Zemani-Fodil et al., J Pharmacogenomics Pharmacoproteomics 2014, 5:1
DOI: 10.4172/2153-0645.1000124

Journal of Pharmacogenomics & Pharmacoproteomics

Research Article Open Access

Factor 8 Gene Mutations and Risk of Inhibitor Development in Hemophilia A Algerian Patients

Faouzia Zemani-Fodil[1#], Meriem Abdi[1#*], Mostefa Fodil[1], Meriem Samia Aberkane[1], Naima Mesli[2], Mohamed Belazaar[3], Malika Mehalhal[4], Yasmina Rahal[5], Hadj Touhami[5], Nadhira Saidi-Mehtar[1], Abdallah Boudjema[1]

[1]*Laboratoire de Génétique moléculaire et Cellulaire, Université des Sciences et de la Technologie d'Oran -Mohamed Boudiaf- (USTOMB), Oran, Algeria*
[2]*Service d'hématologie, Centre Hospitalo-universitaire de Tlemcen, Algeria*
[3]*Service d'hématologie, Centre Hospitalo-universitaire de Sidi Bel Abbes, Algeria*
[4]*Service d'hématologie, Centre Hospitalo-universitaire de Mascara, Algeria*
[5]*Service d'hématologie, Centre Hospitalo-universitaire d'Oran, Algeria*
[#]*The authors have contributed equally to the study.*

Abstract

Background: Neutralizing inhibitors development toward factor VIII is one of the most challenging complications in the treatment of hemophilia A. Several studies have suggested that genetic factors influence the development of factor VIII inhibitors such as ethnicity, family history, mutations in the factor 8 gene and in genes of the immune system. The aim of the present study was to analyze the relationship between inhibitor development and F8 gene mutation types in a sample of hemophiliac patients from West Algeria.

Methods: To study the molecular predisposition for inhibitor development, we genotyped 24 hemophiliac patients with and without inhibitors. A conventional Fisher's exact test was used for statistical analysis. A p-value<0.05 was considered to indicate statistical significance.

Results: A total of seven patients had developed inhibitors, while seventeen had not. Six patients with inhibitors (86%) were classified as low responder; whereas, one patient (14%) was categorized as high responder with the Bethesda inhibitor level above five unit.

Among the inhibitor-positive patients, we identified 4 with intron 22 inversion, 1 with a nonsense mutation (c.322A > T, p.Lys108*) and 2 without an identified F8 mutation.

We showed that there was any association of the F8 gene mutations and the inhibitor development in our study-group. However, these findings should be confirmed in a larger group of patients.

In conclusion, genetic factors are not the only determinant of inhibitor development in HA; environmental factors play an important role.

Keywords: Hemophilia A; Inhibitor; F8 gene mutations; West Algeria

Introduction

The development of inhibitor antibodies against factor VIII (FVIII) is the most challenging complications in the treatment of hemophilia A (HA) for patients and their treating physicians, as it increases the bleeding tendency while it renders treatment with therapeutic factor VIII concentrates ineffective [1].

FVIII inhibitor formation occurs in approximately 10-15% of unselected hemophiliac patients and in about 20-30% of patients with severe form of the disease [2]. According to the residual plasma FVIII coagulant activity (FVIII: C), HA can be classified into 3 forms: severe (FVIII: C<1%), moderate (1%<FVIII: C<5%), and mild (5%<FVIII: C<40%) [3]. Inhibitor development is a multifactorial process that involves both genetic and/or environmental factors [4]. Several studies have suggested that genetic factors influence the development of factor VIII inhibitors such as family history, mutations in the factor 8 (F8) genes and in genes of the immune system [5]. The type of mutation in the F8 gene is an important risk factor for inhibitor development [6-8]. Patients with severe molecular defects (e.g. large deletions, nonsense mutations, intron 22 inversion) have seven to ten fold higher inhibitor prevalence than patients with milder gene defects (e.g. missense mutations, small deletions, splice-site mutations) [9].

However, other host-related factors have been shown to influence inhibitor development in HA, such as the ethnic origin [10], indicating the importance to report studies on population specific estimations of the inhibitor risk factors worldwide.

Up to our knowledge, in Algeria, there are any research articles evaluating the correlation between inhibitor formation and F8 gene mutations in patients with HA. In this study, we evaluated for the first time the relationship between inhibitor development and F8 gene mutation types, and their location in hemophiliac patients from West Algeria.

Materials and Methods

We have studied a total of 24 HA patients from West Algeria (22

***Corresponding author:** Abdi Meriem, Laboratoire de Génétique Moléculaire et Cellulaire, Université des Sciences et de la Technologie d'Oran Mohamed Boudiaf (USTOMB), BP. 1505 El M'Naouer 31000, Oran, Algeria, Tel: 00213555555715; E-mail: meriem.rym.abdi@gmail.com; meriem.abdi@univ-usto.dz

Received January 15, 2014; **Accepted** February 18, 2014; **Published** February 26, 2014

Citation: Zemani-Fodil F, Abdi M, Fodil M, Aberkane M S, Mesli N, et al. (2014) Factor 8 Gene Mutations and Risk of Inhibitor Development in Hemophilia A Algerian Patients. J Pharmacogenomics Pharmacoproteomics 5:124. doi:10.4172/2153-0645.1000124

with severe and 2 with moderate form), aged between 5 and 47 years old (median age: 26 years). Patients were diagnosed as having HA according to the *International Consensus Criteria of the 2001 International Society on Thrombosis and Haemostasis* (ISTH). A written informed consent in accordance with the Declaration of Helsinki was obtained from patients or their parents. This study was approved by the ethical committee of Oran Hospital.

Patients were recruited between 2011 and 2012 from treatment centers of HA in the West of Algeria. F8 gene mutations and inhibitor status of each patient were previously established in Abdi et al. [11], but any statistical analysis between the two groups of HA patients (Inhibitor-positive) and (Inhibitor-negative) was realized. Briefly, patients with severe form of HA were first screened for intron 22 inversion using a Long Rang PCR as described by Liu et al. Then, the intron 1 inversion was assessed by multiplex PCR as previously described by Bagnall et al. After excluding subjects with intron 22 and 1 inversions, all 26 exons including flanking intronic sequences, promoter and 3'UTR were amplified using primers and PCR condition described in the HAMSTeRS database [14]. The amplified fragments were purified and sequenced (forward and reverse sequences) using ABI PRISM 310 with BigDye Terminator v3.1 kit (AB Applied Biosystems, Carlsbad, California, USA).

Inhibitor detection for all patients was performed using the Bethesda assay once every 3 months [15]. A positive inhibitor titer was defined as equal to or more than 0.6 BU/mL. Patients who presented a >5 BU/mL value were classified as having high titer inhibitors.

Data were analyzed by Epi Info software, version 7.1.2.0, using Fisher's exact test, and exact p-values less than 0.05 were considered statistically significant. Patients with inversions in intron 1 or intron 22 were pooled in the same group to reach a sufficient number of cases.

Epitope prediction in one of the mutated proteins was obtained through the IEDB Analysis Resource [16] and the BEPIPRED software.

Results and Discussion

In the present study, we first examined the relative frequencies of F8 gene mutations in patients developing inhibitor. Then, we evaluated the association of these F8 mutations with the development of FVIII inhibitors in 24 patients with HA. The hematological and genetic characteristics of every patient are presented in Table 1.

7 patients had developed inhibitory antibody against FVIII including 6 with severe type of HA (85.71%). Among this sub-group, 14% were high responder; whereas, 86% were low responder.

From this, we documented that 29.16% of unselected HA patients and 27.27% of patients with severe HA have developed inhibitors. This result is different with what we have reported previously; where inhibitors were seen in 11% of patient overall and in 12.5% of patient with severe HA [11].

Furthermore, frequency of inhibitor in all hemophiliac patients is higher than that reported by Astermark et al. [2], who obtained a prevalence of 10-15% for the development of inhibitory antibodies against FVIII in all types of patients with HA. However, the frequency of inhibitor development in severe type of HA in our study is in agreement with what was reported by them (20-30%) [2].

Gene mutations were identified in 20 patients of the studied group. Significant heterogeneity in inhibitor prevalence considering the location of the patients' mutation was found, with higher frequencies in carriers of the intron 22 inversion (4/7). One nonsense mutation (c.322A>T, p.Lys108*), one polymorphism (c.3780G>C, p.Asp1260Glu) and an unknown mutation were found in three patients developing inhibitor.

Patient ID	Severity	FVIII:C	FVIII product	F8 gene mutation	Inhibitor
HA1	Severe	<1%	Plasmatic	Intron 22 inversion	Negative
HA2	Severe	<1%	Plasmatic	Intron 22 inversion	Positive (>5BU)
HA3	Severe	<1%	Plasmatic	Intron 22 inversion	Negative
HA4	Severe	<1%	Plasmatic	Intron 22 inversion	Positive (<5BU)
HA5	Severe	<1%	Plasmatic	Intron 22 inversion	Negative
HA6	Severe	<1%	Plasmatic	Intron 22 inversion	Negative
HA7	Severe	<1%	Plasmatic	Intron 22 inversion	Negative
HA8	Severe	<1%	Plasmatic	Intron 22 inversion	Positive (<5BU)
HA9	Severe	<1%	Plasmatic	Intron 22 inversion	Positive (<5BU)
HA10	Severe	<1%	Plasmatic	c.2189G>A	Negative
HA11	Severe	<1%	Plasmatic	c.5219G>A+1	Negative
HA12	Severe	<1%	Plasmatic	c.322A>T	Positive (<5BU)
HA13	Severe	<1%	Plasmatic	c.200A>C	Negative
HA14	Severe	<1%	Plasmatic	Intron 22 inversion	Negative
HA15	Severe	<1%	Plasmatic	Intron 22 inversion	Negative
HA16	Severe	<1%	Plasmatic	Intron 22 inversion	Negative
HA17	Severe	<1%	Plasmatic	c.3780G>C*	Positive (<5BU)
HA18	Severe	<1%	Plasmatic	Unknown mutation	Negative
HA19	Severe	<1%	Plasmatic	Intron 22 inversion	Negative
HA20	Severe	<1%	Plasmatic	c.5953C>T	Negative
HA21	Severe	<1%	Plasmatic	Intron 1 inversion	Negative
HA22	Severe	<1%	Plasmatic	Unknown mutation	Negative
HA23	Moderate	2%	Plasmatic	c.6545G>A	Negative
HA24	Moderate	3%	Plasmatic	Unknown mutation	Positive (<5BU)

*Polymorphism; the c.3780G>C is a polymorphism not a disease-causing mutation

BU: Bethesda Unit

Table 1: Clinical and genetic characteristic of patients studied.

F8 gene abnormality	Inhibitor-positive	Inhibitor-negative	Total (n=24)	*p*	Odds ratio	IC 95%
Total of inversions	4	10	14	1	0.93	0.11-8.50
Intron 22 inversion	4	9	13	1	1.18	0.14-10.64
Intron 1 inversion	0	1	1		Undefined	
Point mutations	2	5	7	1	0.96	0.06-8.9
Missense	0	3	3		Undefined	
Polymorphism	1	0	1		Undefined	
Missense+Polymorphism	1	3	4	1	0.77	0.06-9.07
Splice site	0	1	1		Undefined	
Nonsense	1	1	2	0.50	2.66	0.02-222.13
Splice site + nonsense	1	2	3	1	1.25	0.01-28.27
Unknown mutations	2	2	4	0.55	0.33	0.02-6.03

n: number of patient, ***p***: significance, **IC**: confidence interval

Table 2: Distribution of F8 gene abnormalities in HA patients with and without inhibitors.

Among inhibitor positive patients group, frequency of high-risk mutation type is equal to 71.42%. This high risk mutation group include four subjects with intron 22 inversions and one with a nonsense mutation (c.322A>T, p.Lys108*) in exon 3.

Table 2 shows the distribution of the F8 gene mutations in HA patients with and without inhibitors, giving the probability values for the differences. The differences in frequencies between patients with and without inhibitors were small and did not reach statistical significance for all mutations tested (p>0.05). No relationship between the location of F8 mutations and inhibitor development was detectable in this population, because of the obviously small number of patients in each subgroup.

We determined an equal frequency of severe molecular defects in patients with and without inhibitor. This result isn't in agreement with what was reported in previous articles that documented a higher susceptibility for inhibitor development in patients with severe molecular defects compared with less severe defects [7,8]. This difference could be explained by the fact that these patients did not receive the same dose and schedule of commercial replacement products. Some of them were treated only on request.

The two novel mutations (c.2189G>A, p.Cys711Tyr) and c.5219+1G>T that were previously detected in our population [11] are not associated with inhibitor development. Bioinformatics analysis of the missense mutations previously reported (c.2189G>A, p.Cys730Tyr; c.200A>C, p.Lys67Thr; c.6545G>A, p.Arg2182His) confirmed the absence of formation of a B-cell epitope in the protein. This result was in agreement with the fact that patients presenting these mutations have not developed inhibitors.

Conclusion

Whilst any statistical significance was found in our study due to the reduce number of patients, intron 22 inversion was the most common mutation and the mostly presented in patient developing inhibitors. This observation should be confirmed in a larger study group including the same mean age of both sub-groups (inhibitor positive and negative). Furthermore, we plan to study the implication of immune system genes and environmental factors in inhibitor development. In fact, environmental factors (e.g. age at first treatment, intensity of treatment, continuous infusion and multiple product switches) play an important role in FVIII inhibitor development [17-19]. Results of such studies will provide valuable information to the Algerian hematologists on the locally specific risks for developing inhibitors in patients with HA.

Authors' Note

ZFF was the primarily responsible for this work and corrected the article. AM realized statistical analysis, discussed results, and wrote the article. All authors read and approved the final manuscript.

References

1. Di Minno MN, Di Minno G, Di Capua M, Cerbone AM, Coppola A (2010) Cost of care of haemophilia with inhibitors. Haemophilia 16: e190-e201.
2. Astermark J, Oldenburg J, Paviova A, Bemtorp E (2006) Polymorphism in the IL10 but not in the IL1 beta and IL4 genes are associated with inhibitor development in patients with hemophilia A. Blood 107: 3167-3172.
3. White GC 2nd, Rosendaal F, Aledort LM, Lusher JM, Rothschild C, et al. (2001) Definitions in hemophilia. Recommendation of the scientific subcommittee on factor VIII and factor IX of the scientific and standardization committee of the International Society on Thrombosis and Haemostasis. Thromb Haemost 85: 560.
4. Astermark J, Lacroix-Desmazes S, Reding MT (2008) Inhibitor development. Haemophilia 14: 36-42.
5. de Alencar JB, Macedo LC, de Barros MF, Rodrigues C, Cadide RC, et al. (2013) Importance of immune responsegenes in hemophilia A. Rev Bras Hematol Hemoter 35: 280-286.
6. Boekhorst J, Lari GR, D'Oiron R, Costa JM, Nováková IR, et al. (2008) Factor VIII genotype and inhibitordevelopment in patients withhaemophilia A: highestrisk in patients withsplice site mutations. Haemophilia 14: 729-735.
7. Oldenburg J, El-Maarri O, Schwaab R (2002) Inhibitor development in correlation to factor VIII genotypes. Haemophilia 8: 23-29.
8. Gouw SC, van den Berg HM, Oldenburg J, Astermark J, de Groot PG, et al. (2012) F8 gene mutation type and inhibitor development in patients with severe hemophilia A: systematic review and meta-analysis. Blood 119: 2922-2934.
9. Oldenburg J, Schröder J, Brackmann HH, Müller-Reible C, Schwaab R, et al. (2004) Environmental and genetic factors influencing inhibitor development. Semin Hematol 41: 82-88.
10. Viel KR, Ameri A, Abshire TC, Iyer RV, Watts RG, et al. (2009) Inhibitors of factor VIII in black patients with hemophilia. N Engl J Med 360: 1618-1627.
11. Abdi M, Zemani-Fodil F, Fodil M, Aberkane MS, Touhami H, et al. (2013) First molecular analysis of F8 gene in Algeria: identification of a novel mutation. Clin Appl Thromb Hemost.
12. Liu Q, Nozari G, Sommer SS (1998) Single-tube polymerase chain reaction for rapid diagnosis of the inversion hotspot of mutation in hemophilia A. Blood 92: 1458-1459.
13. Bagnall RD, Waseem N, Green PM, Giannelli F (2002) Recurrent inversion breaking intron 1 of the factor VIII gene is a frequent cause of severe hemophilia A. Blood 99: 168-174.
14. Kemball-Cook G, Tuddenham EG (1997) The Factor VIII Mutation Database on the World Wide Web: the haemophilia A mutation, search, test and resource site. HAMSTeRS update (version 3.0). Nucleic Acids Res 25: 128-132.
15. Verbruggen B, Novakova I, Wessels H, Boezeman J, van den Berg M, et al. (1995) The Nijmegen modification of the Bethesda assay for factor VIII:C inhibitors: improved specificity and reliability. Thromb Haemost 73: 247-251.

16. http://tools.immuneepitope.org/tools/bcell/iedb_input

17. Franchini M, Frattini F, Crestani S, Bonfanti C (2013) Alloantibodies in previously untreated hemophilia A patients: the role of environmental factors. Hematology 18: 183-190.

18. Gouw SC, van der Bom JG, Ljung R, Escuriola C, Cid AR, et al. for the PedNet and RODIN Study Group (2013) Factor VIII Products and Inhibitor Development in Severe Hemophilia A. N Engl J Med 368:231-239.

19. Gouw SC, van den Berg HM, Fischer K, Auerswald G, Carcao M, et al. (2013) Intensity of factor VIII treatment and inhibitor development in children with severe hemophilia A: the RODIN study. Blood 121 : 4046-4055.