ORIGINAL ARTICLE

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Polygenic Risk Score of Adolescent Idiopathic Scoliosis for Potential Clinical Use

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ABSTRACT

Adolescent idiopathic scoliosis (AIS) is a common disease causing three-dimensional spinal deformity in as many as 3% of adolescents. Development of a method that can accurately predict the onset and progression of AIS is an immediate need for clinical practice. Because the heritability of AIS is estimated as high as 87.5% in twin studies, prediction of its onset and progression based on genetic data is a promising option. We show the usefulness of polygenic risk score (PRS) for the prediction of onset and progression of AIS. We used AIS genomewide association study (GWAS) data comprising 79,211 subjects in three cohorts and constructed a PRS based on association statistics in a discovery set including 31,999 female subjects. After calibration using a validation data set, we applied the PRS to a test data set. By integrating functional annotations showing heritability enrichment in the selection of variants, the PRS demonstrated an association with AIS susceptibility ($p = 3.5 \times 10^{-40}$ with area under the receiver-operating characteristic [AUROC] = 0.674, sensitivity = 0.644, and specificity = 0.622). The decile with the highest PRS showed an odds ratio of as high as 3.36 ($p = 1.4 \times 10^{-10}$) to develop AIS compared with the fifth in decile. The addition of a predictive model with only a single clinical parameter (body mass index) improved predictive ability for development of AIS (AUROC = 0.722, net reclassification improvement [NRI] 0.505 ± 0.054, $p = 1.6 \times 10^{-8}$), potentiating clinical use of the prediction model. Furthermore, we found the Cobb angle (CA), the severity measurement of AIS, to be a polygenic trait that showed a significant genetic correlation with AIS susceptibility (rg = 0.6, $p = 3.0 \times 10^{-4}$). The AIS PRS demonstrated a significant association with CA. These results indicate a shared polygenic architecture between onset and progression of AIS and the potential usefulness of PRS in clinical settings as a predictor to promote early intervention of AIS and avoid invasive surgery. © 2021 American Society for Bone and Mineral Research (ASBMR).

KEY WORDS: HUMAN ASSOCIATION STUDIES; ORTHOPEDICS; SKELETAL MUSCLE; STATISTICAL METHODS

Introduction

Adolescent idiopathic scoliosis (AIS) is a disease with three-dimensional spinal deformity defined as the Cobb angle (CA) of 10° or more on plain radiographs in the standing position[1,2] (Supplemental Fig. S1*A*). AIS is a common disease affecting 2% to 3% of female adolescents[3,4] and progresses during rapid growth periods. Patients with AIS suffer from physical,[5] mental,[6] and respiratory[7] problems as the disease progresses in severity. These severely affected patients finally require orthopedic surgery with the use of spinal implants, which burden patients and their families with physical, psychological, and economic stress.[8] The effectiveness and importance of early interventions by the use of orthosis to prevent progression of AIS have been reported.[9,10] Therefore, early intervention based on early detection of patients who need treatment is warranted.[10] However, there is no good prediction tool for AIS prognosis at present. AIS is currently evaluated only by periodic radiographs. Frequent screening with radiographs raises concerns on the cumulative radiation exposure of screened individuals. Development of a predicting tool for the onset and progression of AIS has been expected in the clinical setting.

Genetic[11,12] and environmental factors[13] are involved in AIS. A negative association between AIS and body mass index (BMI) was reported in our previous study.[13] The concordance rate of AIS in monozygotic twins is as high as 0.73 to 0.92 and its heritability is estimated at 87.5%, indicating that genetic factors explain a large fraction of its onset.[14–17] We have previously reported a total of 20 loci associated with AIS through genomewide association studies (GWAS) in Japanese.[18–22] Although these loci are informative about the polygenic architecture of AIS and describe a portion of it (4.6% of variance), predicting AIS by using such a limited number of loci is challenging.

Prediction of AIS progression by genetic measurements is also needed in clinical settings.[23–25] Recent studies have revealed that the polygenic architecture of a trait below the *p* value threshold of GWAS significance explains a substantially larger part of its heritability compared with a limited number of GWAS significant variants.[26–29] Remarkable predictive abilities were obtained by taking polygenic architecture into account in some diseases, including coronary artery disease.[30] Our recent GWAS study on AIS susceptibility provided further evidence supporting the polygenic nature of this disease[22] and motivated us to leverage these findings to construct a polygenic risk score (PRS) for possible prediction of AIS onset. In addition, to our knowledge, we conducted the first GWAS and prediction for CA using the aforementioned scores.

Materials and Methods

Subjects

This study was approved by the ethical committees at all collaborating institutions including RIKEN. Informed consent was obtained from subjects and/or parents of subjects who were minors. Three independent AIS cohorts were collected at different time points. Subjects in cohorts 1,[18,19] 2,[20] and 3[22] have been described previously. In all cohorts, the case subjects were recruited using the same protocol in collaborating hospitals (Japanese Scoliosis Clinical Research Group). They underwent clinical and radiologic examinations by expert scoliosis surgeons and were included in the case group when they had idiopathic

scoliosis with CA > 10° during the ages of 10 to 18 years. The control subjects were randomly selected from the BioBank Japan project.[31]

Evaluation of repeatability of the Cobb angle

CA was obtained together with the Risser sign[32] by evaluating the most recent radiographs for non-operated cases and from clinical records at last examination before surgery for operated cases. The interobserver agreement of CA was more than 0.95 in all data sets (details are shown in Supplemental Materials and Methods).

PRS for AIS susceptibility

We used the results of our latest GWAS of AIS susceptibility using three cohorts[22] (details are shown in Supplemental Materials and Methods). The study design is shown in Fig. 1*A*. We used cohort 1 as our discovery data set because its sample size was the largest among the three cohorts and hence it enabled the most accurately approximate coefficients of variants. Cohort 2 was used as a validation data set to assess the predictive ability of PRS models and to determine the threshold of *p* values for variants included for the construction of the PRS. Cohort 3 was used as a test data set to evaluate the performance of the PRS. Because female sex is highly predominant in AIS,[3,4] we included only female subjects. Only overlapping single-nucleotide polymorphisms (SNPs; $n = 7{,}101{,}421$) among the three cohorts were included. SNPs with a minor allele frequency <0.01 or variants with an imputation quality score (Rsq) <0.3 for each cohort were excluded. We applied the pruning and thresholding method, the standard method of analysis, to construct the PRS.[30] Specifically, we used the clump function of PLINK version 1.90b[33] to generate eligible SNPs with the setting 250 kb window. We set seven linkage disequilibrium pruning thresholds (r^2) of 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9, and a total of 20 thresholds of *p* values in GWAS (P_T) (5×10^{-8}, 5×10^{-7}, 1×10^{-6}, 5×10^{-6}, 1×10^{-5}, 5×10^{-5}, 5×10^{-4}, 0.05, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1). For each, r^2 were used to generate different PRSs. As weights, we used the natural logarithms of the GWAS odds ratios (ORs) for AIS across all three data sets. The SNP alleles used in the PRS were aligned to risk alleles for AIS susceptibility. All the effect sizes were set into a positive value and the effect alleles were defined accordingly. The PRS was the sum of the weighted allele counts (by their respective GWAS effect sizes) across all the SNPs included in the PRS according to the following formula:

$$PRS_i = \sum_{k=1}^{n} \beta_k X_{k,i}$$

where *i* denotes each subject, *n* denotes the number of variants passing a threshold, β_k denotes an effect size of k-th SNP, and $X_k i$ denotes genotype dosage of *k*-th SNP in individual *i*.

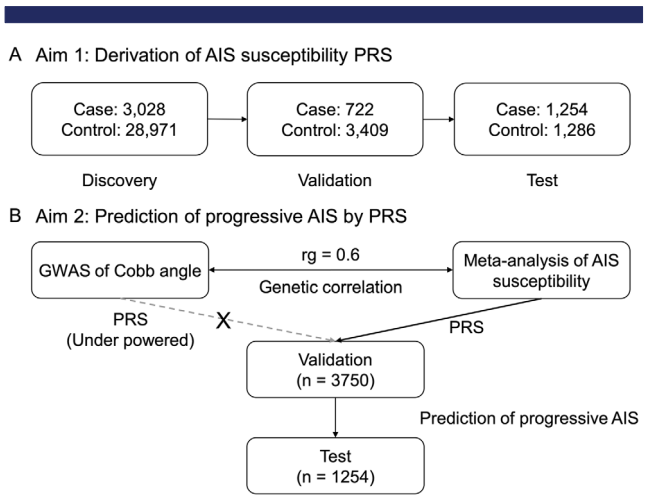

Fig 1. Study design. (*A*) Adolescent idiopathic scoliosis (AIS) susceptibility polygenic risk score (PRS). Three AIS cohorts are collected. Because the sample size of cohort 1 is the largest, we set the data set with the largest sample size as a discovery data set. We infer the effect size of each variant from a discovery data set. The clumping and *p* value thresholding method is used to generate the PRS. Twenty type *p* value thresholds are used. The *p* value threshold for the PRS is chosen based on the maximum area under the receiver-operating characteristic (AUROC) and Nagelkerke's pseudo-R^2 metric in the validation data set. We subsequently used a test data set. (*B*) Prediction of progressive AIS by PRS. We conducted a GWAS of the Cobb angle. While there is no genomewide association signal of Cobb angle GWAS, based on the positive genetic correlation between AIS susceptibility and the Cobb angle, the effect sizes for AIS susceptibility are used to generate the PRS for evaluation of the Cobb angle.

Performance of PRS

After we selected the best PRS from the validation data set, a logistic regression model was applied to calculate the association between the PRS and susceptibility to AIS for the subjects in the test data set. The area under the receiver-operating characteristic (AUROC), sensitivity and specificity of each model, was generated by the R package pROC version 1.13.0. R version 3.4.2 was used to conduct all analyses and generate the plots. To assess the fit of our model, Nagelkerke's pseudo-R^2 metric[34] was calculated. Because inclusion age is different between the AIS case subjects and the control subjects, to incorporate BMI in the model, BMI distribution of the control group was adjusted to adolescent BMI distribution using published Japanese government statistics (https://www.e-stat.go.jp). This was done to prevent overestimation of the association of BMI considering increased BMI after adolescence. Specifically, we randomly extracted subjects from the control cohort in the test set, created 10 subsets adjusted to the distribution of adolescent BMI, and conservatively calculated the AUROC of the model for each subset. Nagelkerke's pseudo-R^2 metric was also used to calculate the proportion of variance explained for showing model improvement as the previous study reported.[30] This proportion was calculated for the full model by including the PRS plus BMI minus R^2 for BMI alone, therefore showing an estimate of the explained variance attributable to the PRS. We also calculate net reclassification improvement (NRI) between the full model including PRS plus BMI and the BMI alone using R package PredictABLE version 1.2.1. We split the data set into 20 quantiles or deciles according to each individual's PRS to evaluate the performance of the PRS to distinguish case and control. The tenth quantile in 20 quantiles and fifth in decile were used as the reference for quantile analysis or as the average risk group of the population, respectively.[35] We also compared the top group (tenth in decile and 20th in 20 quantiles) with the remaining groups (including the first to the ninth in decile and the first to 19th in 20 quantiles) to evaluate our result more conservatively. We

applied logistic regression by comparing the remaining subgroups with the reference by using R.

Evaluation of Cobb angle by PRS

We calibrated 140 PRSs for CA (PRS_{CA}) in the same manner for the PRS of AIS susceptibility. The effect size of each SNP in the meta-analysis of AIS susceptibility GWAS was used to construct the PRS_{CA} (Fig. 1*B*). The linear regression model was applied to evaluate associations between the score and the CA of each individual in the validation data set to choose a threshold with the most significant result among 140 PRS_{CA}s or the best-fitting model of PRS to CA. We used the logarithm of CA as a dependent variable in the linear regression analysis to fit normality. We evaluated the performance of PRS using the test data set and estimated the correlation between that score and the CA of each individual by the Spearman correlation test. We separated the case subjects into progressive and non-progressive groups, then set the criteria to separate the two groups based on the previous study[36] and AIS's progression and clinical outcomes.[37–39] We defined CA ≥40° as the progressive (case) group. Two criteria to define the non-progressive (control) group were used: control 1 (CA ≤20°[40] regardless of skeletal maturity) and control 2 (CA ≤30°[36] regardless of skeletal maturity). We analyzed the difference between these two groups under a logistic regression model and evaluated the performance of each model in the validation and test data sets of CA by measuring AUROC as a predictor. We confirmed the predictive ability restricting to non-progressive subjects with skeletal maturity. Skeletal maturity was defined by the Risser sign (grade 4 or more).[32] The Risser sign is a widely used indicator of skeletal maturity that can be judged by a plain radiograph. We split the data set into quartiles and evaluated the performance of the PRS to distinguish progressive and non-progressive AIS in the same manner as the PRS for susceptibility.

Results

PRS for AIS susceptibility

We used AIS GWAS data comprising 38,670 female subjects. The overall study design and the sample size for each cohort are shown in Fig. 1 and Supplemental Table S1, respectively. We applied the pruning and thresholding method to construct the PRS.[30] The discovery data set was used to define the effect sizes of variants used for PRS calculation. We constructed a total of 140 PRS settings with different r^2 and P_T variants to be included for the PRS (details are described in Materials and Methods). Then these 140 PRSs were applied to the validation data set to optimize the thresholds of r^2 and P_T. As a result, the model with P_T of 0.5 and r^2 of 0.9, containing 840,269 SNPs, reached the highest Nagelkerke's pseudo-R^2 metric (0.106) (Fig. 2*A* and Supplemental Table S2) and AUROC (0.693; 95% confidence interval [CI] 0.671–0.715; Figs. 2*C* and 3*A*, Supplemental Fig. S1*A*, Table 1, and Supplemental Table S2). In this setting, the sensitivity and specificity were 0.677 and 0.628, respectively (Supplemental Table S6). We noticed that variants with p values between 1.0×10^{-5} and 0.05 contributed primarily to the improvement of the fit of the model (Fig. 2*A*, *C* and Supplemental Table S2). This trend was consistently observed throughout all r^2 thresholds (Fig. 2*A*), suggesting causal variants highly enriched in this range of p values in comparison with variants with p values >0.05.

The performance of PRS in the test data set

We verified the performance of the PRS with the test data set. The PRS was significantly associated ($p = 9.6 \times 10^{-39}$) with AIS susceptibility with the AUROC of 0.665 (95% CI 0.642–0.688; Table 1 and Fig. 3*A*), sensitivity of 0.655 and specificity of 0.576 (Supplemental Table S6), explaining 10.5% of the variance (Table 1 and Supplemental Fig. S1*A*). These results indicate that the PRS of AIS can be used to efficiently predict the onset of AIS.

Next, we evaluated the performance of the PRS to efficiently distinguish subjects at very high risk of developing AIS. We divided the subjects into 10 groups according to the PRS and analyzed the relative risk of AIS in each group by referring to the fifth group considered as the average-risk group. We found a consistent increase in effect sizes of PRS to predict the onset of AIS in the deciles. The highest decile showed 2.63 of OR (95% CI 1.68–4.26; Fig. 4*A* and Supplemental Table S3). This performance was also observed in 20 quantiles (OR = 2.41, 95% CI 1.42–4.14; Fig. 4*B* and Supplemental Table S4). We also observed a consistently high relative risk for the highest group by referring to the remaining quantiles in 20 quantiles (OR = 3.96, 95% CI 2.42–6.72) and in deciles (OR = 2.83, 95% CI 2.01–4.00), respectively (Supplemental Table S5).

Adding clinical information to PRS

In the previous study, low BMI at adolescence was epidemiologically shown to be associated with AIS.[13] Since BMI can be easily measured in a noninvasive manner, we considered BMI as a good clinical predictor to increase the accuracy of the AIS prediction model. We included BMI in the model in a conservative way (see Materials and Methods) and our model (PRS and BMI) improved AUROC from 0.665 to 0.714 (standard deviation [SD] ±0.009) in the test data set (Table 1). We also confirmed our model's improved performance in comparison with a model using only BMI (from 0.653 ± 0.007 to 0.714, the proportion of variance explained [%] 6.087 ± 1.194, NRI 0.425 ± 0.042, $p = 1.2 \times 10^{-6}$; Table 1).

Select SNPs to improve predictive ability

Next, we addressed the possible improvement of the performance of the PRS by prioritizing SNPs included in the predictive model. Since we showed significant heritability enrichment of AIS in the regulatory regions in six cell types,[22,41] we prioritized SNPs overlapping with any of the regulatory regions of the six cell types in addition to lead variants in GWAS significant loci (see Supplemental Materials and Methods, Supplemental Fig. S2*A*). Adopting the same manner for generating the PRS described above, we found the model with r^2 of 0.7 and P_T of 0.6 included 187,633 SNPs, a much smaller number of variants than in the model described above (840,269 SNPs) (Fig. 2*B*, *D* and Supplemental Fig. S2*B*), reached the comparable AUROC (0.674, 95% CI 0.651–0.696 in the test data set) (Fig. 3*B*, Supplemental Fig. S1*C*, Table 1, and Supplemental Table S6). The sensitivity and specificity were 0.644 and 0.622, respectively (Supplemental Table S6). Compared with the reference group as mentioned above, the highest decile showed 3.36 of OR (95% CI 2.33–4.88) (Fig. 4*C* and Supplemental Table S3). This performance was also observed in 20 quantiles (OR = 4.04, 95% CI 2.39–6.96) (Fig. 4*D* and Supplemental Table S4). Furthermore, in comparison, the highest group in the other quantiles showed consistent high relative risk in 20 quantiles (OR = 2.92, 95% CI 1.84–4.8), in decile (OR = 2.96, 95% CI 2.11–4.23), respectively

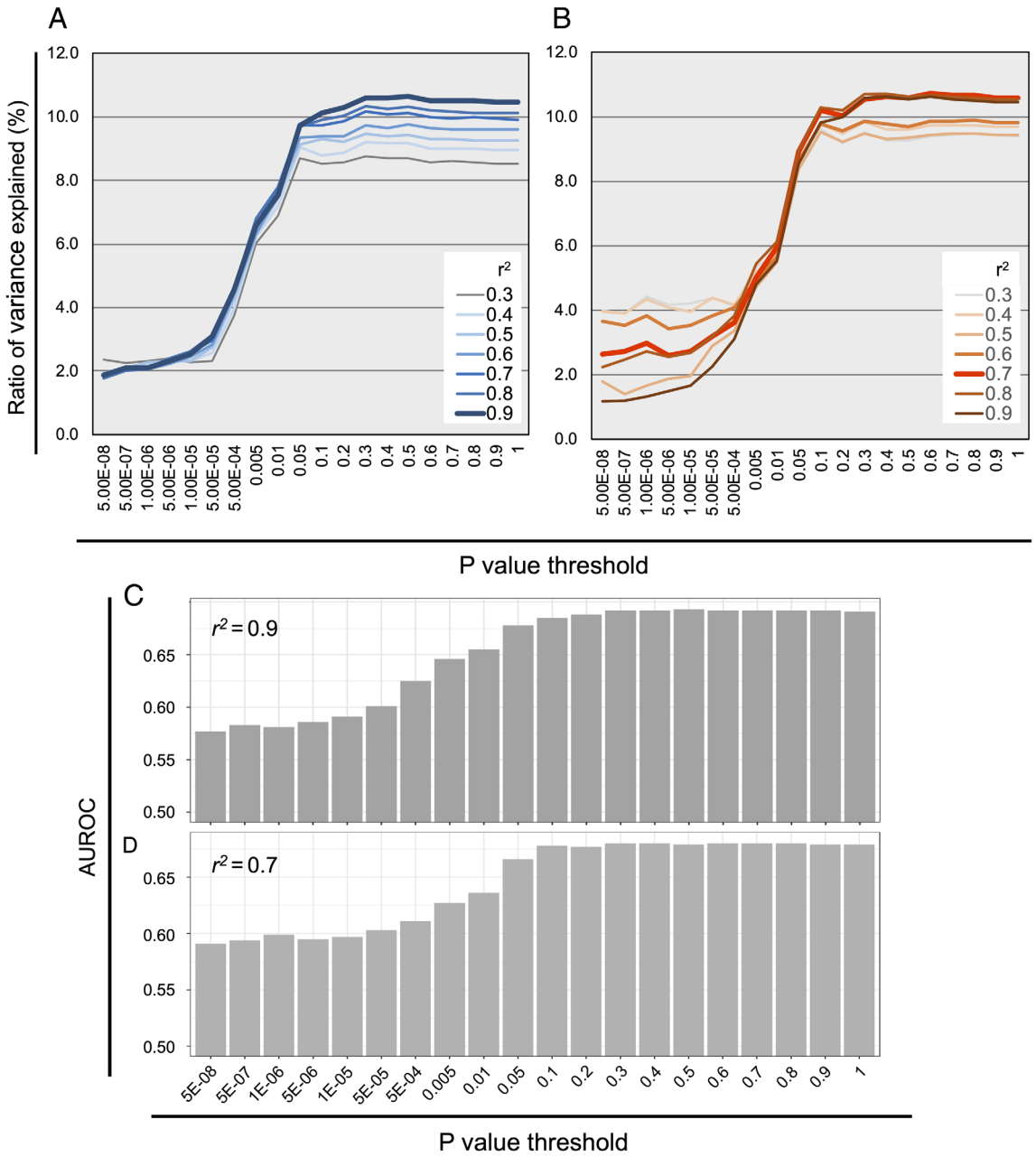

Fig 2. Determination of tuning parameter of the polygenic risk score (PRS) in the validation data set. To determine the best parameter, the linkage disequilibrium pruning threshold (r^2) and threshold of *p* values (P_T) are used. The *x* axis represents P_T of GWAS. The *y* axis represents ratio of variance explained (%) calculated by Nagelkerke's pseudo-R^2 metric (*A*, *B*) and the area under the receiver-operating characteristic (AUROC) (*C*, *D*). The highest ratio of variance explained (%) are (*A*) 10.64 under $r^2 = 0.9$ and P_T of GWAS of 0.5 in the conventional method and (*B*) 10.72 under $r^2 = 0.7$ and P_T of GWAS of 0.6 in prioritized method. The highest AUROC are also observed in the same parameters: (*C*) 0.693 (95% CI 0.671–0.715) under $r^2 = 0.9$ and P_T of GWAS of 0.5 and (*D*) 0.68 (95% CI 0.657–0.702) under $r^2 = 0.7$ and P_T of GWAS of 0.6.

(Supplemental Table S5). When we included BMI into the prediction model, AUROC improved to 0.722 (SD ±0.009) and the PRS contributed to a significant improvement of the model (proportion of variance explained [%] 7.101 ± 1.231, NRI 0.5 ± 0.054, $p = 1.6 \times 10^{-8}$) in the test data set (Table 1 and Supplemental Table S6). These results indicated that integrating with PRS functional annotations in which disease heritability is enriched can help us to efficiently prioritize variants and reduce the number of SNPs to construct a prediction model, improving the clinical application of the PRS.

PRS using LDpred

The LDPred computational algorithm[42] was also used to generate the PRS (see Supplemental Materials and Methods). As a result, the best model (ρ = 0.01, 200,889 variants) showed

Table 1. Predictive Ability of the Polygenic Risk Score (PRS) in Validation and Test

Method	Parameter	SNP number	Data set	Logistic regression				AUROC					Proportion of variance explained (%) (± SD)	NRI (± SD)
				PRS				PRS	95% CI	BMI	SD	PRS + BMI	SD	
				OR	95% CI	*p* Value	R^2							
Conventional	$r^2 = 0.9$	840269	Validation	1.01	1.007–1.009	1.06×10^{-47}	0.106	0.693	0.671–0.715	0.647	0.006	0.733	0.010	12.870 (±1.190)	0.560 (±0.068)
	$P_T = 0.5$		Test	1.01	1.006–1.008	9.63×10^{-39}	0.105	0.665	0.642–0.688	0.653	0.007	0.714	0.009	6.087 (±1.194)	0.425 (±0.042)
Prioritized	$r^2 = 0.7$	187633	Validation	1.03	1.027–1.036	4.10×10^{-46}	0.107	0.68	0.657–0.702	0.647	0.006	0.725	0.008	12.116 (±2.092)	0.464 (±0.046)
	$P_T = 0.6$		Test	1.03	1.025–1.034	3.52×10^{-40}	0.105	0.674	0.651–0.696	0.653	0.007	0.722	0.009	7.101 (±1.231)	0.505 (±0.054)
LDPred	$\rho = 0.01$	200889	Validation	2.71	2.350–3.151	8.19×10^{-41}	0.093	0.672	0.649–0.695	0.647	0.006	0.718	0.009	10.577 (±1.651)	0.458 (±0.063)
			Test	2.62	2.270–3.034	9.64×10^{-39}	0.116	0.671	0.648–0.693	0.653	0.007	0.714	0.009	5.477 (±1.095)	0.427 (±0.057)

SNP = single-nucleotide polymorphism; OR = odds ratio; CI = confidence interval; R^2 = Nagelkerke's pseudo-R^2 metric; AUROC = area under the receiver-operating characteristic; BMI = body mass index; SD = standard deviation; NRI = net reclassification improvement.

"Conventional" represents pruning and thresholding method and "prioritized" represents the PRS generated by selected SNPs based on partitioned heritability enrichment analysis in addition to lead variants in genomewide association study (GWAS) significant loci. Linkage disequilibrium pruning threshold (r^2), *p* value in discovery GWAS (P_T) study in pruning and thresholding and tuning parameter to model the proportion of variants assumed to be causal (ρ) in LDPred algorithm are used to generate different PRS.

AUROC of PRS + BMI and NRI represent the average of the AUROC calculated by 10 subsets. Proportion of variance explained is calculated for the full model including the PRS plus the BMI minus R^2 for the BMI alone. NRI is calculated between the full model including the PRS plus the BMI and the BMI alone.

comparable results with the models above (AUROC 0.671, 95% CI 0.648–0.693; sensitivity 0.641 and specificity 0.627 in the test data set) (Table 1 and Supplemental Table S6). The highest decile showed 2.27 of OR (95% CI 1.53–3.4). This performance was also observed in 20 quantiles (OR = 2.98, 95% CI 1.68–5.48, data not shown). When we included BMI in the prediction model, AUROC significantly improved to 0.714 (SD ±0.009) and the PRS contributed to a significant improvement of the model (proportion of variance explained [%] 5.477 ± 1.095, NRI 0.42 ± 0.057, $p = 7.1 \times 10^{-6}$) (Table 1).

Summarizing these results, the model with prioritized SNPs showed the best fit and highest result for AIS susceptibility using the smallest number of variants. This indicates our method could efficiently and correctly select SNPs with a reasonable effect size for predicting AIS (Supplemental Fig. S2*C* and Table 1).

PRS comparably fitting male subjects

We evaluated the performance of the model (constructed with female patient data) in male subjects (cases 323, controls 38,478). The best PRS in female subjects generated by prioritized SNPs was significantly associated ($p = 1.9 \times 10^{-19}$) with AIS susceptibility with 0.655 of AUROC (95% CI 0.624–0.686), sensitivity of 0.653 and specificity of 0.583 (Supplemental Fig. S3*A* and Supplemental Table S6). The highest decile showed 2.76 of OR (95% CI 1.72–4.6) (Supplemental Fig. S3*B*). This performance was also observed in 20 quantiles (OR = 3.56, 95% CI 1.82–7.61; Supplemental Fig. S3*C*). In comparison, the highest group in the other quantiles also showed consistent high relative risk in 20 quantiles (OR = 2.47, 95% CI 1.64–3.57), in decile (OR = 2.15, 95% CI 1.56–2.91), respectively (Supplemental Table S5). Furthermore, the inclusion of BMI in the model showed 0.721 of AUROC (SD ±0.002) and the PRS contributed to a significant improvement (proportion of variance explained [%] 4.653 ± 0.258, NRI 0.437 ± 0.025, $p = 1.2 \times 10^{-12}$) (Supplemental Table S6). These results indicate our model showed reasonable predictive ability not only in females but also in males.

GWAS and evaluation of Cobb angle

When conducting early interventions in an effort to avoid spinal corrective surgery,[9] detecting AIS patients at high risk for progressive cases is quite important. Thus, next, we focused on CA and conducted a meta-analysis of GWAS for CA using a total of 4465 subjects (see Supplemental Materials and Methods, Supplemental Table S8, and Supplemental Fig. S4*A*, *B*). No signals exceeded a GWAS significant level (Supplemental Fig. S4*C*, *D*). Rs35333564, previously reported as a risk factor of severe cases,[36] showed a trend for association ($p = 0.025$). Despite the lack of significant variants, we observed the heritability of CA as 21% (standard error 10%) and a genetic correlation with AIS susceptibility (rg = 0.6, $p = 3.0 \times 10^{-4}$) (see Supplemental Materials and Methods). These suggest that genetic components, especially associated with AIS onset, contribute to the progression of AIS and that lack of significant signals for CA in the current study was due to insufficient power.

Evaluation of PRS for Cobb angle

As expected, when we constructed the PRS_{CA} based on the GWAS results of CA (as for AIS susceptibility), we did not observe a significant correlation (data not shown). Thus, based on its genetic correlation to AIS susceptibility and acceptable contribution of AIS susceptibility polygenic components to CA, CA was

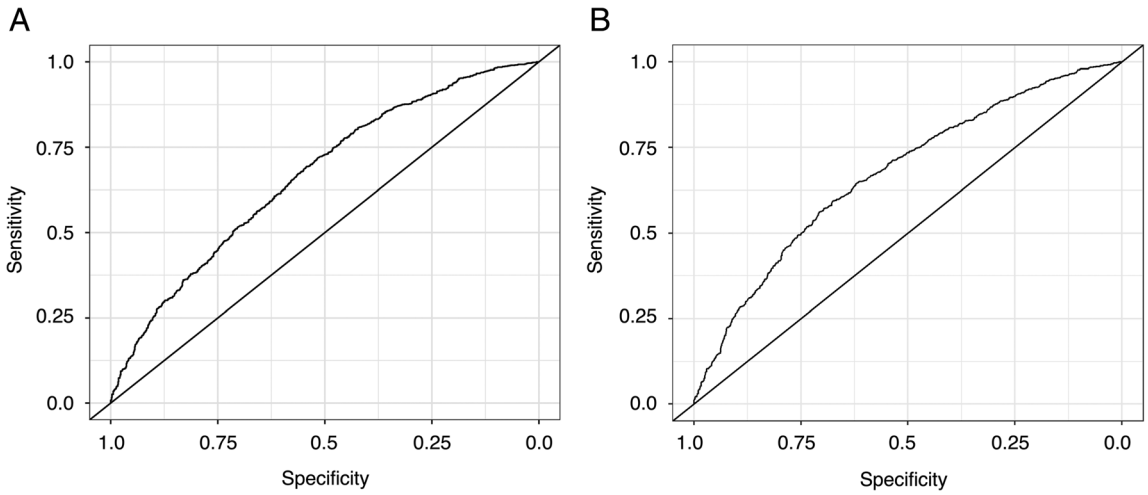

Fig 3. Performance of adolescent idiopathic scoliosis (AIS) susceptibility based on the polygenic risk score (PRS) in the test data set. Receiver-operating curve (ROC) for AIS based on PRS of (*A*) conventional and (*B*) prioritized method. The area under the receiver-operating characteristic (AUROC) is 0.665 (95% CI 0.642–0.688) in the conventional method and the AUROC is 0.674 (95% CI 0.649–0.696) in the prioritized method.

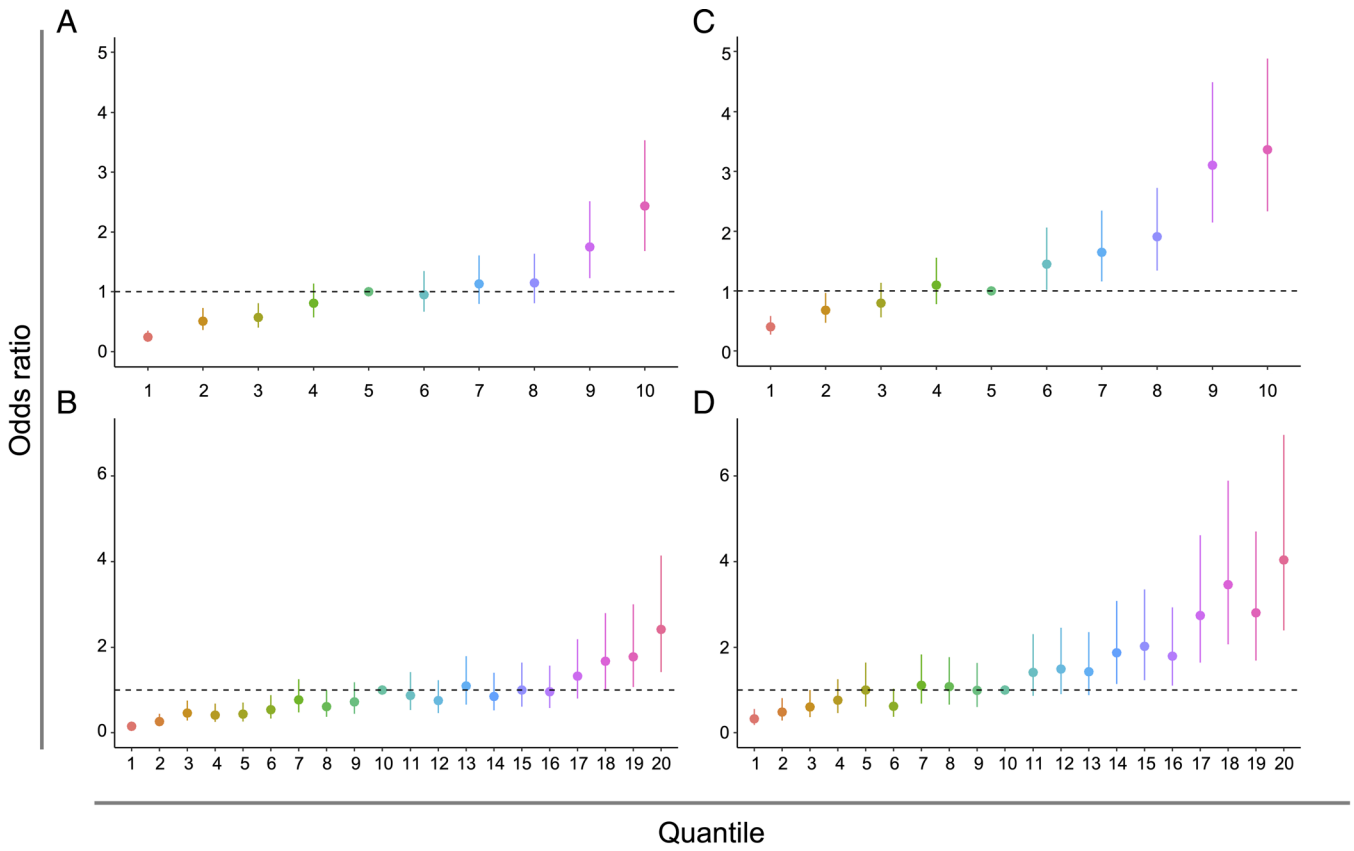

Fig 4. Predictive ability of the polygenic risk score (PRS) for adolescent idiopathic scoliosis (AIS) susceptibility in the test data set. Odds ratio (OR) of quantiles defined by PRS to detect AIS is indicated in (*A*, *B*) conventional method and (*C*, *D*) prioritized method. Bars indicate 95% confidence intervals. OR is calculated in each quantile compared with the fifth in decile and the tenth quantile in 20 quantile analyses, respectively.

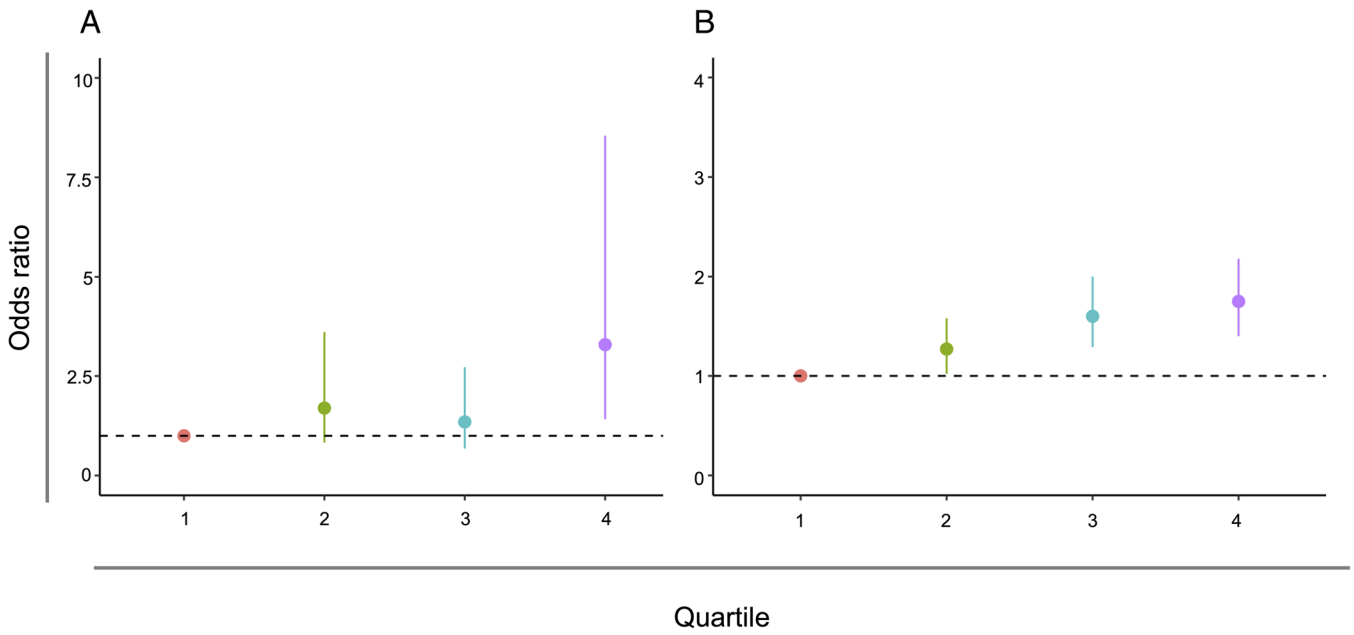

Fig 5. Predictive ability of the polygenic risk score (PRS) for adolescent idiopathic scoliosis (AIS) progression in the test data set. The test data sets are split into quartiles according to each individual's PRS. Odds ratio of quartiles defined by PRS to detect progressive subjects with having a Cobb angle more than 40° is indicated. Non-progressive groups are defined by Cobb angle, (*A*) Cobb angle ≥40° versus Cobb angle ≤20° and (*B*) Cobb angle ≥40° versus Cobb angle ≤30°. Bars indicate 95% confidence intervals. OR is calculated in each quartile compared with the lowest quartile.

correlated with the PRS calculated based on AIS susceptibility. When we applied the best PRS for AIS susceptibility, we obtained a significant association in the test data set (ρ: 0.09, $p = 3.9 \times 10^{-3}$) (Supplemental Table S7), indicating that our PRS was useful to predict not only onset but also the progression of AIS.

To optimize PRS_{CA}, we tested the 140 PRSs (mentioned above) with different thresholds of r^2 and *p* values (Fig. 2; see Materials and Methods). As a result, the PRS ($P_T = 0.6$ and $r^2 = 0.9$) showed the most significant ($p = 2.9 \times 10^{-13}$) by linear regression and the best correlation by Spearman correlation test in the validation data set (ρ: 0.13, $p = 2.0 \times 10^{-13}$) (Supplemental Table S8). We further analyzed how efficiently we could distinguish subjects with progressive AIS from those with non-progressive AIS by AUROC (Supplemental Table S9). We compared subjects with CA ≥40°(progressive group, cases) and those with CA ≤20° and CA ≤30°(non-progressive group, controls) (see Materials and Methods). Consistent with the results of the linear regression and Spearman correlation test, the best values were obtained with the same threshold for both non-progressive groups; AUROC = 0.619 (95% CI 0.586–0.653), sensitivity was 0.579 and specificity was 0.603 using the non-progressive group with CA ≤20°; AUROC = 0.573 (95% CI 0.551–0.595), sensitivity was 0.556 and specificity was 0.561 using the non-progressive group with CA ≤30°, respectively. The test data set confirmed a significant correlation (ρ: 0.12, $p = 1.5 \times 10^{-4}$; Supplemental Table S8), which was comparable to that in the best PRS for AIS susceptibility. Taken together, the PRS based on AIS susceptibility can quantitatively predict AIS progression without calibration for CA.

We compared subjects with CA ≥40° (progressive group, cases) and those with CA ≤20° (non-progressive group, controls) regardless of skeletal maturity in the test set. The AUROC of the best PRS for AIS susceptibility was 0.601 (95% CI 0.526–0.677). Sensitivity and specificity were 0.560 and 0.579. When BMI was included in the model, the AUROC was 0.635 (95% CI 0.561–0.710) (Supplemental Table S10). Subgrouping into quartiles revealed the reasonable discerning ability of the progressive group (OR = 3.29, 95% CI 1.42–8.55; Fig. 5 and Supplemental Table S11). The best PRS generated by the conventional method for CA showed AUROC of 0.670 (95% CI 0.597–0.742), sensitivity of 0.711 and specificity of 0.579. The AUROC including BMI was 0.698 (95% CI 0.630–0.766) (Supplemental Table S10). OR of 8.27 (95% CI 3.15–28.47) was also shown in quartile analysis (Supplemental Fig. S6). The non-progressive group with CA ≤30°[36] resulted in similar results in comparison with the result of the best PRS for susceptibility (Fig. 5 and Supplemental Tables S10 and S12). When we restricted to subjects with evidence of skeletal maturity (to avoid the confounding of future progression), we obtained similar results for the performance of the PRS and its subgroups (Supplemental Tables S10–S12 and Supplemental Fig. S5). Taken together, these results showed reasonable ability to predict the onset and progression of AIS based on the PRS.

Discussion

In the current study, we took advantage of the largest, to date, AIS data set (case n = 5327) and showed that we can efficiently predict AIS onset with the use of the PRS constructed by prioritized SNPs with functional annotation. Combining PRS with BMI resulted in better performance. In addition, we demonstrated that CA is also a polygenic trait that shares genetic components with AIS susceptibility and that we can quantitatively and

qualitatively predict CA with the use of the same PRS (or applying a slightly different PRS).

The PRS alone demonstrated the AUROC of ~0.67 for AIS susceptibility. Although the maximum AUROC of the PRS is shown to highly correlate with SNP heritability of traits in the previous study of the UK Biobank,[43] our result of the Nagelkerke pseudo-R^2, which here is 10%, cannot reach the expected SNP-heritability h^2 metric, which is 44% for AIS.[22] Because the SNP heritability fails to capture substantial heritability estimated in twin studies, our PRS has the potential to improve through the inclusion of variants with rare allele frequencies and low LD genotyped in sequencing in the future.[44] Importantly, the acceptable AUROC and OR of ~4.0 to predict AIS in comparison between the highest and reference PRS groups were purely derived from the PRS and not dependent on any other risk factors (including BMI). When we include BMI into the model, the value of OR did not change remarkably (data not shown). Integrating functional annotations could reduce the number of variants to construct PRS by ~88% to attain the best predictive performance. Since a limited number of cell-specific functional annotations in AIS-related tissues are available, in-depth analyses of connective tissues, bones, and spinal discs would lead to constructing a better PRS based on a smaller number of variants. Despite rigorous conservative analysis, we also improved the performance of our prediction model including BMI, a known clinical risk factor. This promises that further identification for environmental factors related to AIS would improve predictive accuracy.

Because of sex imbalance in AIS, we excluded male subjects in constructing the PRS. However, our model showed reasonable predictive ability for male subjects. While we reported potential sex-specific variants to AIS susceptibility,[22] the findings in the current study suggest that genetic components of AIS susceptibility are highly shared between males and females and that we may use the same prediction model for both sexes.

Although we observed no significant signals in the GWAS of CA, we found CA as a heritable trait. These indicate that our current data sets do not have enough power to identify GWAS-significant signals (including rs3533564) despite the large data set. The positive genetic correlation between AIS susceptibility and CA indicates that the higher the risk for AIS, the more potential exists for its progression, which is in line with previous studies.[36,45]

Applying the PRS with the best performance in AIS susceptibility to CA showed the PRS was associated with CA, indicating the possibility to use a single PRS to predict both AIS susceptibility and progression. On the other hand, the PRS with annotated SNPs showed less predictive ability than that of the conventional method. This suggests that the genetic architecture in terms of critical cell type–specific regulatory regions is different between susceptibility and severity (CA). On the other hand, when BMI was included in the model, the degree of improvement in AUROC of PRS_{CA} was slightly inferior to that of susceptibility. From these results, BMI may have more impact on onset than on the severity of AIS.

Because CA is a heritable and polygenic trait, it is essential to continue recruiting samples to obtain more accurate coefficients of variants and to identify novel susceptibility loci to obtain better prediction accuracy in the future. In the clinical aspect, to obtain the best performance of prediction (in addition to genetics), the incorporation of appropriate covariates would lead to better results.

In spite of showing a modest performance of the PRS to predict the development and progression of AIS, the current study has some limitations. Because GWASs generally utilize controls that contain a small fraction of possible cases, our control may contain potential scoliosis cases. This is because the frequency of adolescent idiopathic scoliosis, which is generally 2% to 3% in control, does not affect the statistical significance of GWAS (a GWAS that compares cases and controls containing 2% to 3% cases would result in only a slight decrease of statistical power). Because the current study is a retrospective study, the replication of our results is needed using another independent cohort in the future. A prospective study will be necessary to show the clinical utility of our model including the PRS because our results did not suggest strong evidence or association for clinical utility in the current study. In addition, a prospective study will be able to make more accurate measurement of phenotypes such as the Cobb angle and of exposures such as BMI. For such a study, the PRS tuned by a p value threshold of 0.05 should also be evaluated for its predictive ability because p values between 1.0×10^{-5} and 0.05 will contribute primarily to improving the model. Depending on the results of a future prospective analysis, the number of SNPs used to generate the PRS could be simplified. However, in this study, we firmly evaluated the accuracy of the PRS using three independent cohorts as discovery, validation, and test data sets. Accuracy was not significantly different between the validation and test data sets; therefore, the predictive ability of the PRS is expected to be robust. The current study primarily focused on female subjects due to the sex imbalance of AIS. Further research may be necessary to construct PRS for prediction in males. Because the current study is limited to the Japanese population, the prediction ability of our PRS to other populations is also still unknown. As previous research suggested a common AIS genetic architecture between Japanese patients and other populations,[46] it would be interesting to analyze whether a PRS made from Japanese samples can predict AIS in other populations.

In summary, we performed the PRS approach for the first time in AIS on an unprecedented scale and revealed that the PRS was beneficial for predicting the onset and severity of AIS. We also demonstrated that the progression of AIS is a heritable and polygenic trait that shares a large portion of genetic architecture in common with AIS susceptibility. This PRS approach for AIS is promising to consider for early intervention from the standpoint of preventive medicine. Ultimately, it is expected that more AIS patients can avoid spinal correction surgery through prediction based on PRS.

Disclosures

The authors declare no competing financial interests.

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Authors' roles: CT, SIk, W-CC, and KoW supervised the project. NO and CT designed the project and provided overall project management. NO, H-FL, and CT drafted the manuscript. YM and MKu performed the genotyping for the GWAS. CT, NO, H-FL, YKa, YKo, and MKo analyzed the GWAS data and performed integrative analyses. MNakajima and IK contributed to preparing the manuscript. NO, KT, YO, YoT, SM, NK, KU, MI, KeW, TKa, HY, HT, KH, YuT, TKo, TT, TSato, KKaneko, HSu, AI, SIn, TSakuma, NF, MY, MNakamura, KC, KKo, TSu, TA, KN, KKakutani, HShi, TI, SD, RS, NH, EO, MM, and KoW collected and managed clinical data.

Author contributions

Nao Otomo: Conceptualization; data curation; formal analysis; investigation; project administration; validation; visualization; writing-original draft; writing-review & editing. **Hsing-Fang Lu:** Formal analysis; methodology; visualization; writing-original draft; writing-review & editing. **Ikuyo Kou:** Funding acquisition; investigation. **Kazuki Takeda:** Data curation; resources. **Masaru Koido:** Data curation; methodology. **Yukihide Momozawa:** Data curation. **Michiaki Kubo:** Data curation. **Yoichiro Kamatani:** Supervision. **Yoji Ogura:** Data curation; resources. **Yohei Takahashi:** Data curation; resources. **Masahiro Nakajima:** Data curation. **Shohei Minami:** Resources. **Koki Uno:** Resources. **Noriaki Kawakami:** Resources. **Manabu Ito:** Resources. **Tatsuya Sato:** Resources. **Kei Watanabe:** Resources. **Takashi Kaito:** Resources. **Haruhisa Yanagida:** Resources. **Hiroshi Taneichi:** Resources. **Katsumi Harimaya:** Resources. **Yuki Taniguchi:** Resources. **Hideki Shigematsu:** Resources. **Takahiro Iida:** Data curation; resources. **Satoru Demura:** Resources. **Ryo Sugawara:** Resources. **Nobuyuki Fujita:** Resources. **Mitsuru Yagi:** Resources. **Eijiro Okada:** Resources. **Naobumi Hosogane:** Resources. **Katsuki Kono:** Resources. **Masaya Nakamura:** Resources. **Kazuhiro Chiba:** Resources. **Toshiaki Kotani:** Data curation; resources. **Tsuyoshi Sakuma:** Resources. **Tsutomu Akazawa:** Resources. **Teppei Suzuki:** Resources. **Taichi Tsuji:** Resources. **Hideki Sudo:** Resources. **Akira Iwata:** Data curation; resources. **Kazuo Kaneko:** Resources. **SatoshiInami Inami:** Resources. **Yuta Kochi:** Supervision. **Wei-Chiao Chang:** Supervision. **Morio Matsumoto:** Funding acquisition; resources. **Kota Watanabe:** Project administration; resources; supervision; writing-review & editing. **Shiro Ikegawa:** Project administration. **Chikashi Terao:** Conceptualization; project administration; supervision; writing-review & editing.

Peer review

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Data availability statement

GWAS for Cobb angle will be available in our website soon after publishment.

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