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The Guangzhou Twin Project: An Update

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The Guangzhou Twin Registry is a population-based registry of twins residing in Guangzhou City. The initial registry database included 9,700 pairs of young twins recruited from the Official Household Registry of Guangzhou City. The registry is designed to provide a resource to identify the genetic and environmental causes of common diseases with an initial focus on eye diseases. From 2006 onward, phenotype and DNA collection have been completed for more than 1,200 twin pairs and their parents or siblings. Most of the young twins have come back for an annual examination of the progressive traits, such as refraction, ocular biometry, weight, and height. Genome-wide association scans have been completed recently. This article gives an update of the study design, cohort profile, previous findings, and future directions. Results from the Guangzhou Twin Project may contribute to the understanding of gene-environmental interplay for complex diseases in both adults and children.

■ **Keywords:** twins, registry, genetics, environment, Chinese

History of the Guangzhou Twin Project

The challenge for genetics in the new millennium is the genetic dissection of complex traits. This challenge must be met with new analytic methods based on powerful genetic samples. The Guangzhou Twin Project, one of China's rich sets of genetic resource, is a longitudinal cohort study of major eye diseases among Chinese twins residing in urban southern China (He et al., 2006). The project offers a unique opportunity to disentangle genetic and environmental influences on common eye trait variation. Participants in the project were enrolled from the Guangzhou Twin Registry, a population-based registry (initiated in 2005) including 9,700+ pairs of young twins recruited from the Official Household Registry of Guangzhou City, southern China. Both the project and registry have been maintained by the research investigator (MH) at the Zhongshan Ophthalmic Center (ZOC) of Sun Yat-sen University. The research team has been securing domestic and international funding to invite study participants to take part in the baseline and follow-up examinations. The initial focus of the project was to estimate heritability and identify new risk factors for eye phenotypes, particularly the myopia- and glaucoma-related quantitative traits. From 2006 onward, clinic-based data collection and DNA extraction have been completed for more than 1,200 young twin pairs aged 7–15 years and their parents or siblings (see Table 1). Most of the young twins have come back for annual examinations of progressive phenotypic data, such as refraction, ocular biometry, weight, and height. The study participants were served by a dense network of public transport services (e.g., bus, metro) and most of them had a 1-hour travel time to ZOC using public transport. The project has been recently developed into a multidisciplinary platform for genetic research of both eye and general diseases in both adults and children.

What Has Been Measured?

The Guangzhou Twin Project is particularly interested in the genetic determinants of myopia- and glaucoma-related eye traits in children and adolescents. The details of workflow and examination procedures for the young twin cohort have been published elsewhere (He et al., 2006). In brief, the study was conducted in Guangzhou, the capital city of Guangdong province and one of the top three metropolitan cities in China. A twin eye clinic was established at ZOC in 2006. Young twins aged 7–15 years have been consecutively recruited for the twin registry since then. All invited twins were provided information about the project through letter or telephone. For those who gave consent, a date was then set

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TABLE 1

Characteristics of Young Twins And Their Parents

Young twin pairs	Number (pair)	Age in 2012 mean (*SD*)
MZ male	368	13.6 (3.2)
MZ female	409	14.0 (3.3)
Total MZ	777	13.8 (3.2)
DZ male	113	13.4 (3.4)
DZ female	95	14.2 (3.4)
DZ opposite sex	230	13.4 (3.3)
Total DZ	438	13.6 (3.3)
Total twin pairs	1,215	13.7 (3.3)
Triplet	6	14.3 (2.3)
Young twins' parents	Number (person)	Age in 2012 mean (*SD*)
Father	1,178	46.9 (5.5)
Mother	1,200	43.7 (5.0)

Note: MZ = monozygotic; DZ = dizygotic.

for a physical examination at ZOC. Several sophisticated eye tests were conducted at the baseline visit (see Table 2). These included visual acuity test, intraocular pressure (IOP), ocular biometry, corneal thickness, optic disc tomography, peripheral refraction, and retinal photography. Measurements of systemic conditions included height, weight, body fat mass, skinfold thickness, and blood pressure. Data on rapidly progressive phenotypes such as visual acuity, refraction, and ocular biometry as well as body height and body weight were collected at each annual visit. These data have been automatically synchronized between the machines and the central server system and subsequently stored into an electronic database. In addition to these objective measurements, each participant received a short questionnaire to update socio-economic and medical information, and a structured interview by trained staff to provide lifestyle activities associated with the development and progression of myopia. Twins' parents completed a parental questionnaire regarding basic demographic information, birth records, parental education, and socio-economic status (see Table 2). In addition, parents of the twins underwent the same physical and eye examination, and DNA collection procedure that the twins did. Data collected from questionnaires were manually entered into an electronic database. Any data outliers and missing values were checked on the original forms by a team of data coordinators. A blood sample was collected at the baseline visit and venous blood was taken for zygosity test, DNA extraction, and biochemistry tests (e.g., blood lipid and glycosylated hemoglobin). A sub-group of DNA samples (stored at –40 °C) have been used for genome wide association studies (GWASs) using Affymetrix Axiom genotyping arrays.

Zygosity was determined by genotyping with 16 polymorphic markers in all same-sex twin pairs. A written informed consent was obtained from the parents or guardians of the young twins after a careful explanation of the study details, as well as the potential risks and benefits. All the examinations were conducted in accordance with the Tenets of the World Medical Association's declaration of Helsinki. Ethical approval and DNA data using approval were obtained from Sun Yat-sen University Ethical Review Board and the Ethical Committee of ZOC.

A subset of twin pairs ($N = 100$) is being invited to participate in a MRI phenotyping project, which will provide a non-invasive opportunity to identify novel three-dimensional structural traits in brain and eyes. In addition, another randomly selected group of twins are receiving a comprehensive assessment on cognitive function (e.g., IQ scores, imitation ability, pro-social behavior, and emotional perception). This part of the work has been conducted by the collaborators from Peking University and the Chinese Academy of Sciences.

Who is in the Sample?

Between 2006 and 2012, a total of 1,221 twin pairs aged from 7 to 15 years participated in the study. All participants have been invited for an annual eye examination. One hundred percent of the twin participants are of Chinese Han ethnicity; this ethnic homogeneity excludes possible confounding or effect modification by race in the analysis. As of 2011, 77% of twins had at least one follow-up exam. The twin cohort is representative of the general population for many measures (Sanfilippo et al., 2011), as evidenced by a structural equation modeling analysis showing that the mean and variance of major eye traits (e.g., spherical equivalence) and behavior risk factors (e.g., near-work activities) were very similar between the twin individuals from our cohort and singletons from the general population (Hur et al., 2009). Among the 1,221 young twin pairs, 95.4% had their parents (1,178 were males and 1,200 were females) available for detailed examinations.

In addition to the young twin family cohort, we have recently examined elderly twins aged 50–70 years, who were contacted via the Official Household Registry of Guangzhou City. All of these adult twins underwent physical and eye examinations using the same measurement protocol for young twins.

What Has Been Found? Key Findings and Key Publications

The main contribution of the Guangzhou Twin Project has been to test the hypothesis of whether the major eye diseases (e.g., refractive errors) and new ocular traits are genetically determined. In doing so, we have constructed classical twin studies to estimate heritability — the proportion of individual phenotypic variation that can be explained by genetic differences among individuals in a given time in a given population. Specifically, our study is the first to identify the high heritability for many novel ocular biometric measures, including lens thickness, peripheral refraction, iris thickness, pupil size, and iridotrabecular angle width (He et al., 2008a, 2009; Ding et al. 2012; Shen et al., 2012). We have provided

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TABLE 2

Summary of Data Collected In The Guangzhou Twin Eye Study

Phenotype	Measurement method
Eye data	
Spherical equivalence	Autorefractor and subjective refraction
Peripheral refraction	Open-field autorefractor
Astigmatism	Autorefractor
Central cornel thickness	Pentacam Scheimpflug imaging system
Lens thickness	Lenstar
Ocular biometry	IOLMaster
Optic disc parameters	Heidelberg retinal tomography III
Anterior segment parameters	Anterior segment optical coherence tomography
Eye shape	MRI test
Intraocular pressure	Tonopen
Visual acuity	ETDRS acuity testing
Stereoacuity	Randot stereo chart
Color test	Standard color test chart
Major eye diseases (e.g., glaucoma, retinal diseases)	Ophthalmologists' clinical diagnosis
Systemic data	
Height	Seca 202 mechanical telescopic measuring rod
Waist–hip ratio; arm circumference	Girth measuring tape
Triceps skinfold thickness	Skinfold caliper
Weight; body fat mass	TANITA body composition analyzer
Blood pressure	Automatic sphygmomanometer
Blood data	
Complete blood count	Biochemistry test
Glycated hemoglobin	Biochemistry test
Cholesterol level (i.e., total cholesterol, High density lipoprotein (HDL), Low density lipoprotein (LDL), triglyceride)	Biochemistry test
DNA sample	Qiagen DNA extraction kits
Questionnaire-based data (subjective)	
Demographic data	Questionnaire
Zygosity questionnaire	Questionnaire
Birth weight	Questionnaire
Gestational age	Questionnaire
Parental education level	Questionnaire
Ocular and medical history	Questionnaire
Indoor and outdoor activities	Questionnaire

additional evidence to support the genetic influences of many classical ocular traits, such as central corneal thickness, IOP, optic disc parameters, and axial length (AL). In line with the data from the Australian and UK twin registries, the heritability estimates of these traits were very high, ranging from 0.6 to 0.9 (see Table 3; Ding et al., 2012; He et al., 2008a, 2008c, 2008d, 2009; Shen et al., 2012; Zheng et al., 2008a, 2008b, 2009). These data suggest the consistency in levels of heritability across different ethnic groups and environments. The high heritability estimates of these eye diseases and biological markers discovered from our twin project and others have encouraged the search for the genetic pathways that underlie these complex traits.

Many genetically influenced systemic and ocular traits are correlated, which raises a question of whether the comorbidity of these traits arises from shared genetic factors that affect the susceptibility to both conditions. The Guangzhou Twin Project has a wealth of phenotypic data that allow us to document whether the correlation between traits of interest are the consequence of common genetic influences. For example, using Cholesky genetic models, we have quantified the shared genetic components that explain the phenotypic correlations between angle opening distance (AOD), anterior chamber depth (ACD), and AL (He et al., 2008b). We found that 23% of the genetic factors for AOD were shared with those for ACD, and 13% being shared with those for AL. Similarly, 25% of genetic factors for ACD were shared with those for AL. The shared genetic influences between AOD, ACD, and AL support the existence of pleiotropic effect of genes affecting both angle closure and myopia. We have also identified a significant shared genetic component between AL and height (Zhang et al., 2011). In another multivariate Cholesky model, we have identified significant shared genetic components between cardiovascular risk factors (e.g., blood pressure and body mass index) and retinal vascular caliber, an early marker of micro-vascular damage (Zheng et al., 2012). These genetic studies show how common genes can influence ocular biomarkers and systemic conditions in twins, and it seems likely that identifying genes responsible for one trait may

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TABLE 3
Heritability Estimates from the Guangzhou Twin Project

Phenotype	Number of twin pairs MZ	DZ	Mean value (*SD*)	Best-fitting model	Heritability (95% CI)	References
Anterior chamber depth	357	206	MZ: 3.5 ± 0.3 mm DZ: 3.5 ± 0.3 mm	AE	0.90 (0.82–0.92)	He et al., 2008d
Central cornea thickness	297	152	MZ: 551.9 ± 34.0 μm DZ: 551.7 ± 30.3 μm	AE	0.91 (0.89–0.93)	Zheng et al., 2008a
Iris thickness	309	165	MZ: 0.4 ± 0.6 mm DZ: 0.4 ± 0.1 mm	AE	0.59 (0.51–0.65)	He et al., 2009
Pupil size	309	165	MZ: 5.6 ± 0.8 mm DZ: 5.6 ± 0.9 mm	AE	0.63 (0.57–0.69)	He et al., 2009
Intraocular pressure	309	164	MZ: 14.2 ± 2.3 mmHg DZ: 14.2 ± 2.2 mmHg	AE	0.67 (0.61–0.72)	Zheng et al., 2009
Optic disc area	355	202	MZ: 2.0 ± 0.4 mm DZ: 2.0 ± 0.5 mm	AE	0.77 (0.73–0.81)	He et al., 2008c
Cup area	355	202	MZ: 0.5 ± 0.5 mm DZ: 0.5 ± 0.3 mm	AE	0.83 (0.79–0.86)	He et al., 2008c
Cup-disc area ratio	355	202	MZ: 0.2 ± 0.1 DZ: 0.3 ± 0.1	AE	0.78 (0.75–0.82)	He et al., 2008c
Lens thickness	482	286	MZ: 3.5 ± 0.2 mm DZ: 3.5 ± 0.2 mm	AE	0.90 (0.88–0.91)	Shen et al., 2012
Angle opening distance	305	157	MZ: 0.7 ± 0.2 mm DZ: 0.7 ± 0.2 mm	AE	0.70 (0.64–0.75)	He et al., 2008a
Temporal peripheral refraction	72	48	All: –0.3 ± 2.0 D	AE	0.84 (0.77–0.89)	Ding et al., 2012
Nasal peripheral refraction	72	48	All: 0.4 ± 2.2 D	AE	0.76 (0.66–0.84)	Ding et al., 2012

Note: A = additive genetic effect; E = unshared environmental effect; D = diopter.

further illuminate the pathological mechanisms that underlie another complex trait.

The Guangzhou Twin Project has established collections of annual data in twins across a wide range of age categories from childhood to young adulthood. The annual eye examination offers a unique opportunity to identify risk factors associated with clinical onset of eye diseases and to explore phenotypic progression. The value of our longitudinal data is best demonstrated in our analysis of annual phenotypic data on refraction and AL. Based on our young twin cohort, we found that the progression rate of spherical equivalent refraction and the elongation of AL accelerated before the onset of myopia and slowed down thereafter, supporting the hypothesis that an inhibitory mechanism exists after the development of myopia (Xiang et al., 2012). We have also documented a concomitant change of AL and height in our young twin cohort: the longer the eyeball, the greater the body height (Wang et al., 2011). These new data indicate that common pathways may exist for the development of eye size and body size in young people.

What Will Be the New Areas of Research?

The current research focus is the longitudinal cohort eye study of young twins from childhood until young adulthood. The existing annual phenotypic data make this cohort particularly suitable to identify genetic risks and early life environmental exposures impact on onset and progression of eye and systemic diseases. The current cohort has been under annual follow-up (from 2006 onward) for 6 years and the study has a wealth of qualitative and quantitative data to elucidate pathological pathways. Therefore, the priority of our project is to continue the longitudinal follow-up of young twins. In addition, we are planning to extend our research to the following areas in order to maximize the scientific potential of the project:

Quantitative Analysis of Genetic and Environmental Influences on New Ocular Traits

The original research aim -– to determine the extent to which genetic components explain variation in common eye traits (e.g., refractive errors and AL) — has been much elaborated. However, the genetic basis of many novel phenotypes remains to be established. These include live imaging phenotypes generated from advanced ocular imaging devices and new molecular biomarkers (e.g., gene expression levels, degree of methylation of CpG sites, and levels of metabolites) yielded from blood and urine.

Genetic Research Using Family-Based Association Analysis

Insight obtained from twin studies extends beyond traditional estimates of heritability. In the young twin cohort, we have collected detailed phenotypic and genetic information for both the twin individuals and their parents, making it possible to establish an extension of the twin model (which incorporates data from twins and their parents) for genetic research. It is increasingly recognized that newly identified short nucleotide polymorphisms (SNPs) from traditional

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case-control GWASs only explain a small proportion of heritability in disease (van Dongen et al., 2012). By contrast, family-based association analysis (i.e., combining linkage and association analysis) based on the extended twin model offers a new analytic approach to identify cause of human variation. Unlike case-control GWASs, family-based association analysis allows for control of population stratification and provides information on social interactions among relatives (Ott et al., 2011). We have recently completed the GWAS scan for more than 3,000 participants (including twins and their parents or siblings) using Affymetrix Axiom SNP genotyping solution and the family-based association analysis will be performed soon.

Discordant Twins Study

Given that monozygotic (MZ) twins are perfectly matched for age, sex, and genetic background, and partly for early environmental influences, the study of discordant MZ twins may offer novel insight into DNA variant, epigenetic variation and environmental effects associated with eye diseases and quantitative traits (van Dongen et al., 2012). We are working on epigenetic variation for myopia and glaucoma, and in the meantime, looking for funding and collaborators in this area.

Collaboration

We are currently seeking collaboration with other research groups with the goals of employing whole-genome sequencing and other advanced technologies to explore variants of DNA sequence, epigenetic variations, and metabolites associated with systemic and eye diseases. The data collection is still ongoing and growing, and the Guangzhou Twin Project has an open policy with respect to domestic and international collaboration with academic- and industry-based researchers. Request for collaboration should be addressed to Dr. Mingguang He (mingguang_he@yahoo.com).

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