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Potential genetic markers predicting the outcome of brace treatment in patients with adolescent idiopathic scoliosis

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Abstract

Purpose To investigate whether the predisposition genes previously reported to be associated with the occurrence or curve severity of adolescent idiopathic scoliosis (AIS) play a role in the effectiveness of brace treatment.

Method A total of 312 AIS patients treated with bracing were enrolled in this study. The Cobb angle of the main curve was recorded at the beginning of brace treatment as well as at each follow-up. The patients were divided into two groups according to the outcome of brace treatment (success/failure). The failure of brace treatment was defined as a curve progression of more than $5°$ compared to the initial Cobb angle or surgical intervention because of curve progression. Single nucleotide polymorphism (SNP) sites in the genes for estrogen receptor α (ERα), estrogen receptor β (ERβ), tryptophan hydroxylase 1 (TPH-1), melatonin receptor 1B (MTNR1B) and matrillin-1 (MATN1), which were previously identified to be predisposition genes for AIS, were selected for genotyping by the PCR-RFLP method. Differences of genotype and allele distribution between the two groups were compared by the χ^2 test. A logistic regression analysis was used to figure out the independent predictors of the outcome of brace treatment.

Results There were 90 cases (28.8%) in the failure group and 222 cases (71.2%) in the success group. Patients in the failure group were associated with the genotype GA (50.9 vs. 17.9% $p < 0.001$) and the G allele (27.1 vs. 12.0%, $p < 0.001$) at SNP rs9340799 of the ERα gene. Similarly, they were also associated with the genotype AT (33.3 vs. 13.0%, $p = 0.002$) and the A allele (16.7 vs. 9.6%, $p = 0.033$) at SNP rs10488682 of the TPH-1 gene. For MTNR1B, the difference of genotype distribution between the two groups was found to be statistically significant, while the difference of allele distribution between the two groups was found to be marginally statistically significant; for the MATN1 and ERβ genes, we found no significant differences of the genotype or allele distribution between the two groups. In the logistic regression analysis, ERα and TPH-1 were demonstrated to be independent factors predictive of bracing effectiveness.

Conclusions ERα and TPH-1 might be potential genetic markers that could predict the outcome of brace treatment. Patients with the G allele at the rs9340799 site of the ERα gene and the A allele at the rs10488682 site of the TPH-1 gene are prone to be resistant to brace treatment.

Keywords Gene polymorphism · Brace treatment · Adolescent idiopathic scoliosis

Introduction

As the main non-operative treatment for adolescent idiopathic scoliosis (AIS) patients, brace treatment has proved to be effective in preventing curve progression by many studies conducted in different areas or with different ethnic groups [1–3]. Nevertheless, wearing brace is always associated with excessive radiographic exposure, anxiety and indirect cost-of-care caused by absence from work or travel expenses [4, 5]. An investigation into the factors that affect the outcome of brace treatment could help narrow the field of indications and provide further insight into whether brace treatment should be continued so as to avoid overtreatment or not. The prediction of the final outcome of brace treatment has been

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studied by many authors, and several risk factors for curve progression have been documented [6–11]. Lonstein and Winter [10] reviewed 1,020 patients treated with the Milwaukee brace, of whom 229 patients (22%) had to resort to surgery. In their series, Risser sign and curve magnitude were found to be associated with failure of brace treatment. In addition to Risser sign and curve magnitude, the degree of initial correction could also predict the outcome of bracing. In a study of 102 patients treated by the Boston brace, Yrjonen et al. [6] found the association between the degree of initial correction and progression risk. However, as to curve magnitude and chronologic age, no association was found in their study. Curve pattern has also been proposed to be a prognostic factor for brace treatment; nevertheless, the predictive value of different curve patterns varied in three independent series [7, 10, 11]. To sum up, the knowledge of factors that affect the effectiveness of brace treatment is not clear enough to accurately predict its final outcome yet.

The role of genetic factors in the development of AIS has been well supported. Genetic association studies have served as a powerful tool to study genetic predisposition in AIS, [12] and to date, several single nucleotide polymorphisms (SNP) have been found to be associated with AIS. In a case-only study of 304 AIS patients, Inoue et al. [13] first reported that polymorphisms of the oestrogen receptor α (ERα) were associated with curve severity in AIS patients, which was later confirmed by Wu's study [14]. In the following several years, four other predisposition genes were subsequently demonstrated to be involved in the occurrence or curve severity of AIS, including SNP rs1149048 of the matrillin-1 (MATN1) gene, [15] SNP rs1256120 of the oestrogen receptor β (ER β) gene, [16]. SNP rs10488682 of the tryptophan hydroxylase 1 (TPH-1) gene [17] and SNP rs4753426 of the melatonin receptor 1B (MTNR1B) gene [18]. At the 2008 Annual Meeting of the Scoliosis Research Society (SRS), Ward [19] pointed out that surgeons might be able to predict which kind of patients are likely to progress to severe scoliosis by using a panel of genetic markers identified to be associated with curve progression. Inspired by that study, we assumed that genetic factors could have an influence on the outcome of brace treatment as well. To validate this hypothesis, we performed a retrospective study to investigate the possible association between polymorphisms of those five predisposition genes and the effectiveness of brace treatment.

Patients and methods

Subjects

Under the approval of the Ethics Committee of the University, a retrospective study was performed in the authors'

scoliosis clinic. 1788 AIS patients who received brace treatment from January 2004 to September 2008 were reviewed. Inclusion criteria were as follows: initial curve magnitude ranging from 20° to 40°; no treatment prior to bracing; initially skeletal immature (Risser grade 0–3) and aged 10–15 years old; followed up at an interval of 3 months until brace weaning. At each visit during brace treatment, patients and their parents were inquired about the actual hours of brace wearing each day. The ratio of the actual hours to the scheduled hours per day was calculated to determine their compliance with brace treatment. Only those with more than 75% compliance were included in the study. Finally, a cohort of 312 patients fulfilled the inclusion criteria, of whom 219 patients' parents gave their informed consent to DNA analysis.

Brace treatment strategy

The choices of prescription of a Milwaukee brace or a Boston brace to the patients were made according to the curve pattern [20]. The Milwaukee brace was applied to the patient with a major thoracic, a double thoracic, a double major or a triple curve, while for patients with a thoracolumbar or a lumbar curve, the Boston brace was used. At the patient's first visit, the following data were recorded: age, gender, standing height, body weight, curve magnitude and curve pattern. Initially, each patient was instructed to wear the brace for 22 h/day. The patients were allowed to pursue physical exercise and personal hygiene out of the brace for up to 2 h each day. All the patients were followed up with consecutive clinical evaluations and radiographs at an interval of 3 months. Standing posteroanterior radiographs of the whole spine were obtained at each visit and radiographic parameters were recorded, including Cobb angle of the main curve, Risser grade and curve pattern. The scheduled duration of brace-wear per day was adjusted according to Cobb angle evolution and skeletal maturity status recorded at the visit. The outcome of bracing was evaluated as previously described by Qiu et al. [20]. The deformity was considered worsened if the Cobb angle increased more than 5°, and it was considered stable if the change of Cobb angle was <5°, and it was considered improved if the Cobb angle decreased more than 5°. If the deformity kept stable, the daily wearing time of brace was not modified for the first 6 months, and it would be shortened to 18 h/day in the next 6 months; if worsened, daily bracing time remained 22 hours per day; and if improved, or with the Risser grade >3, or over-1-year after menarche, daily bracing time was shortened by 2–4 h/day at each follow-up [20]. The weaning of brace treatment started at skeletal maturity, which could be evidenced by Risser sign (grade 4 for girls and 5 for boys), at least 30 months since the beginning of menarche for girls and

Table 1 Primers and conditions of PCR-RFLP analysis

Gene	SNP	Length (bp)	Alleles	Primer sequences	Tm (°C)	Restriction enzyme
ER α	rs9340799	1,374	A/G	F:5′-CTGCCACCCTATCTGTATCTTTTCCTATTCTCC-3′ R:5′-TCTTTCTCTGCCACCCTGGCGTCGATTATCTGA-3′	60	XbaI
TPH-1	rs10488682	220	A/T	F:5′-GCCCTAAAAGAGCGATTGGT-3′ R:5′-GAAGTTGCACAATGCAGACAA-3′	59	SpeI
MTNR1B	rs4753426	127	T/C	F:5′-AACATATTTGTGATTAATCCAGGC-3′ R:5′-TAACACCTGCAATTTCCACC-3′	56	Hae III
MATN1	rs1149048	224	A/G	F:5′-TGG AGGTGAACGAGGAGAAC-3′ R:5′-GAGCGGAGAAGTGACACAGA-3′	58	MSPI
ER β	rs1256120	300	T/C	F:5′-TGGAACTGGAGAGCTTGG-3′ R:5′-GTGCGGGTGACAAAATCC-3′	59	ALWNI

absence of increase in height for more than 6 months. If curve magnitude progressed beyond 50°, brace treatment was terminated, and patients were advised to receive surgery. The treatment was considered to be a failure if the curve progression was more than 5°, or if patients underwent surgery [21]. If the curve improved or kept stable as mentioned above, the treatment was considered successful [21]. According to the final outcome of brace treatment, the cohort was divided into the success group and the failure group.

SNPs genotyping

Peripheral blood was obtained at the convenience of the patients during bracing follow-up, and genomic DNA was extracted by using DNA extraction kit. Considering the accuracy and the convenience, we chose PCR-RFLP analyses for the genotyping of each polymorphism site. Polymerase chain reaction was carried out to amplify the targeted DNA fragment in a mixture consisting of PCR Taq mix, ddH$_2$O and primers. The sequences of primers were cited from the original literature. The reaction mixture was denatured at 94°C for 2 min, followed by 30 cycles at 94°C for 60 s, annealing temperatures for 45 s, 72°C for 40 s and then by a final extension at 72°C for 5 min. The polymerase chain reaction products were subsequently digested with specialized restriction enzyme devised with NEB Cutter, and genotypes were determined using 2.0–3.0% agarose gel electrophoresis. A repetition of the PCR-RFLP analysis over 50% was done to confirm the results. Sequences of the primers, annealing temperature and restriction enzyme of each SNP site are shown in Table 1.

Statistical analyses

Mean differences were compared using traditional analysis of variance, and student t test was used for two-group comparisons. Hardy–Weinberg equilibrium (HWE) was computed using a goodness-of-fit χ^2 test. Chi-square tests were used to evaluate distribution difference of allele and genotype frequencies of the two groups. A level of $p < 0.05$ was considered to be statistically significant. Logistic regression analysis was used to identify the independent predictors of the outcome of brace treatment. In our statistical analysis, genotypes of ER α were coded as 0 for AA and 1 for GA or GG. Genotypes of TPH-1 were coded as 0 for TT and 1 for AT or AA. Risser sign was coded as 0 for grade 2 or 3 and 1 for grade 0 or 1. The initial curve magnitude was coded as 0 for <30° and 1 for more than or equal to 30°.

Results

41 boys and 271 girls were included in the study. The mean age at the prescription of brace treatment was 12.7 ± 1.5 years old (range 10–15 years). For girls, the mean menarcheal age was 12.6 ± 1.2 years old (range 10–14.5 years). The mean bracing period was 2.5 ± 1.0 years (range 1.2–3.6 years), and the mean duration of follow-up was 1.2 ± 0.4 years (range 0.6–2.2 years). Overall, 90 patients received failure of brace treatment, of whom the mean curve magnitude increased from 27.9° to 40.3°. While for patients successfully treated by wearing brace, the mean curve magnitude kept stable, measured as 28.8° at the beginning and as 24.8° at the final visit. The failure rate of the brace treatment in our study was 28.8% (90/312). In addition, 60 individuals underwent surgery and the overall surgical rate was 19.2% (60/312). The patient in the failure group had a remarkably lower Risser sign (0.8 ± 1.2) than those in the success group (1.4 ± 1.3, $p < 0.001$). In terms of initial curve magnitude and chronologic age, no significant difference was found (Table 2).

Table 2 Comparison of initial curve magnitude, Risser sign and intial age between failure and success group

Groups	N	Initial curve magnitude	Risser sign	Initial age
Failure	90	28.0 ± 7.2	0.8 ± 1.2	12.4 ± 1.6
Success	222	28.7 ± 6.3	1.4 ± 1.3	12.8 ± 1.4
Statistical value		0.352	0.001	0.126

Analysis of the distribution of genotype

The distributions of the genotypes and alleles of the five predisposition gene are presented in Table 3. Hardy–Weinberg equilibrium was calculated and no significant differences of genotype frequencies were found in the cohort.

For SNP rs1149048 in MATN1 gene, the genotype GG in the failure group occupied a higher proportion than in the success group (40.4 vs. 32.5%, $p > 0.05$). The G allele was found to be a bit more frequent in the failure group than in the success group (64.5 vs. 59.6%, $p > 0.05$). For SNP rs1256120 in ERβ gene, the frequency of genotype CC was slightly higher in the failure group than in the success group (5.2 vs. 1.2%, $p > 0.05$) and so was the C allele (64.4 vs. 59.6%, $p > 0.05$). None of these differences were statistically significant.

With regard to ER α and TPH-1, significant association with the outcome of brace treatment was found. Patients in the failure group had more genotype GA (50.9 vs. 17.9%, $p < 0.001$) and G allele (27.1 vs. 12.0%, $p < 0.001$) at SNP rs9340799 of the ERα gene. Similarly, they also had more genotype AT (33.3 vs. 13.0%, $p = 0.002$) and A allele (16.7 vs. 9.6%, $p = 0.033$) at SNP rs10488682 of the TPH-1 gene. For the MTNR1B gene, the frequency of genotype CC was higher in patients with failure of brace treatment (54.4 vs. 35.8%, $p = 0.04$). The C allele was also more frequent in these patients, while the deviation was marginally significant (71.9 vs. 62.7%, $p = 0.074$).

Analysis of previously reported predictive factors

Curve pattern, curve magnitude at the prescription of brace treatment, gender and Risser sign were recorded and analysed to determine their influence on the outcomes of brace treatment, as shown in Table 4. According to the curve pattern, the subject were divided into three subgroups as follows: subgroup I for major thoracic curve ($n = 128$), subgroup II for single thoracolumbar or lumbar curve ($n = 66$) and subgroup III for double major curve ($n = 118$). The rate of curve progression was 32.1% for subgroup I, 30.3% for subgroup II and 24.5% for subgroup

Table 3 Association between potential risk factors and outcome of brace treatment

Items	N	Percentage of curve progression	Percentage of surgery
Risser sign			
0–1	177	35.0%	27.7%
2–3	135	20.7%	8.1%
P value (χ^2)		0.008	P < 0.001
Gender			
Male	41	34.1%	26.8%
Female	271	28.0%	18.0%
P value (χ^2)		0.304	0.203
Curve pattern			
Single thoracic curve	128	32.1%	23.4%
Single thoracolumbar or lumbar curve	66	30.3%	17.6%
Double major curve	118	24.5%	15.2%
P value (χ^2)		0.493	0.263
Initial curve magnitude			
<30°	195	27.2%	12.8%
≧30°	117	31.6%	29.9%
P value (χ^2)		0.113	<0.001

III. The incidence of operation intervention was 23.4% for subgroup I, 17.6% for subgroup II and 15.2% for subgroup III. The patients with a double major curve had a relatively lower rate of curve progression and operation than the other two subgroups, while the differences were not significant ($p = 0.493$, 0.263, respectively).

The primary curve with Cobb angle more than or equal to 30° was considered as a large curve. In our study, 195 patients were above this threshold. Although the incidence of surgery was significantly higher in patients with large curve (29.9 vs. 12.8%, $p < 0.001$), there was no remarkable difference in terms of the percentage of curve progression (31.6 vs. 27.2%, $p = 0.113$).

No subjects had Risser sign beyond grade 3 at the prescription of brace treatment, and 177 patients were of grade 0 or 1. The rate of curve progression was significantly higher in these patients with lower initial Risser sign (35 vs. 25.7%, $p = 0.008$). Similarly, the rate of surgery intervention of such subjects was also significantly higher (27.7 vs. 8.1%, $p < 0.001$).

The ratio of boys to girls was 1:6.5 in the current study. Boys had approximately the same duration of follow-up as girls, and there was no significant difference in terms of Risser sign or initial Curve magnitude. In addition, boys tended to have a higher rate of curve progression and they were more inclined to have surgery, whereas the differences were not significant (Table 3).

Table 4 Genotypes and alleles frequencies of five genes in effective and ineffective groups of brace treatment

Genotype	Success		Failure				Success		Failure		
	n	%	n	%	χ^2 (P)		n	%	n	%	χ^2 (P)
ER α					<0.001	Alleles					<0.001
GG	5	3.1	1	1.8		G	39	12.0	31	27.1	
GA	29	17.9	29	50.9		A	285	88.0	83	72.9	
AA	128	79.0	27	47.3							
MTNR1B					0.04	Alleles					0.074
TT	17	10.5	6	10.5		T	121	37.3	32	28.1	
TC	87	53.7	20	35.1		C	203	62.7	82	71.9	
CC	58	35.8	31	54.4							
TPH-1					0.002	Alleles					0.033
AA	5	3.1	0	0		A	31	9.6	19	16.7	
AT	21	13.0	19	33.3		T	293	90.4	95	83.3	
TT	136	83.9	38	66.7							
MATN 1					0.95	Alleles					0.82
AA	20	13.4	7	11.5		A	127	40.4	43	35.5	
AG	87	54.1	29	48.1		G	197	59.6	71	64.5	
GG	55	32.5	21	40.4							
ER β					0.21	Alleles					0.50
TT	45	27.7	14	24.6		T	205	63.2	68	60.7	
TC	115	71.1	40	70.2		C	119	36.8	46	39.3	
CC	2	1.2	3	5.2							

Table 5 Results of logistic regression analysis

	Regression coefficient	P	Odds ratio	95% CI
Model 3				
ER α	1.269	<0.001	3.559	1.84–6.89
Risser score	0.828	0.014	2.289	1.18–4.43
TPH-1	0.738	0.05	2.092	0.99–4.38

CI Confidence interval

Genotypes of ER α were coded as 0 for AA and 1 for GA or GG; Genotypes of TPH-1 were coded as 0 for TT and 1 for AT or AA; Risser sign was coded as 0 for grade 2 or 3 and 1 for grade 0 or 1; the initial curve magnitude was coded as 0 for <30° and 1 for more than or equal to 30°

Results of logistic regression analysis

A logistic regression model was used to analyse the covariate effects which had been shown to have a significant association with the outcome of brace treatment in the crude analysis. Risser sign, initial curve magnitude and genotypes of ER α and TPH-1 entered into the model as the candidate predictor variables. The results showed that Risser sign of grade 0 or 1 (OR = 2.289, 95% CI = 1.18–4.43), the G allele of ER α (OR = 3.559, 95% CI = 1.84–6.89) and the A allele of TPH-1 (OR = 2.092, 95% CI = 0.99–4.38) had significant associations with the

Table 6 The sensitivity, specificity and accuracy of the regression model at different cut-off point level

Cut-off point	Sensitivity (%)	Specificity (%)	Accuracy (%)
0.1	100	0	27.9
0.2	86.7	30.3	46.0
0.3	56.7	72.3	67.9
0.4	45.3	89	73.3
0.5	41.7	92.3	75.3
0.6	15	98.7	75.3
0.7	0	100	72.1

curve progression under brace treatment. However, with respect to the initial curve magnitude, no significant difference was found (Table 5). Table 6 shows the sensitivity and the specificity of the model at different cut-off levels. With the cut-off point for the probability of curve progression set at 0.50, 78.3% of the patients could be correctly classified. The sensitivity and specificity of the model were 41.7 and 92.3%, respectively.

Discussion

Brace treatment has long been considered as an effective treatment for AIS paitents [8]. Fernandez et al. [22]

conducted a case–control study in which age or menarche status matched AIS patients received either bracing or observation. After a mean duration of 3.3 years, they found that the risk of curve progression in observation only patients was about three times that of braced patients. Lange et al. [1] evaluated the long-term outcome of the Boston brace in AIS patients. The pre-brace curve magnitude was 33.4° on average. At weaning and at the last follow-up, the corresponding values were 28.3° and 34.2°, respectively. The results showed that long-term results were satisfactory in most patients treated with the Boston brace. Lonstein and Winter reviewed 1,020 patients treated with the Milwaukee brace and concluded that the rates of both curve progression and surgery were lower than those expected from natural history [10]. In the current study, 28% of the well-braced patients had curve progression of more than 5° and 19% of them received surgery, which was comparable to previous studies [6, 8, 10]. For all its effectiveness, wearing a brace simultaneously brings about several side effects, including patients' repeated exposure to radiation, psychological trauma and direct cost-of-care. Besides, some patients still receive a failure outcome, most of whom have to be confronted with surgery despite the fact that they have fully abided by the surgeon's instructions on brace-wear. Discussing the potential benefit and the expected outcome of bracing with the child and the family is essential to avoid frustration. Hence, it would be very beneficial if surgeons could accurately predict the type of patient prone to be resistant to brace treatment.

Factors that affect the final outcome of bracing have been extensively discussed in the literature [7, 9, 23, 24]. Curve magnitude and Risser sign of patients were usually analysed as prognostic factors of failure outcome. Lonstein and Winter [10] reported that, of 1,020 patients treated with the Milwaukee brace over a 25-year period, the failure rate was significantly higher in the subgroup that had a curve magnitude between 30° and 39° and a Risser sign of 0 or 1. In the current study, Risser sign was found to be strongly predictive of bracing effectiveness. In patients with a Risser sign of 0 or 1, the failure rate was 35% and the rate of surgical intervention was 27.7%, both significantly higher than in patients with a Risser sign of 2 or 3. The rate of surgical intervention was also higher in patients with larger curve, as indicated by a Cobb angle of more than or equal to 30°. In addition to Risser sign and curve magnitude, the age of the patient at diagnosis, the curve pattern and gender were also reported to affect bracing effectiveness. Emans et al. [11] reviewed a total of 295 patients treated with the Boston brace. The authors reported that young age and a large curve magnitude were the most prognostic indicators for surgery. Yrjonen et al. [6] compared the results of brace treatment between males and females, revealing that the overall results of treatment of boys were inferior in contrast with matched girls. However, Yrjonen et al. [6] did not support the association between treatment failure and the age of the patient, curve pattern or curve magnitude. Katz et al. [7] investigated the effectiveness of brace treatment in larger curves (>35°) and concluded that patients with a double major curve were significantly more likely to have unsuccessful brace treatment. In terms of other curve patterns and Risser sign, they drew the same conclusions as Yrjonen. In the current study, we did not find a significant association between the risk of brace failure and the age of the patient, curve pattern or gender. Namely, the influence of these factors on the effectiveness of brace treatment remains obscure.

The role of genetic factors in the development of AIS is widely accepted [25, 26]. AIS is best understood as a complex phenotype that results from the interaction of multiple genetic loci with each other and the environment. Genetic markers associated with the occurrence or progression of AIS have the potential to be used as prognostic factors [27]. At the 2008 Annual SRS Meeting, Ward [19] investigated the predictive value of a panel of genetic markers for AIS patients, suggesting that a risk model of patients' natural history could be developed using this DNA test. However, whether the model could be applied to Asian populations or be used to predict the outcome of brace treatment remains unclear [28].

Five predisposition genes reported to be associated with the occurrence or severity of AIS were investigated in our study, including ERα, ERβ, TPH-1, MTNR1B and MATN1. The genotype distributions of the predisposition genes were compared between the two groups (failure/success). We found that genotype GA of ERα and genotype AT of TPH-1 indicated a significantly higher probability of curve progression under brace treatment. The G allele of ERα and the A allele of TPH-1 could be considered as risk factors leading to the failure of brace treatment. MTNR1b had a marginal association with the outcome of brace treatment, with the p value just equal to 0.05. As for ERβ and MATN-1, no significant association was found.

To analyse the covariate effects which have been shown to have a significant association with the failure of brace treatment in the crude analysis, a stepwise logistic regression model was created. The results showed that Risser sign and genotypes of ERα and TPH-1 were independent predictors of the effectiveness of brace treatment. The odds ratios of ERα and TPH-1 were 3.56 and 2.09, respectively. With the cut-off point set at 0.50, the sensitivity and specificity of the model were 41.7 and 92.3%, respectively. When the cut-off point was set at 0.2, the sensitivity of the model rose to 86.7%, while the specificity of the model dropped to 30.3%. Namely, due to the insufficiency of the predictor that could be enrolled in the regression model, the current model was not powerful enough for its application

to the clinical practice. To clarify which kind of patients may fail the brace treatment, further investigation for more predictors should be carried out.

The association between ERα and AIS has been demonstrated in previous studies [13, 14]. Inoue et al. [13] reported that patients with the genotypes GG and GA had a higher risk of receiving operative treatment than those with the AA genotype. This finding was confirmed in our study. Our results showed that patients with a G allele had about a threefold higher risk of brace failure. The TPH-1 gene encodes for the rate-limiting enzyme TPH for serotonin synthesis, which is an intermediary in the pathway of melatonin biosynthesis. Polymorphisms in the TPH-1 gene could be indirectly associated with a deficiency of melatonin [17]. Recently, Machida et al. [29] reported that melatonin deficiency plays a role in the prognosis of AIS. In the current study, we found that the A allele of the TPH-1 gene was associated with a higher risk of bracing failure, which was partially in line with Machida's finding [29]. Interestingly, although reported to be associated with the curve severity, [16, 18] MATN1 and ERβ were not found to have a significant association with the outcome of brace treatment in our study. Furthermore, MTNR1b was found to be marginally associated with bracing failure in our study; therefore, enlargement of the sample size is necessary in future study to unveil its association with the outcome of brace treatment.

In our study the curve size at initial bracing was compared between boys and girls, and the differences was not significant. Bias might be introduced here as a result of large age range (10–15) and the large Risser spread (0–3) of the enlisted patient. Patients with age ≤13 or Risser sign <2 were reported to be more likely to be resistant to brace treatment [10, 11]. Namely, patients with age >13 or Risser sign ≥2 could be more likely to receive a successful outcome of brace treatment. To do the logistic regression analysis, each variant should be stratified into two categories and coded as 0 or 1, respectively. Curtailing the age range and Risser spread might help avoid the bias mentioned above. However, it could also decrease the amount of the cohort simultaneously, affecting the predicting power of the regression model. Weighing the pros and cons between the bias and the power, we think that keeping the age range and Risser spread adopted by the current study is important for a convincing result of the regression analysis.

Accurate prognosis for the outcome of bracing could spare some patients from excessive stress on both body and psychology. The logistic model created in our study shows that patients with a combination of a low Risser sign of 0 or 1, the G allele of ERα and the A allele of TPH-1 have a remarkably higher risk of failing brace treatment. For patients without these risk factors, wearing brace might bring about a more positive outcome. However, at present,

it is impossible to predict the efficacy of bracing with total accuracy. Thus, the model presented in this study should be interpreted with some caution.

In the current study, we retrospectively investigate the possibility to predict the outcome of brace treatment in AIS patients, using a logistic regression model with genetic markers and Risser sign included in. All the subjects of the study were enrolled under strict inclusion criteria and exclusion criteria. However, out of the inherent defects of retrospective study, it is very difficult to define why some patients in the excluded cohort discontinued their visits, which could possibly affect the outcomes of this study. A prospective study should behave better in this aspect. Another limitation of our study mainly lies in the relatively short duration of follow-up. As recommended by the SRS committee [21], a minimum two-year follow-up beyond skeletal maturity for each patient should be taken into account in brace studies. Some of our patients failed to reach this threshold, which was partly attributed to the short history of the application of brace treatment in Chinese AIS patients. A recently published systematic review concluded that bracing is effective in the long term [30]. However, there is still a lack of evidence in Chinese AIS patients, and further study should be carried out to investigate the influence of these predisposition gene polymorphisms on the long-term outcome of brace treatment. A third limitation of our study concerns the compliance with brace treatment. To exclude its influence on the effectiveness of brace treatment, we only enlist those patients with the compliance more than 75%, most of them nearly full per cent. However, it is quite difficult to avoid the bias derived from subjective reports from the patients or their parents, although they had been well instructed about the importance of wearing brace with full compliance. Nowadays temperature sensor has been applied to determine the adherence of the patients, which is totally objective [31]. In the future prospective study this new technique should be adopted, so as to evaluate the effectiveness of brace treatment more validly.

Conclusion

The current study investigated the association between SNPs of five predisposition genes and the effectiveness of brace treatment. This is the first study, to our knowledge, to show that polymorphisms of the ERα and TPH-1 genes could significantly affect the final outcome of brace treatment. Patients with the G allele at the SNP rs9340799 site of the ERα gene and the A allele at the SNP rs10488682 site of the TPH-1 gene are prone to be resistant to brace treatment.

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