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ORIGINAL ARTICLE

Low-dose immune tolerance induction in children with severe hemophilia A with high-titer inhibitors: Type of factor 8 mutation and outcomes

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Abstract

Background: No studies evaluated the role of *F8* mutations in outcomes for low-dose immune tolerance induction (ITI) in people with severe hemophilia A (SHA) with high-titer inhibitors.

Objectives: To explore the association between *F8* mutation types and low-dose ITI outcomes in children with SHA with high-titer inhibitors.

Methods: Children SHA with high-titer inhibitors who received low-dose ITI therapy at least for 1 year were included in this study. Based on the risk of inhibitor development, *F8* mutations were classified into a high-risk group and a non–high-risk group. Rapid tolerance and the final ITI outcomes were assessed at the 12th and 24th month of treatment, respectively, and the predictor of outcomes was analyzed.

Results: Of 104 children included, 101 had *F8* mutations identified. The children with non–high-risk mutations presented a higher rate of rapid tolerance than those with high-risk mutations (61.0% vs. 29.2%; $p = 0.006$). Among 72 children beyond 24 months of ITI, 55 children (76.4%) achieved success, 3 (4.2%) achieved partial success, and 14 (19.4%) failed. The children in the non–high-risk group showed a higher success rate (86.8% vs. 43.8%; $p = 0.001$) and a shorter time to success (mean time, 9.3 months vs. 13.2 months; $p = 0.04$) compared to those in the high-risk group. In multivariable logistic regression, *F8* mutations were an independent predictor of ITI success (non–high-risk group vs. high-risk group, adjusted odds ratio [OR], 20.3; 95% confidence interval [CI], 3.5–117.8), as was the interval from inhibitor diagnosis to ITI

start (adjusted OR, 0.95; 95% CI, 0.90–0.99). They remained the significant predictors when success time was taken into account in a Cox model.

Conclusions: Types of *F8* mutation were a key predictor of outcomes for low-dose ITI in children with SHA with high-titer inhibitors. It can help to stratify the prognosis and guide clinical decisions.

KEYWORDS
F8 mutation, high-titer inhibitor, immune tolerance induction, low-dose, predictor, severe hemophilia A

Essentials

1. No study evaluated the *F8* mutation role in outcome for low-dose immune tolerance induction (ITI).
2. The predictor of ITI outcome in severe hemophilia A (SHA) with high-titer inhibitors was analyzed.
3. Non–high-risk *F8* mutations were strongly associated with low-dose ITI success and time to success.
4. *F8* mutations were a key predictor of outcomes for low-dose ITI in SHA with high-titer inhibitors.

1 | INTRODUCTION

Alloantibodies (inhibitors) against coagulation factor VIII (FVIII) usually develop in 25%–35% of people with severe hemophilia A (SHA) (FVIII clotting activity of <1%) during the initial 50 exposure days, and two thirds of these people develop persistent and high-titer inhibitors, which is the most serious and challenging issue in the management of people with SHA.[1–2] These people face a higher risk of disability, worse quality of life, and death than those without developing inhibitors. At present, immune tolerance induction (ITI) is the only strategy to eradicate high-titer inhibitors for patients by frequent exposure to FVIII concentrates. The predictors of outcome and time to success have been developed based on the available data from ITI registries and other publications.[3–9] The *F8* genotypes are a major risk factor for inhibitor development.[10–11] Based on a review involving dozens of single- and multiple-center cohort studies, Garagiola et al.[11] classified *F8* mutation types into a high-risk group of inhibitor development (large deletions or insertions in multiple exons and nonsense mutations in the light chain) and non–high-risk group (low- or medium-risk mutations including large deletions or insertions in a single exon, nonsense mutations in the heavy chain, inversions, small deletions or insertions, missense, and splicing-site mutations).

The efficacy of low-dose ITI was confirmed to be comparable to that of other regimens.[12–13] As far as we know, none have reported the association between *F8* mutations and outcomes of low-dose ITI regimen, although a few studies[7–8] have assessed the role of *F8* mutations in ITI response. This study aimed to elucidate the predictors of outcome for a low-dose ITI regimen in people with SHA with high-titer inhibitors in China to address the issue.

2 | MATERIALS AND METHODS

2.1 | Participants

The single-center retrospective cohort study was conducted in children with SHA with high-titer inhibitors who were admitted from January 2015 to May 2022. The study was approved by the Ethics Review Committee of Beijing Children's Hospital, and the informed consent form was acquired from the patients/guardians appropriately.

Inclusion criteria were as follows: (i) children with an established diagnosis of SHA;[14] (ii) children aged younger than 14 years old at the first visit to our center; (iii) children with inhibitor titers of 5 Bethesda units (BU)/ml or higher on at least one occasion from our medical records; (iv) children who received a low-dose ITI regimen alone or with immunosuppressants for more than 1 year; (v) people who had *F8* gene analysis at our center. Exclusion criteria were as follows: (i) children who had acquired hemophilia; (ii) children with SHA with comorbidity of autoimmune or chronic infectious disease; (iii) children who refused ITI therapy.

2.2 | Clinical data collection and definition of ITI outcome

All patient-related and treatment-related data were obtained from medical records from the hemophilia comprehensive care center, including history of inhibitor development, time interval between inhibitor diagnosis and ITI initiation, age, inhibitor titer at ITI start, and treatment information.

All patients received a low-dose ITI regimen (plasma-derived FVIII/von Willebrand factor concentrate at 50 FVIII IU/kg every other day) alone or combined with the immunosuppressants rituximab and prednisone (ITI-IS). Patients with the following conditions were treated with the ITI-IS regimen: (i) patients with historical peak inhibitor titer of ≥100 BU/ml or titer of ≥40 BU/ml at the onset of ITI; (ii) patients on ITI alone were switched to ITI-IS if the peak titer of ≥40 BU/ml during ITI or if the inhibitor decline was <20% in the first 3 months after the initial peak titer on ITI.

The FVIII inhibitor titers were determined using the Nijmegen modification of the Bethesda assay. During ITI, inhibitor assay was performed every 2 weeks until a clear downward trend after the initial peak titer, then monthly until normal FVIII recovery, and thereafter every 3 months for monitoring. FVIII recovery was assessed when two consecutive inhibitor assays gave values of <0.6 BU/ml.

ITI outcome was reviewed according to the following criteria: (i) partial success: achieving inhibitor elimination (FVIII inhibitor titer of <0.6 BU/ml in at least two consecutive assays), but persistently abnormal FVIII recovery; (ii) success: negative inhibitor titer and FVIII recovery of ≥66% of expected values; (iii) failure: partial success and success were not achieved within 24 months of treatment. Furthermore, rapid tolerance was defined as patients achieving ITI success within 1 year.

2.3 | Molecular genetic analysis and *F8* mutation classification

F8 genetic tests were performed using a combination of molecular techniques including long-distance polymerase chain reaction, next-generation sequencing, and multiplex ligation-dependent probe amplification according to the manufacturer's protocols. The interpretation of sequence variants was performed according to the American College of Medical Genetics and Genomics guidelines.[15] Pedigree verification was conducted using corresponding molecular assays. The types of *F8* mutations identified were stratified into two classes according to Garagiola's research[11] mentioned above: a high-risk group and a non–high-risk group.

2.4 | Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics version 26.0 for Windows (IBM Corp.). Count data were expressed in frequencies (*n*) and percentages (%), and measurement data were expressed as median (interquartile range [IQR]). Differences between continuous variables were analyzed using the *t* test or Mann–Whitney *U* test, and categorical variables were evaluated by chi-square test or Fisher's exact test. Odds ratio (OR) and 95% confidence interval (CI) were appropriately calculated. Adjusted OR and 95% CI were obtained using logistic regression models controlling for variables with *p* values derived as less than 0.2 in the univariate analysis. Hazard ratio (HR) and 95% CI were derived from a Cox model, which accounted for both success time and success rate. *P* values less than 0.05 were considered significant.

3 | RESULTS

3.1 | Patients' characteristics and clinical information

A total of 104 unrelated children with SHA with high-titer inhibitors were included in this study. The ethnicities of all participants were 100 Han, 3 Zhuang, and 1 Tujia. All patients investigated received ITI therapy at a median age of 3.2 years, after a median interval time of 5.3 months from inhibitor diagnosis. Among them, 60 cases (57.7%) underwent the ITI-IS regimen. The detailed clinical information is shown in Table S1. Over a median follow-up time of 24.4 months (IQR, 17.5–31.9), 51.9% (54/104) of patients achieved rapid tolerance. Of the 72 patients treated for more than 2 years, 55 patients (76.4%) were successful with a median time to success of 9.5 months (IQR, 6.0–13.1), only 3 cases (4.2%) achieved partial success, whereas the remaining 14 cases (19.4%) failed.

Three patients (5.5%) relapsed with, a mean time of 7.2 months (range, 3.5–9.9) after achieving success initially due to rapid ITI dose reduction or irregular post-ITI FVIII prophylaxis. One patient on the ITI-alone regimen reestablished success without relapse after repeating the original ITI dose (50 FVIII IU/kg every other day). Two patients on the ITI-IS regimen were managed with an additional course of rituximab (375 mg/m^2 weekly for 2 weeks), one of whom achieved success again without relapse, while the other sustained low-titer inhibitor over 9.4 months at the time of data analysis.

3.2 | Relationship between *F8* variant types and ITI outcomes

A total of 101 patients had *F8* mutations identified in this study, including 42 cases (40.4%) of intron 22 inversions, 22 cases (21.2%) of nonsense mutations (6 cases in the light chain and 16 cases in the heavy chain), and 20 cases (19.2%) of large deletions or insertions (18 cases with multiple exons and 2 cases with one exon), and the remaining variants as shown in Figure 1. Three patients with unknown variants were excluded in the analysis, of whom two cases achieved success after a treatment time of 12.3 and 18.6 months, respectively, and ITI failed in the remaining one case.

The patient characteristics were comparable in the two mutation groups stratified on the basis of the risk of inhibitor development, as illustrated in Table 1. The patients in the high-risk group developed significantly higher historical peak inhibitor titers (median titer, 59.4 BU/ml vs. 28.8 BU/ml; $p = 0.05$), higher pre-ITI titers (median titer, 44.8 BU/ml vs. 18.2 BU/ml; $p = 0.01$), and higher peak titers during ITI (median titer, 74.6 BU/ml vs. 23.4 BU/ml; $p < 0.001$). In addition, the patients carrying high-risk variants received a higher

rate of combined immunosuppressant therapy (87.5% vs. 48.1%; $p = 0.001$).

The patients carrying *F8* variants associated with the non–high risk of inhibitor development presented a significantly higher rate of rapid tolerance than those carrying high-risk mutations (61.0% vs. 29.2%; $p = 0.006$). When outcomes were assessed in 69 patients treated for more than 2 years, a significantly higher success rate (86.8% vs. 43.8%; $p = 0.001$) and a shorter time to success (mean time, 9.3 months vs. 13.2 months; $p = 0.04$) were observed in non–high-risk group, as seen in Table 2.

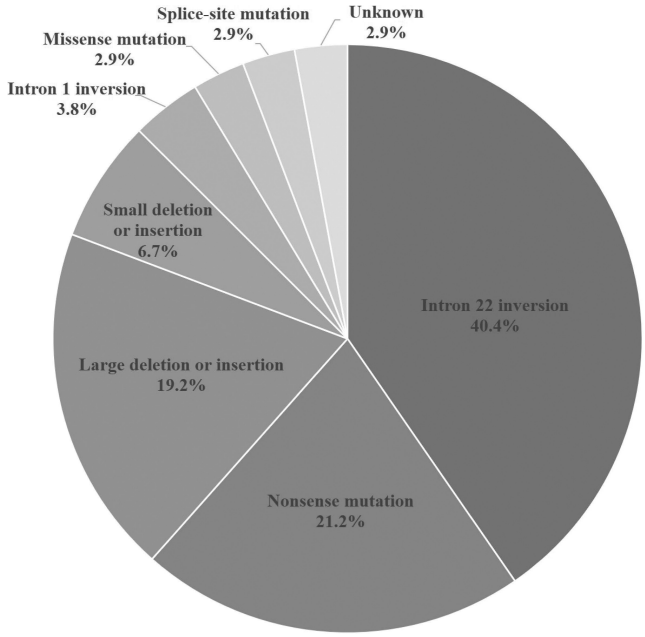

FIGURE 1 Distribution of *F8* variant types in a cohort of 104 children with severe hemophilia A with high-titer inhibitors

3.3 | Predictors for ITI success

The variables with *p* values less than 0.2 from the univariate logistic analysis were included in the multivariable logistic regression, including *F8* mutation types, age at ITI initiation, time interval from inhibitor diagnosis to ITI start, and peak inhibitor titer during ITI. Upon the multivariable analysis, non–high-risk *F8* mutations were found as an independent predictor of ITI success (non–high-risk group vs. high-risk group: adjusted OR, 20.3; 95% CI, 3.5–117.8), as was the time interval from inhibitor diagnosis to ITI start (adjusted OR, 0.95; 95% CI, 0.90–0.99). The detailed results are presented in Table 3. When time to success was taken into account, in a Cox model, *F8* mutations (non–high-risk group vs. high-risk group: hazard ratio [HR], 5.5; 95% CI, 2.3–13.1; Figure 2) and time interval from inhibitor diagnosis to ITI start (HR, 0.97; 95% CI, 0.95–0.99) were also found to be the significant predictors of outcomes, as seen in Table S2.

4 | DISCUSSION

Although a few studies[7–8] have demonstrated the role of *F8* mutations on ITI response, the association of *F8* mutations with the outcome of low-dose ITI remains unclear. To our knowledge, this research was the first large ITI cohort study that analyzed the predictors of the outcomes for low-dose ITI and specifically the association between *F8* genotypes and the outcomes in people with SHA with

TABLE 1 Comparison of clinical characteristics between the *F8* mutation classes

Variables	Non–high-risk group ($n = 77$)	High-risk group ($n = 24$)	OR/median difference (95% CI)
Age at inhibitor diagnosis, years, median (IQR)	2.3 (1.3–3.8)	2.5 (1.4–5.4)	0.3 (−0.5 to 1.3)[a]
Eds of inhibitor development, days, median (IQR)	29 (16–48)	31 (12–50)	0.0 (−10 to 12)[a]
Titer at inhibitor diagnosis, BU/ml, median (IQR)	12.0 (3.6–31.0)	15.5 (6.5–44.9)	2.4 (−4.6 to 11.8)[a]
Historical peak inhibitor titer, BU/ml, median (IQR)	28.8 (16.4–72.1)	59.4 (22.4–138.5)	16.6 (0.2 to 42.2)[a]
Time interval between inhibitor diagnosis and ITI start, months, median (IQR)	6.6 (1.0–25.2)	2.4 (0.5–31.8)	−0.5 (−3.9 to 2.0)[a]
Age at ITI start, years, median (IQR)	3.2 (2.2–6.2)	3.0 (1.7–8.0)	0.1 (−1.0 to 1.6)[a]
Pre-ITI inhibitor titer, BU/ml, median (IQR)	18.2 (9.0–36.2)	44.8 (17.5–96.1)	17.9 (3.1 to 41.1)[a]
Peak inhibitor titer during ITI, BU/ml, median (IQR)	23.4 (8.1–64.7)	74.6 (36.5–144.2)	42.3 (19.2 to 67.8)[a]
Treatment regimen, *n* (%)			
ITI alone	40 (51.9)	3 (12.5)	7.6 (2.1 to 27.5)[b]
ITI-IS	37 (48.1)	21 (87.5)	

Abbreviations: BU, Bethesda units; Eds, exposure days; IQR, interquartile range; IS, immunosuppressant; ITI, immune tolerance induction; OR, odds ratio.

[a]95% CI for difference between medians of continuous variables across two groups.

[b]95% CI for OR across two groups (non–high-risk/high-risk).

TABLE 2 Distribution of ITI response for *F8* mutation classes in the current study population

ITI response	Non–high-risk group	High-risk group	OR/mean difference (95% CI)
Rapid tolerance assessed at 12th month ($N = 101$)[c]			
Rapid tolerance, *n* (%)	47 (61.0)	7 (29.2)	3.8 (1.4 to 10.3)[a]
Tolerance time, months, mean ± SD	7.2 ± 3.0	7.6 ± 2.4	0.4 (−2.0 to 2.8)[b]
ITI outcomes assessed at 24th month of treatment ($N = 69$)[d]			
Success, *n* (%)	46 (86.8)	7 (43.8)	8.4 (2.4–30.0)[a]
Partial Success, *n* (%)	2 (3.8)	1 (6.3)	0.6 (0.05–6.94)[a]
Failure, *n* (%)	5 (9.4)	8 (50.0)	0.1 (0.03–0.40)[a]
Success time, months, mean ± SD	9.3 ± 4.5	13.2 ± 4.7	3.9 (0.2–7.6)[b]

Abbreviations: CI, confidence interval; ITI, immune tolerance induction; OR, odds ratio; SD, standard deviation.

[a]95% CI for OR across two groups (non–high-risk/high-risk).

[b]95% CI for difference between mean of continuous variables across two groups.

[c]Non–high-risk group: large deletions or insertions with one exon ($n = 2$), nonsense mutations in the heavy chain ($n = 16$), intron 22 inversions ($n = 42$), intron 1 inversions ($n = 4$), small deletions or insertions ($n = 7$), missense mutations ($n = 3$), splicing site mutations ($n = 3$); high-risk group: large deletions or insertions with multiple exons ($n = 18$), nonsense mutations in the light chain ($n = 6$).

[d]Non–high-risk group: large deletions or insertions with one exon ($n = 0$), nonsense mutations in the heavy chain ($n = 14$), intron 22 inversions ($n = 27$), intron 1 inversions ($n = 3$), small deletions or insertions ($n = 4$), missense mutations ($n = 3$), splicing site mutations ($n = 2$); high-risk group: large deletions or insertions with multiple exons ($n = 11$), nonsense mutations in the light chain ($n = 5$).

TABLE 3 Logistic regression analysis on predictors of ITI success

	Univariate logistic regression		Multivariable logistic regression	
Variables	**Crude OR**	**95% CI**	**Adjusted OR**	**95% CI**
Patients carrying non–high-risk variants	8.4	2.4–30.0	20.3	3.5–117.8
Time interval from inhibitor diagnosis to ITI start, months	0.97	0.94–0.99	0.95	0.90–0.99
Age at ITI start, years	0.82	0.69–0.98	0.9	0.7–1.2
Peak inhibitor titer during ITI <100 BU/ml	2.3	0.7–7.7	2.4	0.5–12.4
Historical peak inhibitor titer <200 BU/ml[a]	2.8	0.6–14.2	–	–
Pre-ITI inhibitor titer <10 BU/ml[a]	0.9	0.2–3.2	–	–
Pre-ITI inhibitor titer <5 BU/ml[a]	0.9	0.2–4.9	–	–
Treatment regimen of ITI-IS[a]	0.5	0.2–1.7	–	–

Abbreviations: BU, Bethesda units; CI, confidence interval; IS, immunosuppressant; ITI, immune tolerance induction; OR, odds ratio.

[a]These variables with *p* values >0.20 in the univariate analysis were excluded from multivariable logistic regression analysis.

high-titer inhibitors. *F8* variant types were established as the most important predictors of low-dose ITI response in the current study.

This project focused on subjects with SHA with high-titer inhibitors. Although the children enrolled in this retrospective cohort study were managed with low-dose regimen, the overall success rate of ITI (76.4%) was comparable to that from other large studies.[16–17]

F8 genotype was not only associated with inhibitor formation but also with the outcome of ITI because the high-risk mutations of inhibitor development were more likely to have a poor prognosis in ITI.[8] The ITI data from our center further confirmed that the type of *F8* mutation was a strong predictor of ITI success and time to success. Several cohort studies[7–9,18] also provided information on *F8* mutations in patients with an ITI regimen. Peyvandi et al.[18] reported that patients with large deletions had the lowest success rate (1/8) of ITI, and a similar finding was revealed that large deletions in *F8* were associated with ITI unresponsiveness.[7] The Italian ITI registry observed that small insertions/deletions and missense mutations were linked to complete inhibitor eradication and a shorter time to ITI success.[8–9] This study showed that the patients carrying non–high-risk mutations associated with inhibitor development were more likely to be successful in ITI and resulted in a shorter time to success. Interestingly, patients carrying high-risk variants were associated with higher inhibitor titers, including historical peak titers, pre-ITI titers, and peak titers during ITI, as well as higher rates of combined immunosuppressive treatment. Higher inhibitor titers in patients with high-risk *F8* mutations may reflect actual stronger immune stimulation, and thus may have resulted in a lower chance of success or longer time to success.[8,19]

Previous studies suggested that the shorter time from inhibitor development to ITI start, the greater chance of success.[3,20] Liu et al.[21] also proposed a rationale to support the above conclusion that an early ITI can suppress the maturation of immune response

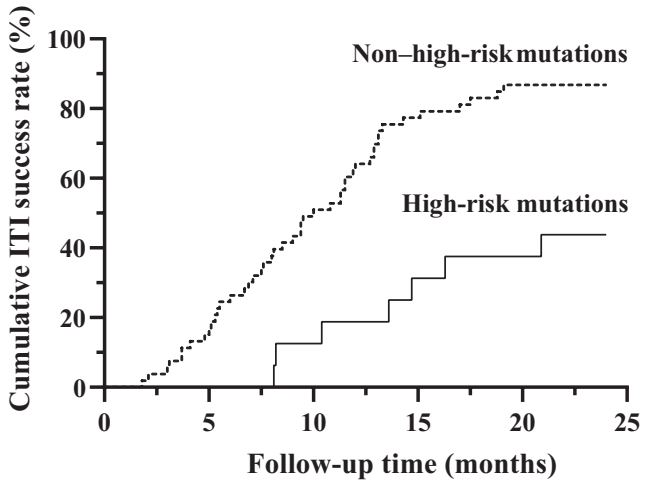

FIGURE 2 Cumulative ITI success rates for different *F8* mutation risk classes over the treatment time. ITI, immune tolerance induction

and the generation of long-lived plasma cells. In this cohort, we also confirm the role of the time interval between inhibitor diagnosis and ITI initiation as predictors of ITI response and further discovered that the shorter the interval, the shorter time to success. On the other hand, pre-ITI inhibitor titer, historical peak titer, and peak titer during ITI had no significant impact on the outcomes from our data analysis. However, some studies involving multivariable analysis to evaluate outcomes emphasized the role.[9,16,22] Particularly the inhibitor titer of less than 10 BU/ml at the initiation of ITI, historical peak titer of less than 200 BU/ml, and peak titer of less than 100 BU/ml during ITI were identified as predictors of ITI success.[9,22] The reason for this inconsistency between our results and previous studies may have been that the combination of immunosuppressive therapy in patients with relatively high inhibitor titer may have improved the chance of ITI success, thereby affecting the potential role of the above variables in outcomes of ITI.

Establishing predictors of ITI success using multivariable analysis remains critical for optimizing the selection, treatment strategies, and follow-up for people with inhibitor. Despite the limitations of this single-center retrospective study (e.g., patients lacked half-life data of FVIII products during ITI due to relatively poor compliance; there was a limited number of subjects harboring high-risk mutations, and the discrepancy in subject number between the two groups may slightly decrease the power of statistics; further investigation with a larger sample size was necessary to confirm and generalize the conclusion), the centralized review for outcomes, the homogeneous collection of data, and the long-term follow-up provided evidence for predicting the outcomes of low-dose ITI regimen.

5 | CONCLUSION

The types of *F8* mutation were a key predictor of the success for low-dose ITI therapy and time to success, and the high-risk genotypes (large deletions or insertions in multiple exons and nonsense mutations in the light chain) were associated with a relatively poor prognosis. An early ITI therapy improved prognosis and shortened time to tolerance in children with SHA with high-titer inhibitors. This study provided a solid context for future research, which can combine *F8* genotyping with clinical predictors to develop tools, such as a clinical scoring system, that can identify high-risk patients with poor prognosis to optimize clinical choices.

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 and completed the experiment; KH, DA, and GL performed the research; XX and HG reviewed the manuscript; GL and WY provided a critical and detailed revision of the manuscript; and 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 stated that they had no conflicts of interest or bias.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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