
A Prospective Twin Registry in Southwestern China (TRiSC): Exploring the Effects of Genetic and Environmental Factors on Cognitive and Behavioral Development and Mental Health Wellbeing in Children and Adolescents

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The goals, prospects and methods of the Prospective Twin Registry in Southwestern China (TRiSC) are described. The aim of this study is to measure children's behavioral development and psychopathology from phenotypic, genetic and environmental perspectives. It focuses on measuring children's behavior and psychopathology from child self-reports, as well as parental and teacher informant reports, and relating it to the children's general cognitive abilities, and to the parenting style in the family. Other variables of interest such as children's temperament and parental health status are discussed, as well as plans for further research.

Keywords: twin registry, China; children's behavioral development, genetics

Twin studies have been a valuable source of information about the social/environmental and genetic basis of complex traits (Kendler et al., 1999). Population-based twin registers, especially those that collect twin pairs prospectively, are very valuable for epidemiological research owing to the lack of ascertainment bias, and for future in-depth study of the etiology of disease phenotypes. Until very recently, almost all twin studies have been based in developed countries, mainly in North American and Europe (Baker et al., 2002; Kendler et al., 2005; Willemsen & Boomsma, 2007). In developing countries, the first population-based twin register was established in Sri Lanka in 1997 (Sumathipala et al., 2003), which recruited adult twins for a study on common mental disorders (Siribaddana et al., 2006). The Chinese National Twin Registry, which is the first population-based twin registry in China, was initiated in 2001 (Yang et al., 2002). The

pioneering twin studies by Li and his colleagues (Pang et al., 2006; Yang et al., 2002) had a special focus on adult chronic disease such as cardiovascular disease and related traits in Qingdao and Beijing, two cities in northern China. However, twin studies for quantitative behavioral genetic research, especially in children and adolescents in developing countries, are still lacking.

Developmental processes play an important role in determining the importance of genetic and environmental influences on children's behavior and psychopathology (Knafo & Plomin, 2006a, 2006b). It has been shown that stability in ADHD symptom scores is mainly due to shared genetic influences in a longitudinal study of British twins ($N = 9,400$ pairs) aged 2, 3, 4, and 7. However, additional genetic influences, as well as child-specific environmental influences, that are not shared with those acting earlier on, emerge at later age periods (Kuntsi et al., 2005). Longitudinal genetic analyses showed that genetics account for both change and continuity in children's behavior and that the environment not shared by twins contributes mainly to change (Knafo & Plomin, 2006b). Evidence from twin studies also suggests that there are important roles for family environmental factors, parental positive feelings and discipline, which correlate positively with children's behavior, an effect attributed mainly to the environment shared by family members (Knafo & Plomin,

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2006a). Furthermore, genetic influence was greater for adolescent antisocial behavior when parenting was more negative or less warm (Feinberg et al., 2007).

However, these findings raised additional issues that could not be answered by the questionnaire based reports (Knafo & Plomin, 2006a, 2006b). What are the 'new' genetic effects that are responsible for change as children grow up? For example, evidence from 670 twin pairs aged 5 to 17 suggests that shared environmental effects had significant influence in younger children's depressive symptoms, which become increasingly heritable as children grow into adolescence (Scourfield et al., 2003). Could they be traced back to genetic influences on cognitive abilities, temperament, or both (Russell et al., 2003; Zahn-Waxler et al., 1982)? What is the role of parents' health status and parenting style in the development of children's behavior? Therefore, a developmental study using age-appropriate assessment methods and different measures of children's behavior is needed. These considerations have led to the establishment of the prospective Twin Registry in Southwestern China (TRiSC).

The main purpose of the TRiSC project is to create a twin register of 2,000 twin pairs for use in social, genetic and developmental research in China, and subsequently, to explore the effects of genetic and environmental factors on cognitive and behavioral development and mental health well-being in children and adolescent. It is hoped that the TRiSC project will advance the research basis in China, through initiating a quality longitudinal children and adolescent twin database to be followed in later ages in order to study the behavioral development of children and adolescents.

Main Research Questions of the TRiSC

1. What are the phenotypic, genetic, and environmental relations among different modalities of children's behavioral development? This includes children's problematic behavior and prosocial behavior. An important distinction has been made in the scientific literature between two types of behavioral problems in child psychopathology: internalizing behavioral problems (such as depression and anxiety) and externalizing behavioral problems (such as attention deficit and hyperactivity and conduct problems) (Gjone & Stevenson, 1997). Twin studies have shown that there is considerable covariance between both of these behavioral traits, and most of this is accounted for by common environmental components (Gjone & Stevenson, 1997). For the most common childhood-onset behavioral problem, attention deficit and hyperactivity disorder, the mean heritability of various twin studies was shown to be 0.77 (Kuntsi et al., 2005); For adolescent substance use, lifetime use of any drug showed a heritability of 45%, with shared environment accounting for 47% of the variation (Maes et al., 1999); Meanwhile, evidence from twin

studies has shown increasing genetic influence on depressive symptoms as children grow into adolescence (Rice et al., 2003). A question we would like to answer, for example, is: Do individual differences in different kinds of behavior load on the same genetic factors? To what extent do environmental factors influence these behaviors?

2. What is the role of continuity and change in the development in a wide range of aspects of child behavior and psychopathology? As children grow from childhood into adolescence, dramatic changes in children's cognitive abilities and their environment occur. Could these developments account for the increase in genetic influence around this age in several domains of behavior, such as depressive symptoms, as reported by Scourfield et al. (2003)? There is also evidence of additional genetic influences, which are not shared with those acting earlier on, emerging at later age periods of ADHD (Kuntsi et al., 2005). Age differences in the extent of different aspects of children's behavior and psychopathology will be studied in this project, and so will the hypotheses that from the early childhood into adolescence genetic and environmental factors both contribute to change and stability in the development of children's behavior and psychopathology (Knafo & Plomin, 2006b).

3. What is the relationship between children's cognitive abilities and the development of behavior? For children to behave prosocially, children have to identify others' needs (Pearl et al., 1985). They have to perceive social cues calling for prosocial behavior, and to know how to provide others with help, resources, or support, and therefore, cognitive abilities are important. Several studies have also examined the relationship between measures of cognitive ability and children's psychopathology. The association between these two traits has been found to be stable over time and has been reported in clinical as well as in population based samples (Hollaender et al., 1989; Loney et al., 1998; Manikam et al., 1995). Two general population cohort studies independently found that lower childhood cognitive ability was associated with non-psychotic psychopathology in adulthood (Crow et al., 1995; van Os et al., 1997), with the earliest origin of this association in childhood. In addition, there is work linking lower intelligence to specific childhood psychopathologies such as hyperactivity and delinquency (White et al., 1989; Willcutt et al., 1999). Indeed, general cognitive abilities correlate with children's prosocial behavior moderately (Zahn-Waxler et al., 1982). It is also important to study specific cognitive abilities such as WISC-III subtest profile patterns, such as Coding, Symbol Search which have been shown to be diagnostic utility for children with Attention Deficit Hyperactivity Disorder (Snow & Sapp, 2000). The phenotypic, genetic and environmental relationships between children's behavior and cognitive abilities will be studied. For example, it is possible to investigate whether the relationship

between the cognitive ability of attention and conduct behavior is due to common genetic factors, common environmental factors, or both.

4. What is the role of the social context in the development of children's behavior? Studies on the relationship between children's behavior, psychopathology, socioeconomic status (SES), and parents' psychological health have yielded mixed results (Burbach et al., 2004; Eisenberg et al., 1993; Raviv, 1981). We will address this relationship in our study, using a broad set of indicators of children's behavior psychopathology with self, parents and teachers' reports. Another important social context variable is parenting style. Warm, supportive parenting may increase prosocial behavior by providing a caring model for children, and by increasing children's willingness to attend to parental messages and their accuracy in detecting these messages (Knafo & Schwartz et al., 2003). But the effects of parenting, especially of the harsh and negative kind, can partially reflect the child's genetic tendencies, for example, children's temperament also influences parenting (Knafo & Plomin, 2006b). Using our longitudinal-genetic design we will address the genetic and environmental contributions to the relationships between parenting and children's behavior.

Study Design and Recruitment Process

Twinning Rate in China and Research Population

The average incidence of twinning in China is between 5% to 9% of total births (Yu & Zheng, 2001). Twinning is becoming increasingly common in China, with a 30% increase in multiple births over the last decade (Cui et al., 2007). This is probably due to a combination of increasingly advanced fertility and neonatology technologies and increasing age of child-bearing over recent years, resulting in increased use of fertility drugs and In vitro fertilization (IVF).

The focus area of twin collection is Chengdu and Chongqing cities in Southwest China. According to the Chinese National Bureau of Statistics, there are about 8,316 twin births per year in Chengdu and Chongqing. These two cities are chosen as the center of the study sites for two reasons, the first being that Chengdu and Chongqing have 39,600,000 inhabitants spread over 94,690km of both urban and rural areas, and according to economic indices, these areas are very diverse in sociodemographic terms, and are representative of the Western Chinese population. The second reason is that Chengdu and Chongqing are two key areas under the current Western China Development policy of the Chinese government, and provide a window on the effects of the social and economic change in a developing country.

Study Design

The general design of our project is to conduct a longitudinal cohort survey in 2,000 pairs of child and adolescent twins aged 0 to 16 years old in South West

China. We will collect DNA and data in order to explore the influences of genetic, environmental and psychosocial factors on the individual attributes of children and adolescents regarding behaviors and mental health. Data will also be collected on such attributes as height, weight, pubertal development, and chronic and disabling conditions. Data regarding to various environmental and psychosocial factors will be gathered from parents and schoolteachers in all instance, and from the children aged over 11. However, we would also set up the mechanism for long-term follow-up to facilitate further studies as a prospective twin registry so the numbers of twin pairs are open without limitation.

Families in the study area were chosen for in-person assessments through the schoolteacher and the personnel based at the national network for monitoring Maternal and Child Health. In addition to participating in the questionnaire phase of the study, they would be contacted by phone by the research team to ask for participation and enrollment in a parent-training program periodically on how to nurture the twins. Through this parent education program, the research team can keep a close contact with the twin's family longitudinally; in the meanwhile, the parents of the twins can learn some basic knowledge on twin-specific developmental issues. When participants complete all assessments, children receive rewards for participation, and parents are monetarily compensated for their transportation costs.

We plan to call families every 2 years after the baseline investigation. To maintain contact with families, parents are invited between data collection points to enter the dedicated study website (<http://sbt.hxyx.com/>), to browse basic general study results and information about twins, parenting and child development. A newsletter about twins is another method that will be used to communicate with families.

Recruitment Process

All twin pairs aged between 0 to 16 years old dwelling in Chengdu and Chongqing Cities will be included in the TRiSC. The 1989 to 1999 birth cohorts constitute the first tier of the study. The registry of the twins in the TRiSC comes from two resources; the Chinese birth deficiency surveillance network based at the Municipal Population and Family Planning Commission, and the Municipal School board.

Population-based. According to the Population and Family Planning Commission from five urban districts (Jingjiang district, Jingniu district, Qingyang district, Chenghua district and Wuhou district) in Chengdu, Sichuan province, China, 9,339 possible twins pairs (lived in a same address and have the same date of birth) aged between 0 to 16 years old have been identified (Table 2). Currently, 1,600 letters to parents of twins have been posted to invite participation, among them, 60 pairs of twins replied and 55 pairs joined the study, which shows a very low response rate.

School-based. A list of twins was solicited from the primary and secondary schools in Chengdu and Chongqing. Currently, 65 schools were contacted, in which up to 80% of the schools were willing to participate and provided with twins' names and contact details. Altogether 344 pairs of twins in above schools have been invited to participate, and 255 pairs of twins joined the study.

Measures

Zygoty Determination

The zygoty of the twins is determined by examination of sixteen highly polymorphic DNA markers by using the PowerPlex® 16 System (Promega Corporation). Unlike-sex twins and same-sex twins with at least one different genetic marker were classified as dizygous; twins identical for the sixteen markers were classified as monozygous. After the genetic analysis, a probability of monozygoty of 0.999 was reached (Yang et al., 2006).

Demographic Variables

Parents are asked to provide basic information on demographic and health issues (e.g., perinatal data, birthweight and mode of delivery, for the purpose of screening children with extreme developmental problems). They also report the demographic characteristics of the family regarding SES, religion, family size, and so on. Information about the twins' social settings (e.g., whether they go to preschool or not, and for how long) is also recorded.

Children's Behavior

We assess twins' behavior, including internalizing behavior and externalizing behavior from a multi-informants from the parents, children above the age of 11 and from the teachers in schools. Behavioral problems are assessed using standardized parent-rated scores of the five sub-scales of the 25-item Strengths and Difficulties Questionnaire (Goodman, 1997). The clinical validity of the SDQ has been established in several studies (Goodman et al., 2000; Klasen et al., 2000). DSM-IV childhood onset mental disorders are diagnosed according to a semi-structural diagnostic instrument-Development and Well-Being Assessment (DAWBA) (Goodman et al., 2000). All of the questionnaires have been translated into Chinese and back-translated into English to ensure the validity of the translation, and piloted on an independent sample (Du et al., 2006). Mothers fill out questionnaires including several scales describing each of the twins, focusing on their temperament and psychological problems (Table 1).

Temperament

Temperament is measured by using age-appropriate Carey Temperament Scales, the Revised Infant Temperament Questionnaire (McDevitt & Carey, 1978), the Toddler Temperament Scale (Fullward et al., 1984), and the Carey Temperament Scales (3–7 years) (McDevitt & Carey, 1978). The Chinese version of these temperament scales have been validated, the

Table 1

Questionnaire Measures of Children's Behavioral Development

Scale	Source	Wave 1	Wave 2
Psychological problems			
Emotional problems	SDQ	x	x
Conduct problems	SDQ	x	x
Hyperactivity	SDQ	x	x
Peer problems	SDQ	x	x
Mental Disorders (DSM-IV)	DAWBA	x	—
Prosocial behavior	SDQ	x	x
Temperament	Age-appropriate Carey Temperament Scales	x	x

Note: SDQ: The Strengths and Difficulties Questionnaire; DAWBA: Development and Well-Being Assessment
Wave 1: the baseline investigation; Wave 2: follow-up in 2 years.

internal consistency of temperament dimensions, test-retest reliability, descriptions of means and standard deviations have been examined, and the norms of different ages have been published (Hong et al., 1999; 2001; Zhang et al., 1998), and they are widely used nationally.

Family Environment

Because a major interest of this study is in the behavioral development processes, parents are asked to describe the family environment using the Family Cohesion and Adaptability Scale (FACESII; Chinese-version), it has been tested to have good validity and reliability in Chinese cultural background (Phillips et al., 1998). The perceived levels of family functioning and their relationships with children's behavior are recorded for each twin pair. Parental beliefs and child-rearing attitudes toward each of the twin using the Family Life Questionnaire is used for assessing parental warmth, induction and reasoning, autonomy support, corporal punishment, verbal hostility, directiveness, permissiveness and the level of interaction of child and the parents (Vitolo et al., 2005). As parents' mental health issues also influence the children's psychological well-being, and the General Health Questionnaire (GHQ) is the most popular screening instrument for detecting psychiatric disorders in community samples (Goldberg & Hillier, 1979), and the overall heritability of the GHQ as a measure of psychosocial distress was substantial (44%) (Rijsdijk et al., 2003), therefore, we use GHQ-12 for measuring the psychological status of the twin's parents. In addition, family stressful life events are assessed using the Family Stress Questionnaire (Goodman, 1995). It has been shown to be a brief and informative scale in screening out the family stress in a recent survey of Brazilian schoolchildren ages 7 to 14 years.

Cognitive Abilities

Cognitive abilities of children of 6 to 16 years old are assessed with the WISCIII for 6–14 years. As attention

Table 2

Number of Twins and Multiples Registered in the Chengdu Urban Area

Birth year	Twin pairs <i>N</i> (%)			Twins subtotal	Triplet
	male-male	female-female	opposite-sex		
1989	204(33.7)	234(38.6)	168(27.7)	606	1
1990	206(35.8)	190(33.1)	179(31.1)	575	1
1991	192(34.0)	216(38.3)	156(27.7)	564	2
1992	201(36.2)	212(38.1)	143(25.7)	556	0
1993	214(34.2)	219(35.0)	192(30.7)	625	1
1994	202(35.1)	217(37.7)	156(27.1)	575	4
1995	218(35.7)	217(35.5)	176(28.8)	611	1
1996	226(38.8)	210(36.1)	146(25.1)	582	2
1997	208(36.2)	237(41.3)	129(22.5)	574	2
1998	206(35.0)	236(40.1)	147(25.0)	589	1
1999	196(33.5)	220(37.6)	169(28.9)	585	3
2000	197(34.1)	219(37.8)	163(28.1)	579	3
2001	196(38.9)	180(35.7)	128(25.4)	504	3
2002	172(36.1)	183(38.4)	121(25.4)	476	3
2003	157(37.1)	161(38.1)	105(24.8)	423	5
2004	183(38.1)	199(41.1)	98(20.4)	480	5
2005	144(36.5)	143(36.3)	107(27.2)	394	4
Total	3322(35.7)	3493(37.6)	2483(26.7)	9298	41

deficit and hyperactivity behavior being the most common behavioral problem of childhood, and the WISC-IV is more helpful in diagnosing ADHD than the WISC-III (Mayes et al., 2006), especially on Coding A (B) and Symbol Search A (B) subscale, so Coding A (B) and Symbol Search A (B) subscale of WISC-IV for school children are used for providing additional informations on measurement of attention and working memory. For children of 3–7 yrs, WPPSI (Ottem, 2003) is used; for children of 0 to 3 yrs, Bayley Scales of Infant Development, 2nd ed. (BSID II; Glenn et al., 2001) is used for the information on motor and mental development. The Chinese version of all these scales have been tested and normed, and used extensively in China ever since (Cai et al., 1998; Dai et al., 1998; Tang et al., 1998). The validity and reliability of the Chinese version of all these scales have been described elsewhere (Huang et al., 1998; Chen, 2004).

Current Sample

So far, 9,339 pairs of multiple births children aged between 0–16 years have been identified in Chengdu (Table 2 and Table 3). Among them, 9,298 pairs are twins and 41 pairs are triplets. The detailed information on children's behavior development of 324 pairs has been collected in Chengdu and Chongqing, including the DNA sample of each of the twins. Three hundred and ten pairs of twins are between the age of 6 and 16, 14 pairs of twins are below 2 years old. Of the twins collected between the ages of 6 to 16, detailed information of 255 pairs of twins were from schools.

There are some limitations existing in the process of sample recruitment which may result in the imbalances of the twin-register database. Due to the low response rate at the population-based recruitment process, the majority of the twins in our register are recruited from schools. Unavoidably the school-based

Table 3

The Age and Sex Distribution of Registered Twins in the Chengdu Urban Area

Birth year	Twin pairs <i>N</i> (%)			Twin subtotal	Triplet	Total
	Male–male	Female–female	Opposite-sex			
1989–1993	1017(34.9)	1071(36.5)	838(28.6)	2926	5	2931
1994–1998	1060(36.0)	1117(38.0)	754(25.6)	2931	10	2941
1999–2001	589(35.1)	619(36.9)	460(27.4)	1668	9	1677
2002–2005	656(37.0)	686(38.7)	431(24.3)	1773	17	1790
Total	3322(35.6)	3493(37.4)	2483(26.6)	9298	41	9339

process of recruitment would bring bias to the sampling. Because some of children with severe behavioral and mental problems may not go to school, this may cause underestimation of these problems. In addition, the majority of samples are from urban area which may cause bias to the results of future studies. In order to establish a long-term twin registry, we are planning to adopt a gradual progressive design, for example, at the first stage, school-based process will be used in Chengdu urban areas, and then followed in Chongqing urban areas; at the second stage, the twin registry would be extended to both Chengdu and Chongqing rural areas. Instead of letter invitation by mail, we will also use epidemiological randomized sampling methods. For example, home visits may be adopted as it may be more effective for recruitment in the rural areas. For long-term follow up of twin studies, the database in the twin registry will be run under regular management and monitoring continuously.

Additional Research Questions and Potential for Collaboration

The data collected in the TRiSC are expected to provide answers for other important developmental questions regarding children's behavior and other traits of interest. For example, there is evidence that greater heritability of depression seen in adolescence is due to an increase in gene-environment correlation involving negative life events (Russell et al., 2003). Our data will enable an investigation of the extent to which genetic factors are responsible for this association.

The study will lay the foundation for further research after 16 years of age. As data collection continues, it is likely that new research questions will arise, for example, primarily, we will seek to incorporate a molecular genetic aspect within this study. In addition to identifying DNA markers relevant to prosocial behavior, we hope this will constitute a valuable source of information on the genetic influences on children's behavior and psychopathology in other domains. It will also provide a framework for collaboration with researchers from related fields and from other countries interested in children's personality, social, and cognitive development.

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