemophilia*. 1995;1(4):236-42. doi: 10.1111/j.1365-2516.1995.tb00082.x.
33. Sultan Y. Prevalence of inhibitors in a population of 3435 hemophilia patients in France. *ThrombHaemost*. 1992;67(6):600-2
34. Viel KR, Ameri A, Abshire TC, Iyer RV, Watts RG, Lutcher C et al. Inhibitors of Factor VIII in Black Patients with Hemophilia. *N Engl J Med*. 2009 Apr 16;360(16):1618-27. doi: 10.1056/NEJMoa075760. Erratum in: *N Engl J Med*. 2009 Jul 30;361(5):544.
35. Liu Q, Nozari G, Sommer SS. Single-Tube Polymerase Chain Reaction for Rapid Diagnosis of the Inversion Hotspot of Mutation in Hemophilia A. *Blood*. 1998 Aug 15;92(4):1458-9. Erratum in: *Blood* 1999 Mar 15;93(6):2141.
36. Mosaad RM, Amr KS, Rabie EA, Mostafa NO, Habib SA, El-Kamah GY. Genomic alterations in the F8 gene correlating with severe hemophilia-A in Egyptian patients. *Mol Genet Genomic Med*. 2021 Feb;9(2):e1575. doi: 10.1002/mgg3.1575.
37. UKHCDO. Practice Guidelines for the Molecular Diagnosis of HemophiliaA. *Clin Mol Genet Soc* 2008; 1–11.
38. Kloppers JF, Janse van Rensburg WJ. Rapid identification of the intron 22 inversion in haemophilia A. *Haemophilia*. 2017;23(1):e55-e57. doi: 10.1111/hae.13142.
39. Kloppers JF, Marx GM, Rensburg WJJ Van. Intron 22 inversion real-time polymerase chain reaction detection in hemophiliaA families from central South Africa. *South African Med J* 2019;109: 876–879.
40. Yunis LK, Linares A, Cabrera E, Yunis JJ. Systematic molecular analysis of hemophilia A patients from Colombia. *Genet Mol Biol* 2018;41(4):750-757. doi: 10.1590/1678-4685-GMB-2017-0072.
41. Mantilla-Capacho JM, Beltrán-Miranda CP, Luna-Záizar H, Aguilar-López L, Esparza-Flores MA, López-Guido B et al. Frequency of Intron 1 and 22 Inversions of Factor VIII Gene in Mexican Patients With Severe Hemophilia-A. *Am J Hematol* 2007;82(4):283–287. doi: 10.1002/ajh.20865.
42. Keeney S, Mitchell M, Goodeve A; UK Haemophilia Center Doctors' Organization Haemophilia Genetics Laboratory Network. The molecular analysis of haemophilia A: a guideline from the UK haemophiliacentre doctors' organization haemophiliagenetics laboratory network. *Haemophilia*. 2005;11(4):387-97. doi: 10.1111/j.1365-2516.2005.01111.x.

43. Abdulqader AMR, Mohammed AI, Rachid S, Ghoraishizadeh P, Mahmood SN. Identification of the Intron 22 and Intron 1 Inversions of the Factor VIII Gene in Iraqi Kurdish Patients With Hemophilia A. *Clin Appl ThrombHemost.* 2020;26:1-7. doi: 10.1177/1076029619888293.
44. Sattar A, Hussain S, Ullah MI, Mahmood S, Mohsin S. Screening of Intron 1 Inversion of the Factor VIII Gene in 130 Patients with Severe Hemophilia A from a Pakistani Cohort. *Turk J Haematol.* 2017;34(3):278-279. doi: 10.4274/tjh.2017.0031.
45. Bril WS, MacLean PE, Kaijen PH, van den Brink EN, Lardy NM, Fijnvandraat K et al. HLA class II genotype and factor VIII inhibitors in mild hemophiliaA patients with an Arg593 to Cys mutation. *Hemophilia*2004;10 (5): 509–514. doi: 10.1111/j.1365-2516.2004.01011.x.
46. McGill JR, Simhadri VL, Sauna ZE. HLA Variants and Inhibitor Development in Hemophilia-A: A Retrospective Case-Controlled Study Using the ATHNdataset. *Front Med (Lausanne)* 2021;8(663396). doi: 10.3389/fmed.2021.663396.
47. Kim HY, Cho JH, Kim HJ, Chung HS, Kim SH, Lee KO et al. Ethnicity-specific impact of HLA I/II genotypes on the risk of inhibitor development: data from Korean patients with severe hemophilia-A. *Ann Hematol.* 2018;97(9):1695-1700. doi: 10.1007/s00277-018-3358-x.
48. Schwarz J, Astermark J, Menius ED, Carrington M, Donfield SM, Gomperts ED et al. F8 haplotype and inhibitor risk: Results from the Hemophilia Inhibitor Genetics Study (HIGS) Combined Cohort. *Hemophilia*2013;19 (1): 113–118. doi: 10.1111/hae.12004.
49. Elmahmoudi H, Belhedi N, Jilizi A, Zahra K, Meddeb B, Ben Ammar Elgaaied A et al. Factor VIII haplotypes frequencies in Tunisian PwH A. *DiagnPathol.* 2011;6(54). doi: 10.1186/1746-1596-6-54.
50. Astermark J, Oldenburg J, Pavlova A, Berntorp E, Lefvert AK; MIBS Study Group. Polymorphisms in the IL10 but not in the IL1beta and IL4 genes are associated with inhibitor development in patients with hemophilia-A. *Blood* 2006;107(8):3167-72. doi: 10.1182/blood-2005-09-3918.
51. Mafisa L, Dlova AN, Moodley V. The profile of patients with haemophilia managed at a haemophilia treatment centre in Pretoria, Gauteng. *S Afr Fam Pract* 2022;64(1):e1-e7. doi: 10.4102/safp.v64i1.5551.