**Contribution of Genome-Wide Significant Single Nucleotide Polymorphisms in Myopia Prediction in Children：Findings from A 10-year Cohort Study of 1073 Chinese Twin Children**

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### ABSTRACT

**Background:** Given the current limitations and side effects of the interventions for myopia control, myopia prediction is critical to identify children at increased risk of developing high myopia. Recent genome-wide association studies have identified a number of statistically significant single nucleotide polymorphisms (SNPs) associated with myopia, however, the value and contribution of these SNPs in myopia prediction has not yet been identified. We aimed to determine the added predictive ability of genome-wide significant SNPs in myopia prediction in children, and to investigate the earliest age threshold for an accurate prediction of high myopia.

**Methods:** We used data from a prospective longitudinal study with a total of 1073 first-born twins followed annually between 2006 and 2015 from in China. The exposures were genetic factors (parental myopia, SNPs) and environmental factors (near work, outdoor activity). The primary outcome measure was spherical equivalent (SE) at the last visit among all subjects, and the secondary outcome measure was the presence of high myopia at the age of 18 years. The outcomes were analyzed with four linear mixed-effect models consisting of different combinations of age, gender, environmental and genetic exposures. An area under curve (AUC) > 0.95 was defined as an accurate prediction.

**Findings:** Mean age of the study population was 10.5+2.2 years at baseline and 48.6% were male. In all the models, age (P<0.001), parental SE (P<0.001) and SNPs (P<0.05) showed significant fixed-effect whilst gender, outdoor and near-work time were not significant. Incorporating more follow-up data into the model showed better performance across all models. In the prediction of the presence of high myopia at 18 years old, the model consisting of only age and gender showed a good performance (AUC=0.95), while in the addition of SNPs did not further enhance the model performance significantly. The AUC for predicting high myopia was >0.95 after age of 13 years for participants with single visit data, and after the age of 11 years for those with data from 2 or more visits.

**Conclusions:** Current genome-wide significant SNPs related to refractive error have little add-on value for myopia prediction in children. The earliest age threshold to accurately predict high myopia at 18 years old was during early adolescence.

**Key words:** Single nucleotide polymorphisms, myopia, prediction

### INTRODUCTION

With an increasing prevalence in recent decades, myopia is the most common eye disorder worldwide.[[1](#_ENREF_1), [2](#_ENREF_2)] The prevalence of high myopia, a severe form of myopia, is also increasing dramatically.[[3](#_ENREF_3), [4](#_ENREF_4)] It’s estimated that by 2050, 49.8% of the world population will have myopia and 9.8% will have high myopia.[[5](#_ENREF_5)] The tremendous expenditure of optical correction and eye care, costing over 3.8 billion USD annually in the US,[[6](#_ENREF_6)] and the irreversible vision loss due to myopia-related complications, such as glaucoma and myopic macular degeneration,[[7](#_ENREF_7), [8](#_ENREF_8)] currently pose a huge burden to public health.

Atropine and orthokeratology have been suggested to be the most effective interventions to control myopia progression.[[9](#_ENREF_9), [10](#_ENREF_10)] However, these interventions are not without side effects - photophobia and loss of accommodation can result from the application of atropine eye drops,[[11](#_ENREF_11), [12](#_ENREF_12)] whilst corneal inflammation and conjunctivitis are associated with the long-term use of orthokeratology.[[13](#_ENREF_13), [14](#_ENREF_14)]. An effective and reliable disease prediction model of myopia could help triage and identify the children with greatest risk of developing high myopia, and therefore justify the use of the aforementioned treatments.

Myopia is widely acknowledged as a complex disease process affected by both genetic and environmental factors. Recent developments in genome-wide association studies (GWAS) have led to the discovery of a growing number of common single nucleotide polymorphisms (SNPs) for refractive error and myopia, and studies have started to incorporate such genetic information into various prediction models with varying success.[[15-18](#_ENREF_15)] Previously, various prediction models have been built to predict the onset of myopia, [[19-22](#_ENREF_19)] whereby refraction at childhood, parental history of myopia, and ocular biometry were identified as robust myopia predictors., while little information of SNPs in the myopic prediction model was provided. Mojarrad et al. reported that genetic risk score (including both insertion or deletions and SNPs) as a predictor can improve the ability of detecting children at risk of myopia, comparing to number of myopic parents, in a retrospective study.[[23](#_ENREF_23)] However, in their study, only non-cycloplegic autorefraction was analyzed, and information of parental myopia was collected with questionnaires, leading to an insufficient strength of evidence. Uncertainties surrounding the predictive value of SNPs for myopia prediction still exist.

The purpose of this study was to assess the add-on benefit of including 39 myopia-related SNPs previously identified in GWAS studies into the prediction model for myopia progression and high myopia onset and to further investigate the earliest age threshold for an accurate prediction of high myopia based on a longitudinal cohort of children in Southern China.

### METHODS

**Study populations**

The study participants were recruited from the Guangzhou Twins Eye Study (GTES), and details of the sampling and methodology have been reported elsewhere. [[24](#_ENREF_24), [25](#_ENREF_25)] In brief, The Guangzhou Twins Eye Study (GTES) is a longitudinal study including over 1200 pairs of twins and their parents or siblings living in Guangzhou, China. The baseline age of subjects ranged from 7 to 15 years. Participants were invited for annual follow-up examinations. Data in the present study were attained from 1073 elder twins, with available follow-up data between 2006 and 2015.

This study obtained ethical committee approval from the Zhongshan University Ethical Review Board and Ethics Committee of Zhongshan Ophthalmic Center, and all examinations were conducted in accordance with the Tenets of the World Medical Association's declaration of Helsinki. Written informed consent was obtained from all participants, their parents or statutory guardians.

**Measures**

Autorefraction was performed per the same protocol for each twin at baseline and at every follow-up visit. An autorefractor (KR8800, Topcon Corp, Tokyo, Japan) was used to measure refraction of both eyes after cycloplegia. Cycloplegia was induced with 2 drops of 1% cyclopentolate (1% Cyclogyl, Alcon Labs, Fort Wroth, Texas) instilled 5 minutes apart, and a third drop was administered after 20 minutes. The biological parents of the twins were also invited to undertake autorefraction measurements in both eyes without cycloplegia. Time of outdoor activity and near work were collected using an interviewer-administered questionnaire. Details of questionnaire and calculations of time outdoor and near work duration have been described elsewhere.[[26](#_ENREF_26), [27](#_ENREF_27)] Blood samples were taken from all twins at baseline to extract deoxyribonucleic acid (DNA). DNA samples were genotyped using an Affymetrix high-density SNP array (Affymetrix Gene Titan), and further imputed by IMPUTE2 (v 2.3.0) using the 1000-Genomes Project reference panel (Phase1, Nov 2010 release) and a stringent quality control procedure.[[27](#_ENREF_27)] Data of 39 SNPs with genome-wide significant associations for refractive error that have been identified from two previous large GWAS were extracted for analysis (Table S1).[[17](#_ENREF_17), [18](#_ENREF_18)]

**Statistical methods**

The right eyes of the first-born twins were arbitrarily selected for analysis. **Spherical equivalent (SE, defined as the sum of sphere and 1/2 cylinder power) at last observation was the primary outcome measure of this study. The presence of high myopia (SE<-6.00 Diopters [D]) at the age of 18 years was the secondary outcome measure.**

The study population (N=1073) was divided into 2 separate groups for model building and model validation. The validating dataset consisted of all participants aged >18 years at their last follow-up examination (N=384), including 36 high myopes. In consideration of the limited number of highly myopic cases, approximately one third (N=18) of the 53 highly myopic participants aged <18 years at their last examination were also selected through random sampling and included in the validating dataset, thus leaving a total of 402 participants in the validating set. The modeling dataset consisted of all participants aged <18 years at their last examination, excluding the 18 high myopia patients that were selected for the validating dataset (N=671). Group t-tests, Wilcoxon tests and Chi-squared tests were used to compare the baseline characteristics of participants in the modeling and validating sets.

A linear mixed-effect model was used in this analysis because repeated measurements were potentially correlated in the same individual. [[28-30](#_ENREF_28)]

, (1)

where represents the SE of the individuals in the visiting, and denotes as the covariates, such as age, gender, outdoor and near work time, parental SE, and so on. We assume the random terms follows multivariate normal distributions, that is and , where.

We built 4 linear mixed models based on the modeling dataset with SE as the outcome variable and choose different covariate**Model 1 included age and gender as fixed effect variables. Model 2 included age, gender, parental SE, outdoor and near work time as fixed effect variables. Model 3 included age, gender, SNPs, outdoor and near work time as fixed effect variables, and Model 4 included all the variables mentioned above (Table 1).**The missing measure rates of paternal SE, maternal SE and SNPs were 20.8%, 9.3% and 18.6%, respectively. Imputation of these three variables were made with miss-Forest package in R (version 3.4.3, <https://www.r-project.org/>) before modeling.

Three groups were derived from the validating dataset for prediction: 1) all baseline participants, 2) participants with 1 follow-up visit, and 3) participants with at least 2 follow-up visits. Personalized prediction was performed based on the joint distribution and correlation structure of multiple measurements of SE on the same individual. Detailed derivation about the personalized prediction modeling can be founded in the Supplementary note.Mean squared error (MSE), R square and Akaike Information criterion (AIC) were used to assess the performance of these models in predicting SE at last visit. Receiver operating characteristic (ROC) and area under curve (AUC) were used to evaluate the ability of these models in identifying the presence of high myopia at 18 years old. Good accuracy was considered when the AUC >0.95. P values of <0.05 were defined as significant. All the analysis was conducted using R software.

### RESULTS

A total of 1073 first-born twins were included in the current study with a mean age of 10.5+2.2 years at baseline. Almost half (48.6%) were male. Of all the baseline participants, 58.2% were examined more than 6 times and 16.2% completed all examinations from 2006 to 2015.The distribution of follow-up times can be found in supplementary Figure 1. Based on the methods described earlier, 402 participants (37.47%) were included in the validation group and 671 (62.53%) were included in the modeling group (supplementary Figure 2). Participants in the modeling group were younger (P<0.001), more hyperopic (P<0.001), had less near-work time (P=0.001) and more myopic parents (P=0.002 for father; P=0.008 for mother), compared to participants in the validating dataset (Table 2).

The parameters of fixed effect for the four models were summarized in Table 3. Age (P<0.001), parental SE (P<0.001) and SNPs (P<0.05) were consistently significant in all models. Gender, outdoor and near-work time were non-significant in all models. Prediction of SE at the last visit was performed in three groups (subjects with only baseline data, baseline data plus 1 follow-up visit, and baseline data plus at least 2 follow-up visits) derived from the validating dataset, using age-specific SE and mentioned variables in each model. Table 4 showed the AIC, R2 and MSE parameters. Incorporating more follow-up data into the model resulted in a greater R2 and less MSE for all four models, while within the same dataset, the performance of these four models were not significantly different.

The prediction performance of the four models for the presence of high myopia at 18 years old were also assessed. ROCs were plotted for these models as per the three groups from the validating dataset. The AUC values were similar across all groups, ranging from 0.94 to 0.97 (Figure 1). Model 1 showed a better performance than Model 4 based on SE at baseline (P=0.03), while their performance based on more follow-up data were similar (Table 5).

**To identify the earliest age for accurate high myopia prediction, we further analysed Model 1 using data at certain ages (9 to 17 years respectively), as well as data at certain ages plus one following visit.** Table 6 showed the data at each specific age, revealing that R2 increased with age, and was more than 0.95 after the age of 14 years and above. MSE narrowed as the age increased, and remained under 1.0 after 14 years old. For participants with 1 visit, the AUC was 0.82 to 0.94 for younger participants (9 to 12 years old), and reached >0.95 after the age of 13 years. For participants with at least 2 visits, the AUC increased to 0.95 and above after 11 years old.

### DISCUSSION

In this twin cohort, the contribution of genome-wide significant SNPs in predicting myopia progression and the development of high myopia at 18 years old was of minimal add-on value. Age and its relevant refraction was the most significant determinant, and refractive status during early adolescence can accurately predict the risk of developing high myopia.

The 39 loci included in this study were identified based on the findings from the recent GWAS meta-analysis of nearly 45,000 individuals, most of whom were adults.[[17](#_ENREF_17" \o "Kiefer, 2013 #63), [18](#_ENREF_18" \o "Verhoeven, 2013 #302), [22](#_ENREF_22" \o "Chen, 2016 #5323)] In 2016, the CREAM Consortium assessed the age-of-onset associations between these 39 loci and refractive error in 5200 children, and found that in total, they could explain 0.6% and 2.3% of the variance in refractive error at age 7 and age 15, respectively. [[27](#_ENREF_27" \o "Fan, 2016 #5444)] Our results show that adding these 39 SNPs does not improve the performance of a myopia prediction model. There are several possible reasons for this: (1) the “missing heritability problem” which implies the contribution of GWAS significant SNPs is far less than the heritability estimation identified by genetic epidemiology studies, including the classic twin study;[[31-33](#_ENREF_31)] (2) there is low penetrance and effect size for the GWAS significant SNPs related to myopia or refractive error, in contrast to other SNPs such as the CFH in age-related macular degeneration which may have a stronger predictive ability;[[34](#_ENREF_34)] (3) these SNPs were taken from two GWAS that examined mostly adults from Caucasian populations,[[17](#_ENREF_17), [18](#_ENREF_18)] whilst our analysis included twins aged 7-15 years of Chinese ancestry; and (4) the simple baseline model that included age, relevant refraction and gender already achieved excellent prediction performance, thus limiting the potential additional value from add-on genetic information.

Previous longitudinal studies have reported that parental myopia is strongly associated with myopia onset,[[35](#_ENREF_35), [36](#_ENREF_36)] and the acceleration of myopic progression.[[26](#_ENREF_26)] However, the current study has shown that although parental myopia is associated with childhood myopia, it is not an important predictor for future myopic SE. The inclusion of parental myopia into the original model revealed an improved performance when predicting myopia using data at the baseline only, but this benefit no longer existed after SE at follow-up visits were included. Similarly, Chua et al. found in a prediction model of high myopia[[21](#_ENREF_21)] that parental myopia only improved the AUC minimally when compared to age of myopia onset. The heritability of myopia obtained from parent-offspring correlations has varied in previous reports, particularly in the setting of major differences in environmental exposures between generations.[[37](#_ENREF_37), [38](#_ENREF_38)] Another possible explanation may be that the children’s age and associated refraction have already incorporated the effect of parental myopia. As an example, children with myopic parents may have more severe myopia that occurs earlier in their lifetime, and therefore the associations of age and SE would already include the effect of parental myopia. The lack of benefit in adding parental SE to the prediction model appears to support that notion that the addition of genetic information may not be useful for predicting future SE changes.

The benefit of adding near-work and outdoor time into the prediction model was also limited. The average off-school time outdoors in this cohort was 1.3 hours, which is inadequate to protect children from the onset of myopia.[[39](#_ENREF_39), [40](#_ENREF_40)] In addition, the performance of the model with only age, gender and baseline SE with two or more follow-up visits already provided excellent predictive accuracy, with limited room for improvement. Evidences from many population-based studies suggest that children do not become more myopic with age without exposure to the appropriate risk factors[[41](#_ENREF_41), [42](#_ENREF_42)]. Therefore, age may represent a parameter that incorporates both genetic and environmental exposures and omits the contributions of other risk factors in prediction model. [[37](#_ENREF_37), [43](#_ENREF_43), [44](#_ENREF_44)] Based on our analysis, a mixed effect linear model with age-specific SE on multiple visits plus gender information may suffice in accurately predicting myopia in a real-world clinical setting, therefore avoiding the need to collect additional environmental and genomic data.

**Our results have demonstrated that incorporating more follow-up visits in the linear mixed effect model can improve the performance of prediction. Most previous reports on myopia prediction have established a prediction model based on a single visit at baseline, followed by the prediction of myopia or high myopia at a later visit.[[19-21](#_ENREF_19" \o "Zadnik, 2015 #4985), [35](#_ENREF_35" \o "Jones-Jordan, 2010 #4936)] In other words, subjects with two visits and those with multiple visits did not differ in terms of the accuracy of prediction. The current study adjusted the prediction values using the expectation variance generated by additional follow-up data, allowing a self-learning system that improves the accuracy of prediction alongside the increasing amounts of data. To the best of our knowledge, this is the first prediction model for myopia development that incorporates adjustment from multiple follow-up visit data.**

Age at 13 years appeared to be the earliest age threshold for accurate prediction of high myopia for participants with baseline data only, whilst this age dropped to 11 years for participants with more follow-up data. Below these cutoffs, the AUC values dropped from 0.95 to 0.90 and 0.92, respectively. For Asian children, myopia typically develops at 7 years old, progresses during school years and stabilizes around 16 years of age[[45](#_ENREF_45)]. Our analysis suggests that the best time to estimate the risk of developing high myopia at 18 years may be as early as 11 to 13 years old in a young Asian population.

Strengths of this study include the fact that that the prediction model was constructed based on a population-based design which included a large sample size and a long follow-up time. Furthermore, all refraction data in children were confirmed with cycloplegia in order to maximise accuracy. The genetic data was derived from a large number of recently reported SNPs from GWAS, and the homogeneous genetic background of the study population provides reliable information on the genetic susceptibility analysis. Limitations include a relatively small number of highly myopic cases, and a potential recall bias from parent-reported near-work and outdoor time, since these data were collected by self-reported questionnaire.

In conclusion, statistically significant SNPs derived from GWAS association studies related to myopia or refractive error had little add-on value in terms of myopia prediction. The highest accuracy for prediction required only age and its relevant SE. The earliest time to accurately predict the risk of developing high myopia by 18 years was during early adolescence. This offers practical implications for clinical screening and monitoring of myopic children in a population with high prevalence of myopia.

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Figure legends:

Figure 1.Receiver operating characteristic (ROC) curves of model 1-4 for predicting the presence of high myopia at the age of 18 based on baseline, baseline plus 1-year follow-up and baseline plus 2-year follow-up data of validation dataset.