ORIGINAL ARTICLE

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

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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).
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. 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% 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. 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. Based on the review article by Garagiola et al 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.

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
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. 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 F VIII:C levels, treatment methods and types of F VIII 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 × C table), and the chi-square test of Bonferroni-corrected 2 × 2 table was used for multiple comparisons between groups. All P values were 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 F VIII inhibitors, all male, were included in this study. The ethnicities of all participants included in the analysis were 193 Han, 4 Zhuang, 3 Tuja, 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 F VIII was the primary replacement therapy and was used in 122 cases (60.1%). Recombinant F VIII was administered in 71 cases (35.0%), and 10 cases (4.9%) were treated with both types of F VIII 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.
3.3 | Correlation analysis between F8 mutation risk groups and inhibitor status

Based on the risk categories proposed by Garagiola et al., 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 $\geq 5$ BU, $\geq 25$ BU, $\geq 100$ BU, and $\geq 200$ 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 al.4 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 $\geq 200$ 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 studies16,17 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.
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 al. 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 (≥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.
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.

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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.

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