

ORIGINAL ARTICLE

F8 gene mutation spectrum in severe hemophilia A with inhibitors: A large cohort data analysis from a single center in China

Jie Sun MD^{1,2} | Zekun Li MD^{1,2}  | Kun Huang MD^{1,2}  | Di Ai MD^{1,2} | Gang Li BS² | Xingjuan Xie MS^{1,2}  | Hao Gu MD^{1,2} | Guoqing Liu MD¹ | Yingzi Zhen BS¹ | Zhenping Chen MD, PhD²  | Runhui Wu MD, PhD¹ 

¹Hemophilia Comprehensive Care Center, Hematology Center, Beijing Key Laboratory of Pediatric Hematology-Oncology, National Key Discipline of Pediatrics (Capital Medical University), Key Laboratory of Major Diseases in Children, Ministry of Education, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China

²Hematologic Disease Laboratory, Hematology Center, Beijing Key Laboratory of Pediatric Hematology-Oncology, National Key Discipline of Pediatrics (Capital Medical University), Key Laboratory of Major Diseases in Children, Ministry of Education, Beijing Pediatric Research Institute, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China

Correspondence

Zhenping Chen, Hematologic Disease Laboratory, Hematology Center, Beijing Key Laboratory of Pediatric Hematology-Oncology, National Key Discipline of Pediatrics (Capital Medical University), Key Laboratory of Major Diseases in Children, Ministry of Education, Beijing Pediatric Research Institute, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing 100045, China.
Email: chenzhenping@outlook.com

Runhui Wu, Hemophilia Comprehensive Care Center, Hematology Center, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, No. 56 Nanlishi Road, West District, Beijing 100045, China.
Email: runhuiwu@hotmail.com

Funding information

Capital Health Development Research Project, Grant/Award Number: Capital Development 2022-2-2093; Children's Medicine Research Project of Beijing Children's Hospital, Capital Medical University, Grant/Award Number: YZZD202003; Cultivation Fund Project of the National Natural Science Foundation in Beijing Children's Hospital, Capital

Abstract

Introduction: Type of *F8* gene mutation is the most important risk factor for inhibitor development in people with severe hemophilia A. However, there are few large cohort studies on the *F8* mutation spectrum of people with severe hemophilia A with inhibitors.

Objective: This was the first large cohort study in children with severe hemophilia A with inhibitors from China that aimed to analyze the association between *F8* variant types and inhibitor status.

Methods: The single-center retrospective cohort study was conducted on children with severe hemophilia A with inhibitors admitted from January 2015 to December 2021. The clinical data were collected, and *F8* genetic tests were performed.

Results: Among the 203 patients investigated, a mutation in *F8* was identified in 196 cases. Most patients had deleterious mutations (153; 75.4%), including 82 cases of intron 22 inversions (40.4%); 40 cases of nonsense mutations (19.7%), with 15 cases in the light chain and 25 cases in the heavy chain; and 31 cases of large deletions or insertions (15.3%), with 29 cases involving more than one exon and 2 cases involving one exon. The large deletions or insertions encompassing multiple exons and nonsense mutations residing in the light chain were associated with not only the progression to a high-titer inhibitor ($P < .05$) but also higher peak inhibitor titer ($P < .05$).

Jie Sun and Zekun Li have contributed equally to this work and share first authorship.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Authors. *Research and Practice in Thrombosis and Haemostasis* published by Wiley Periodicals LLC on behalf of International Society on Thrombosis and Haemostasis (ISTH).

Medical University, Grant/Award Number:
GPMS202004

Handling Editor: Dr Henri Spronk

Conclusion: The *F8* gene deleterious mutations, including intron 22 inversions, nonsense mutations, and large deletions or insertions, constitute the main mutation types in people with severe hemophilia A with inhibitors in China, with the latter mutation types (large deletions or insertions in multiple exons, and nonsense mutations in the light chain) signifying for a higher peak titer of inhibitor.

KEYWORDS

China, *F8* gene, hemophilia A, inhibitors, mutation, peak inhibitor titer

Essentials

1. There are limited studies on the *F8* gene mutation spectrum in people with inhibitors.
2. We report clinical data and *F8* genetic testing results from a single-center retrospective study.
3. Intron 22 inversion, nonsense mutation, and large deletion or insertion were the main mutation types.
4. The high-risk *F8* mutation types were associated with a higher peak titer of inhibitor.

1 | INTRODUCTION

Hemophilia A is an X-linked inherited bleeding disease with a prevalence of ≈ 1 in 5000 in males and caused by coagulation factor VIII (FVIII) deficiency attributing to FVIII gene (*F8*) mutations.¹ There are estimated >30 000 people with severe hemophilia A with a FVIII activity level of <0.01 IU/mL in China. People with hemophilia need FVIII replacement therapy for life as prophylaxis and treatment for bleeding; however, inhibitor development against FVIII in 25% to 35%² of people with severe hemophilia A renders the replacement therapy ineffective. Inhibitors usually develop in ≈ 20 to 50 exposure days (EDs) upon initiation of replacement therapy and become the most challenging issue in the management of people with severe hemophilia A.³ Although the exact mechanism underlying the generation of inhibitors is not fully understood, the previous studies suggested that *F8* genotypes were the most important risk factor for inhibitor development.^{4,5} Based on the review article by Garagiola et al⁴ that included 15 published articles involving dozens of single-center and multicenter cohort studies with the sample size ranging from 15 to 206 people with severe hemophilia A with inhibitors, gene mutation types with high risk of inhibitor development were large deletions or insertions in multiple exons and nonsense mutations in the light chain; gene mutation types with medium risk were large deletions or insertions in single-exon, nonsense mutations in the heavy chain, and intron 22 and 1 inversions; and gene mutation types with low-risk were small deletions or insertions, splice-site mutations, and missense mutations.

The current study aimed to retrospectively analyze the *F8* mutation spectrum in a large cohort of 203 people with severe hemophilia A with inhibitors from a single center in China to further explore the relationship between the mutation types and inhibitor status.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

This study was a single-center, retrospective cohort study. We collected clinical data and *F8* genetic testing results of children with severe hemophilia A with inhibitors from January 2015 to December 2021 retrospectively. The study was approved by the Ethics Review Committee of Beijing Children's Hospital, and written informed consent was acquired from the patients/guardians accordingly.

Inclusion criteria included people with an established diagnosis of severe hemophilia A⁶; people aged <18 years old at the first visit to our center; people with FVIII inhibitor titer of ≥ 0.6 Bethesda units (BU) on two consecutive occasions within 1 to 4 weeks; people who had clinical records available before inhibitor development; and people who had an *F8* gene analysis test conducted in our center. Exclusion criteria included people who had acquired hemophilia and people with severe hemophilia A with a comorbidity of autoimmune or chronic infectious disease.

The FVIII coagulant activity (FVIII:C) levels were measured previously using clotting assays based on activated partial thromboplastin time. The FVIII inhibitor titers were determined using the Nijmegen modification of the Bethesda assay. The high-titer inhibitor was defined as titers of ≥ 5 BU on at least one occasion. The low-titer inhibitor was defined as a peak inhibitor titer of <5 BU.

2.2 | Molecular genetic analysis

Genomic DNA was extracted from 2 mL peripheral blood in EDTA using PD348 Kit (Tiangen, Beijing, China). All patients were first screened for intron 22 and 1 inversions by the long-distance

polymerase chain reaction method following the instructions of the Severe Hemophilia A Genotyping kit (MyGenostics, Beijing, China). In people found to be negative for intron 22 and 1 inversions, next-generation sequencing (NGS) assay for the *F8* gene was performed to detect point mutations (nonsense mutations, missense mutations, and splice-site mutations) and small deletions or insertions. In patients who had potential large deletions and duplications or negative by NGS, a multiplex ligation-dependent probe amplification (MLPA) assay was conducted to detect complex rearrangements of *F8* using the SALSA MLPA Probemix P178-B4 *F8* kit (MRC Holland, Amsterdam, the Netherlands) according to the manufacturer's protocol.

The *F8* variants were filtered from the following databases, including the Genome Aggregation Database (<http://gnomad.broadinstitute.org/>), Exome Aggregation Consortium (<http://exac.broadinstitute.org/>), 1000 Genomes Project (<http://browser.1000genomes.org/>), and the Human Gene Mutation Database (<http://www.hgmd.cf.ac.uk/>). Then, the deleterious effects of variants were predicted in silico using a variety of prediction tools (SIFT, <http://sift.jcvi.org/>; Polyphen2, <http://genetics.bwh.harvard.edu/pph2/>; MutationTaster, <http://www.mutationtaster.org/>). Interpretation of sequence variants was performed according to the American College of Medical Genetics and Genomics guidelines.⁷ The identified mutations were verified by Sanger sequencing. In addition, pedigree verification was performed for patients with positive results including MLPA and inversion.

2.3 | Clinical data collection

All clinical data (including baseline FVIII:C levels, treatment methods and types of FVIII concentrate used before inhibitor development, peak inhibitor titer, age, titer, and EDs of inhibitor development) were obtained from the medical records retrospectively. All patients had at least three records of inhibitor titers from the data of our center. Peak inhibitor titer indicated the highest inhibitor titer recorded in the clinical follow-up data, including historical, pre- and post-immune tolerance induction (ITI) (if the patient was placed on ITI treatment) until December 2021.

2.4 | Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 26.0 for Windows (IBM Corp., Armonk, NY, USA). Descriptive analysis of basic characteristics was conducted. Count data was expressed in frequency (n) and percentage (%), and measurement data were expressed as median (interquartile range [IQR]). The Kruskal-Wallis test on multiple independent samples was used to analyze the relationship between *F8* mutation risk groups and inhibitor status. Bonferroni-corrected normal approximation method was used for multiple comparisons between groups. Comparisons of the incidence of peak inhibitor titer of ≥ 5 BU,

≥ 25 BU, ≥ 100 BU, and ≥ 200 BU among mutation risk groups was conducted using Pearson's chi-square test or Fisher's exact test ($R \times C$ table), and the chi-square test of Bonferroni-corrected 2×2 table was used for multiple comparisons between groups. All *P* values are two-sided, and values of $<.05$ were considered significant.

3 | RESULTS

3.1 | Clinical information

A total of 203 cases of children with severe hemophilia A with FVIII inhibitors, all male, were included in this study. The ethnicities of all participants included in the analysis were 193 Han, 4 Zhuang, 3 Tujia, and 1 each of Manchu, Hui, and Mongolian. Before the development of inhibitors, 147 cases (72.4%) were treated only on demand, and 56 patients (27.6%) were receiving regular prophylaxis therapy. Plasma-derived FVIII was the primary replacement therapy and was used in 122 cases (60.1%). Recombinant FVIII was administered in 71 cases (35.0%), and 10 cases (4.9%) were treated with both types of FVIII preparations.

The median age at inhibitor development was 2.6 (IQR, 1.4-5.4; range, 0.1-14.4) years, and the median Eds was 29 (IQR, 17-50; range, 4-555) days. Among all patients, 144 patients (71.0%) developed inhibitors in <50 EDs, 50 cases (24.6%) developed in 50 to 150 EDs, and 9 cases (4.4%) generated in >150 EDs. The median titer at inhibitor development was 10.1 (IQR, 2.6-32.0; range, 0.6-1000.0) BU. The median peak inhibitor titer was 35.2 (IQR, 12.0-106.2; range, 0.9-5529.0) BU. Among 70 patients with low-titer inhibitor at first positive inhibitor test, 43 patients (61.4%) progressed to high-titer status, and only 27 cases (38.6%) maintained persistent low titer.

3.2 | The *F8* mutation spectrum

In study cohort, 196 cases (96.6%) had the *F8* mutations identified, mainly including 82 cases (40.4%) of intron 22 inversions, 40 cases (19.7%) of nonsense mutations (15 cases in the light chain, 25 cases in the heavy chain), and 31 cases (15.3%) of large deletions or insertions (29 cases with multiple exons, 2 cases with one exon) (Figure 1). Of the 143 patients, 45 cases (31.5%) had a family history of hemophilia A by survey, whereas only 18 cases (12.6%) had de novo mutations upon pedigree verification. Among the 92 different mutations detected in this study, 23 mutations in the *F8* gene (25.0%) were neither identified in the European Association for Haemophilia and Allied Disorders *F8* gene variant database nor reported in previous publications. Of the 23 novel mutations, 2 (8.7%) were large deletions, 4 (17.4%) nonsense mutations, 12 (52.2%) small deletions or insertions, 4 (17.4%) missense mutations, and 1 (4.3%) splice-site change. The details of these 23 novel mutations are shown in Table 1.

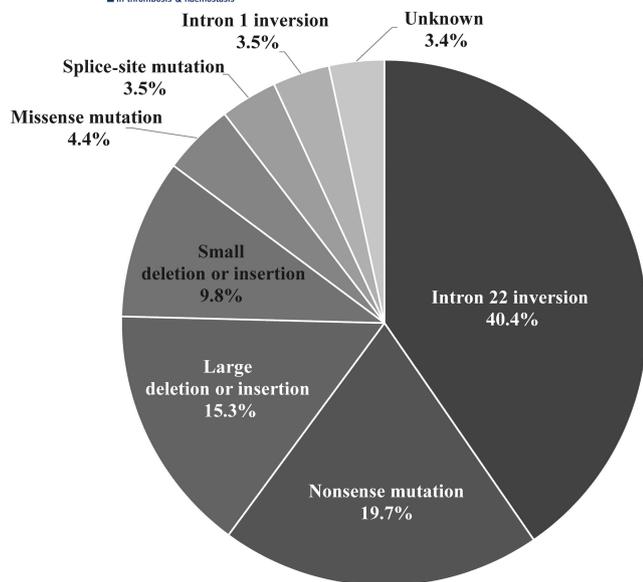


FIGURE 1 Distribution of *F8* gene mutation types in a cohort of 203 children with severe hemophilia A with inhibitors

3.3 | Correlation analysis between *F8* mutation risk groups and inhibitor status

Based on the risk categories proposed by Garagiola et al,⁴ among the 196 people with severe hemophilia A with identified mutations, 44 cases (22.4%) belonged to the high-risk group, 116 cases (59.2%) were in the medium-risk group, and 36 cases (18.4%) were in the low-risk group.

There were significant differences in peak inhibitor titer and the incidence of the progression to a high-titer inhibitor among different *F8* mutation risk groups ($P < .05$); however, there was no difference of EDs and titer at inhibitor development among groups ($P > .05$). The high-risk *F8* mutations were associated with a higher peak inhibitor titer ($P < .05$). Among 67 mutation-identified people with low-titer inhibitor at first positive detection, people with high-risk mutations were more likely to progress to high-titer inhibitor ($P < .05$; Table 2).

There were significant differences in the incidence of peak inhibitor titer of ≥ 5 BU, ≥ 25 BU, ≥ 100 BU, and ≥ 200 BU among different *F8* mutation risk groups ($P < .05$). People with high-risk *F8* mutation types tended to develop a higher peak titer of inhibitor ($P < .05$; Figure 2).

4 | DISCUSSION

The incidence of FVIII inhibitors was the most severe complication in people with hemophilia A receiving replacement therapy, and the *F8* genotype was suggested to be the most important risk factor for inhibitor development.⁴ With the as yet largest cohort of children with severe hemophilia A with inhibitors in China, we found that the high-risk *F8* mutation types of inhibitor development were associated with a higher peak titer of inhibitor.

Consistent with previous studies,^{3,8} the Chinese people with severe hemophilia A with inhibitors investigated in the current study showed that most people developed inhibitors within the first 50 Eds, but the age, titer, and EDs of inhibitor development in this study cohort were slightly higher than those in previous studies.⁹⁻¹¹ The reasons may have been related to China's national situations such as (1) limited by economic factors, most people did not regularly undergo inhibitor assays during on-demand or preventive treatment; when the therapeutic effect was unsatisfactory, the already developed inhibitors with relatively high titer were present before the patients visit; and (2) most local medical centers did not perform inhibitor assays due to limited medical resources.

The main mutation types revealed in the current study were intron 22 inversions, nonsense mutations, and large deletions or insertions, which accounted for 75.4% (153/203) and were all deleterious to the *F8* gene, leading to FVIII deficiency in blood circulation.

The immune response to FVIII replacement treatment may be due to a lack of central tolerance to FVIII protein.¹² In detail, the deleterious *F8* mutations, such as large deletions or insertions and nonsense mutations destructively affect the gene structure, transcription, and translation, resulting in almost complete absence of FVIII in blood circulation, which were mostly associated with the development of inhibitors.^{12,13} Garagiola et al⁴ proposed that the *F8* mutation types could be divided into high-, medium-, and low-risk groups, with the above-mentioned deleterious *F8* mutations boding the highest risk of inhibitor development. The current study with the large patient cohort investigated confirmed that the high-risk *F8* gene mutation types had the highest incidence of high-titer inhibitor and also tended to have higher peak inhibitor titer. This outcome indicated that the *F8* genotype was not only associated with the risk of inhibitor formation but also significantly affected the levels of inhibitor peak titer, particularly in people with large deletions or insertions involving multiple exons and nonsense mutations in the light chain.

Currently, ITI is the only method that can successfully eradicate inhibitors and achieve long-term tolerance. It is widely accepted that a peak historical titer of <200 BU and a peak titer of <100 BU while on ITI are the predictors of ITI success.¹⁴ The Future of Immunotolerance Treatment group believes that a historical pre-ITI peak titer of <25 BU is a very good prognostic indicator but a poor prognosis when it is ≥ 200 BU.¹⁵ Our research further found that patients with high-risk mutation types were more likely to develop inhibitors with a peak titer of >25 , 100, and 200 BU, suggesting a reference for predicting the prognosis.

Meanwhile, the pedigree of 143 patients from this study cohort was verified and found that just 12.6% of patients had de novo *F8* mutations with noncarrier mothers. It clued a higher heritability rate in children with severe hemophilia A with inhibitors compared to previous studies^{16,17} that did not mention the development of inhibitors. Furthermore, the heritability rate in this study was also higher than the data from the spectrum and origin of the 393 Chinese families with sporadic hemophilia A,¹⁸ which showed that 28% patients had de novo mutations with noncarrier mothers. Point mutations (51%) were the predominant mutation types in pedigrees with de novo mutations.

TABLE 1 Description of novel mutations identified in this study

Mutation type	F8 exon/intron	FVIII light/heavy chain	FVIII domain	Nucleotide change	Amino acid change
Large deletion	Exon 4–13	Heavy chain	A1-A2	NA	NA
Large deletion	Exon 3–10	Heavy chain	A1-A2	NA	NA
Nonsense mutation	Exon 14	Light chain	a3	c.5097T>G	p.Y1699*
Nonsense mutation	Exon 14	Heavy chain	B	c.3052A>T	p.K1018*
Nonsense mutation	Exon 14	Heavy chain	B	c.2344G>T	p.E782*
Nonsense mutation	Exon 8	Heavy chain	a1	c.1081G>T	p.E361*
Small deletion/insertion	Exon 7	Heavy chain	A1	c.890_902delinsGAAATT	p.V297Gfs*12
Small deletion/insertion	Exon 17	Light chain	A3	c.5661delinsCTCATGGA	p.R1888Sfs*18
Small deletion	Exon 6	Heavy chain	A1	c.712delG	p.D238Ifs*20
Small deletion	Exon 17	Light chain	A3	c.5739delT	p.N1913Kfs*32
Small deletion	Exon 14	Light chain	A3	c.5136_5140delGAAAA	p.K1712Nfs*19
Small deletion	Exon 14	Heavy chain	B	c.4418delG	p.G1473Vfs*94
Small deletion	Exon 14	Heavy chain	B	c.2640_2641delGA	p.K881Tfs*13
Small deletion	Exon 14	Heavy chain	B	c.2564_2565delAC	p.H855Lfs*15
Small deletion	Exon 11	Heavy chain	A2	c.1740_1741delAA	p.G582Kfs*17
Small deletion	Exon 11	Heavy chain	A2	c.1587delA	p.K529Nfs*5
Small insertion (duplication)	Exon 18	Light chain	A3	c.5843dupT	p.P1949Tfs*22
Small insertion (duplication)	Exon 12	Heavy chain	A2	c.1806_1807dupAA	p.S603Kfs*58
Missense mutation	Exon 17	Light chain	A3	c.5629T>G	p.C1877G
Missense mutation	Exon 14	Heavy chain	B	c.2393T>C	p.M798T
Missense mutation	Exon 13	Heavy chain	A2	c.2020G>A	p.G674R
Missense mutation	Exon 9	Heavy chain	A2	c.1443G>C	p.L481F
Splice-site mutation	Intron 11	NA	NA	c.1752+2T>G	NA

Abbreviations: FVIII, factor VIII; NA, not available.

TABLE 2 Correlation analysis between F8 mutation risk groups and inhibitor states in 196 children with severe hemophilia A with inhibitors

Characteristic	High-risk group (n = 44)	Medium-risk group (n = 116)	Low-risk group (n = 36)	P value
Titer at inhibitor development/BU				
Median (IQR)	10.7 (5.4-46.1)	12.3 (2.0-28.6)	4.4 (1.7-17.5)	.10
EDs at inhibitor development/d				
Median (IQR)	32 (13-55)	25 (17-48)	30 (20-57)	.50
Peak inhibitor titer/BU				
Median (IQR)	80.5 (29.4–259.8) ^a	32.2 (11.7–89.6) ^b	19.9 (5.2–75.2) ^b	<.001
SHA that progressed to high-titer inhibitor (%)	10/10 (100.0) ^a	22/38 (57.9) ^b	10/19 (52.6) ^b	.03

Note: The different annotation with superscript letter a or b indicates that there is a significant difference between groups ($P < .05$), while the same annotation means that there is no significant difference between groups ($P > .05$).

Abbreviations: BU, Bethesda units; EDs, exposure days; IQR, interquartile range; SHA, severe hemophilia A.

Our study was a single-center retrospective cohort study, which included only children with severe hemophilia A with inhibitors. Some clinical data (EDs and historical inhibitor titers) came from local hospitals or the records of the parents, and the accuracy needed to be confirmed. Furthermore, this study lacked data on plasma levels of FVIII antigen (FVIII:Ag), which might also be associated with inhibitor levels. Spena et al¹⁹ found that people with

severe hemophilia A with undetectable FVIII:Ag (<1%) have an increased risk of inhibitor development than people with measurable FVIII:Ag ($\geq 1\%$) and confirmed the protective effect of minute amounts of FVIII. Although no studies have evaluated the association of undetectable FVIII:Ag with higher peak titers of inhibitor, this potential link remains possible and requires further investigations to confirm.

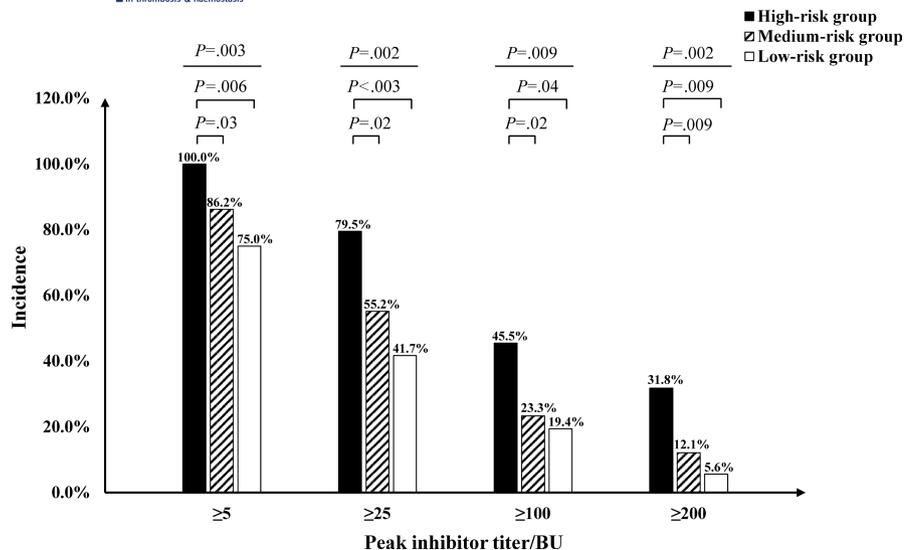


FIGURE 2 Distribution of peak inhibitor titer in F8 mutation risk groups of 196 children with severe hemophilia A with inhibitors

5 | CONCLUSION

This was the first large cohort study of F8 mutation profiles in severe children with hemophilia A with inhibitors in China. Intron 22 inversions, nonsense mutations, and large deletions or insertions focusing on the high- and medium-risk F8 gene mutation types were the main mutation types, accounting for 75.4%. The high-risk F8 mutation types of inhibitor development (large deletions or insertions in multiple exons, and nonsense mutations in the light chain) developed a higher peak titer of inhibitor.

AUTHOR CONTRIBUTIONS

RW and ZC contributed to the study design and preparation of the manuscript. JS collected and analyzed the data and wrote the manuscript. ZL collected the data, performed the analysis, and completed the experiment. KH, DA, and GL performed the research. XX and HG reviewed the manuscript. GL provided a critical and detailed revision of the manuscript. YZ performed literature searches. All authors had full access to the data and participated in the design of the analysis, discussion of results, and revising the draft manuscript.

ACKNOWLEDGMENTS

We thank the patients and their parents. This work was supported by the grants from Capital Health Development Research Project (Capital Development Number, 2022-2-2093), Children's Medicine Research Project of Beijing Children's Hospital, Capital Medical University (YZZD202003), and Cultivation Fund Project of the National Natural Science Foundation in Beijing Children's Hospital, Capital Medical University (No. GPMS202004).

RELATIONSHIP DISCLOSURE

The authors declare no conflicts of interest or bias.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Zekun Li <https://orcid.org/0000-0002-1421-4590>

Kun Huang <https://orcid.org/0000-0003-1641-1472>

Xingjuan Xie <https://orcid.org/0000-0002-3789-9182>

Zhenping Chen <https://orcid.org/0000-0002-5273-145X>

Runhui Wu <https://orcid.org/0000-0003-4030-209X>

REFERENCES

- Guo Z, Yang L, Qin X, Liu X, Zhang Y. Spectrum of molecular defects in 216 Chinese families with hemophilia A: identification of noninversion mutation hot spots and 42 novel mutations. *Clin Appl Thromb Hemost.* 2018;24(1):70-78.
- van den Berg HM, Fischer K, Carcao M, et al. Timing of inhibitor development in more than 1000 previously untreated patients with severe hemophilia A. *Blood.* 2019;134(3):317-320.
- Young G. How I treat children with haemophilia and inhibitors. *Br J Haematol.* 2019;186(3):400-408.
- Garagiola I, Palla R, Peyvandi F. Risk factors for inhibitor development in severe hemophilia A. *Thromb Res.* 2018;168:20-27.
- ter Avest PC, Fischer K, Mancuso ME, et al. Risk stratification for inhibitor development at first treatment for severe hemophilia A: a tool for clinical practice. *J Thromb Haemost.* 2008;6(12):2048-2054.
- Srivastava A, Brewer AK, Mauser-Bunschoten EP, et al. Guidelines for the management of hemophilia. *Haemophilia.* 2013;19(1):e1-47.
- Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405-424.
- Kim JY, You CW. The prevalence and risk factors of inhibitor development of FVIII in previously treated patients with hemophilia A. *Blood Res.* 2019;54(3):204-209.
- Mancuso ME, Fischer K, Santagostino E, et al. Risk factors for the progression from low to high titres in 260 children with severe haemophilia A and newly developed inhibitors. *Thromb Haemost.* 2017;117(12):2274-2282.
- Gouw SC, van der Bom JG, Ljung R, et al. Factor VIII products and inhibitor development in severe hemophilia A. *N Engl J Med.* 2013;368(3):231-239.
- Gouw SC, van der Bom JG, Marijke van den Berg H. Treatment-related risk factors of inhibitor development in previously

- untreated patients with hemophilia A: the CANAL cohort study. *Blood*. 2007;109(11):4648-4654.
12. Gouw SC, van den Berg HM, Oldenburg J, et al. F8 gene mutation type and inhibitor development in patients with severe hemophilia A: systematic review and meta-analysis. *Blood*. 2012;119(12):2922-2934.
 13. Atik T, Işık E, Onay H, et al. Factor 8 gene mutation spectrum of 270 patients with hemophilia A: identification of 36 novel mutations. *Turk J Haematol*. 2020;37(3):145-153.
 14. Nakar C, Shapiro A. Hemophilia A with inhibitor: immune tolerance induction (ITI) in the mirror of time. *Transfus Apher Sci*. 2019;58(5):578-589.
 15. Carcao M, Escuriola-Ettingshausen C, Santagostino E, et al. The changing face of immune tolerance induction in haemophilia A with the advent of emicizumab. *Haemophilia*. 2019;25(4):676-684.
 16. Luna-Záizar H, González-Alcázar JÁ, Evangelista-Castro N, et al. F8 inversions of introns 22 and 1 confer a moderate risk of inhibitors in Mexican patients with severe hemophilia A. Concordance analysis and literature review. *Blood Cells Mol Dis*. 2018;71:45-52.
 17. Lannoy N, Hermans C. Genetic mosaicism in haemophilia: a practical review to help evaluate the risk of transmitting the disease. *Haemophilia*. 2020;26(3):375-383.
 18. Lu Y, Xin Y, Dai J, et al. Spectrum and origin of mutations in sporadic cases of haemophilia A in China. *Haemophilia*. 2018;24:291-298.
 19. Spena S, Garagiola I, Cannavò A, et al. Prediction of factor VIII inhibitor development in the SIPPET cohort by mutational analysis and factor VIII antigen measurement. *J Thromb Haemost*. 2018;16(4):778-790.

How to cite this article: Sun J, Li Z, Huang K, et al. F8 gene mutation spectrum in severe hemophilia A with inhibitors: A large cohort data analysis from a single center in China. *Res Pract Thromb Haemost*. 2022;6:e12723. doi:[10.1002/rth2.12723](https://doi.org/10.1002/rth2.12723)