Twin Research and Human Genetics
Volume 16 ▌ Number 1 ▌ pp. 371–375 ▌ © The Authors 2012 ▌ doi:10.1017/thg.2012.69

The Southern Illinois Twins and Siblings Study (SITSS): Description and Update

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This update on the Southern Illinois Twins and Siblings Study (SITSS) documents some of the follow-up studies that have been conducted and results that have been obtained from this sample. At the current time, 283 twin pairs, 8 triplet families, 98 non-twin sibling pairs, and 287 singletons have been enrolled in SITSS. Twins and triplets are tested as young as 1 year of age and then every year on their birthday through age 5 years. A variety of follow-up studies have been conducted for SITSS children through age 20. Results thus far have demonstrated significant genetic influences on social behaviors such as aggression, victimization, and attention toward facial expressions. Interesting interactions have been documented between the dopamine receptor D4 gene (DRD4) and the social environment (parental sensitivity or peer aggression) as they predict children's aggressive behaviors. In addition, increased difficulty with social interactions has been noted for twins versus singletons. Thus, this multi-trait, multi-method behavior genetic data set contributes to our understanding of the etiology of social behaviors in preschoolers and to predictors of similar behaviors through adolescence.

■ **Keywords:** longitudinal, twins, childhood, adolescence, externalizing, internalizing

The Southern Illinois Twins and Siblings Study (SITSS) is a longitudinal study of young children that examines genetic and environmental effects on early child social development. Initiated in 1993 by Lisabeth DiLalla and first described in DiLalla (2002a), the SITSS is a small yet comprehensive study that explores the areas of early childhood aggression, bullying and victimization, pro-social behaviors, parent–child interactions, and other related constructs. The original 717 twins, siblings, and singletons were tested at age 5. To increase enrollment, twin testing was expanded to include twins at ages 1–4 years. Currently, there are 283 twin pairs (31% monozygotic (MZ), 41% dizygotic (DZ), and 28% opposite-sex dizygotic (OSDZ)), 8 triplet families (6 MZ pairs, 10 DZ pairs, and 8 OSDZ pairs), 98 non-twin sibling pairs, and 287 singletons enrolled. Families in SITSS are relatively well representative of southern Illinois and surrounding regions, although because of the nature of the volunteer sample it is not very racially diverse: 93% Caucasian, 3% African American, and 4% other. Families are from southern Illinois and nearby Missouri and Kentucky, and occasionally farther (e.g., Tennessee, Arkansas) if they move after already enrolling but come back to town for testing.

Rationale for Studies

Although genetic factors have been implicated for some early childhood social behaviors, little is known about the etiology of emotional intelligence, social perspective-taking, and prosocial and aggressive behaviors in preschoolers (DiLalla, 2002b). For example, peer victimization appears heritable in older children and adolescents (Ball et al., 2008), but has not been studied in younger children.

Most interesting is to ask the question of *how* genes influence behaviors, both in terms of neurotransmitter activity in the brain and how genes and environments correlate and interact. Molecular genetic studies have begun to identify particular genes associated with interpersonal, aggressive, and emotional behaviors. For example, the dopamine receptor D4 gene (DRD4) appears to be involved in cognitive and emotional behaviors, including novelty-seeking (e.g., Kluger et al., 2002) and emotional reactivity (Oniszczenko & Dragan, 2005). Serotonin is implicated in mood disturbances; deficits may lead to problems with mood, impulsivity, and aggression in animals and adult humans, but less is known about children (Beitchman et al., 2006). Also,

RECEIVED 23 July 2012; ACCEPTED 2 August 2012. First published online 9 October 2012.

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TABLE 1

Specific SITSS Measures Administered to Various Sub-Samples of Twins, Siblings, and Singletons

Construct	Age (years)	Measures	Method
Cognitive			
General	1–2	Piagetian A-not-B error; self-awareness	Lab tests
	3–4	Counting tasks; hierarchical classification; Stanford–Binet Memory sub-test	Lab tests
Theory of mind	3–4	False-belief tasks	Lab tests
	6–10	False-belief tasks plus new step assessing understanding of non-literal language	Lab tests
Self-concept	5	Harter's Self-Concept Pictorial Scales (Harter & Pike, 1984)	Lab tests
Fantasy play	1–4	Convergent imitation (e.g., pretend to talk on toy telephone) and divergent imitation (e.g., pretend to use plastic banana as phone)	Lab tests
Relationships with others			
Parent–child interaction	1–5	Mother teaches children to sort objects or learn a puzzle task, dependent on age	Lab test
Bullying/victimization	6–17	Multidimensional Peer-Victimization and Bullying Scale (Mynard & Joseph, 2000)	Lab test; mail survey
Peer relations	5	Child plays with an unfamiliar child, same sex and age, for 20 min; Rosenzweig Picture Frustration task with newly developed coding scheme Gheyara et al., 2012; Rosenzweig et al., 1948	Lab tests
	6–16	Self-report on relations with co-twin, siblings, peers; Parent Checklist of Peer Relationships, Teacher Checklist of Peer Relationships (both from Dodge, 1986)	Lab tests; mail survey
Emotional/behavioral	5	Parent-report Child-Behavior Checklist (CBCL; Achenbach & Rescorla, 2001)	MO/LT[a]
	5–16	Parent and self-report Strengths and Difficulties Questionnaire (Goodman, 1997)	Lab test; mail survey
	6–16	Youth Self-Report form (YSR; Achenbach & Rescorla, 2001); CBCL	Lab test
	10–16	Teacher Report Form (TRF; Achenbach & Rescorla, 2001)	Mail survey
Home/social environment			
Family environment	1–5	Confusion, Hubbub, and Order Scale (CHAOS; Matheny et al., 1995)	MO/LT[a]
	5	Family Climate Inventory (Kurdek, et al., 1995) modified for parent report	MO/LT[a]
Family information	1–20	Parents complete a family information sheet (SES; parental education, etc.)	MO/LT[a]
Daycare information	5	Parents complete a Daycare history form	MO/LT[a]
Parental discipline	5–10	Parent-report Parenting Styles and Dimensions Questionnaire (Robinson et al., 2001), Parental Discipline Questionnaire (Bell, 2006); child-reported Parent Perception Inventory (Hazzard et al., 1983)	Lab tests
Temperament/personality			
Child temperament	1–4	Temperament measures: Infant Behavior Questionnaire (Rothbart, 1981) at age 1; Early Child Behavior Questionnaire (Putnam et al., 2006) at ages 2 and 3; Child Behavior Questionnaire (Rothbart et al., 2001) at age 4	MO/LT[a]
	5	Behavioral Style Questionnaire (McDevitt & Carey, 1978)	MO/LT[a]
Parent personality		Saucier's Adjective Checklist (Saucier, 1994), Multidimensional Personality Questionnaire Aggression scale (Tellegen & Waller, 1994)	MO/LT[a]
Biological	First visit	Parents fill out a birth complications form	MO/LT[a]
	1–5	Zygosity parent and rater form (taken from Nichols & Bilbro, 1966); buccal samples for DNA	MO/LT[a]; lab test

Note: [a]Mailed out, then turned in when children are brought to laboratory for testing.

individuals with serotonin-transporter-linked polymorphic region (5-HTTLPR) short alleles have greater activation of the amygdala when they view facial expressions of negative affect, which is associated with social behaviors (Hariri & Holmes, 2006). Thus, we are beginning to understand some of the underlying mechanisms for genetic associations with social behaviors.

The SITSS integrates biological and environmental perspectives in investigating the causes of young children's social development. The early focus was on heritability, but has grown into inclusion of genotype and a number of developmental and environmental variables, such as daycare experience, parent–child interaction styles, and cognitive ability.

Overall Methodology

Twins are recruited from newspaper birth announcements, mothers of twins clubs, and referrals from other families in the study. Preschool twins aged 1–5 years are invited to our campus laboratory for testing every year within approximately a month of their birthday. The general protocol was described in DiLalla (2002a). Testing consists of laboratory tasks as well as mailed questionnaires assessing home environment, birth complications, temperament, and behavior problems. At age 5, testing was completed using the peer play paradigm that was the original basis for SITSS, and instead twins now are tested on a variety of tasks designed to assess interpersonal functioning. Table 1 presents a summary of all SITSS measures.

Follow-up studies on subsets of the SITSS families have been conducted at various intervals. Perhaps most importantly, buccal cell collection (cheeks and gums are rubbed gently for 20 s three times during the test session) began in 2003 and DNA samples (both for specific genes and to assess zygosity) are now available for 149 twin pairs and 6 triplet sets. These data are stored and analyzed by Dr. Andrew Smolen at the Institute for Behavioral Genetics, University of Colorado, and have been used to confirm zygosity status (94.4% agreement between DNA and parent and tester ratings for same-sex pairs). Confirmation of zygosity was determined using a panel of 12 highly polymorphic loci (see http://ibgwww.colorado.edu/genotyping˙lab). We also have genotyped most of these children for several genes that have been shown to have some relation in other

https://doi.org/10.1017/thg.2012.69 Published online by Cambridge University Press

samples to the behaviors in which we are primarily interested (aggression, emotions related to prosocial and negative behaviors), specifically the dopamine receptor genes for D4 (DRD4) and D2 (DRD2), the serotonin-transporter-linked polymorphism (5HTTLPR), and two single-nucleotide polymorphism (SNPs) of the muscarinic cholinergic receptor 2 gene (CHRM2-rs36210735 and CHRM2-rs1824024).

Several follow-up studies have utilized mailed-out questionnaires and telephone interviews or have involved bringing families back to the laboratory when the children are older. Studies have examined victimization, bullying, psychopathology symptoms, health behaviors, and parenting. Each follow-up study also contains extensive information on temperament, family environment, and child behaviors. Currently, we have begun a follow-up study of 6- to 10-year-old twins and triplets that will involve in-depth interviews on emotion skills, theory of mind, and interpersonal functioning, including victimization and bullying.

Current Findings and Implications

We have demonstrated significant genetic influences on early social behaviors, including being victimized, in 20-min novel peer interactions in children as young as age 5 (Gheyara & DiLalla, 2012). Moreover, observed children's behaviors, rather than parent-reported measures of aggressive tendencies or temperament, were predictive of victimization, further supporting an evocative gene–environment correlation hypothesis. In addition, difficult temperament and chaotic family environment predicted social problems during the novel peer play interactions (DiLalla, 2008). Interestingly, a warm home environment, but not child temperament, was predictive of greater prosocial behaviors. We also conducted a small study on attention toward facial expressions in a subset of 5-year-old twins and found it to be heritable. The overall trend of children to preferentially allocate attention to happy and fearful faces is commensurate with theories that such attentional biases are evolutionarily adaptive (Elam et al., 2010). We have not yet examined the relations between facial attention, emotion recognition skills, and social behaviors, but this is a current goal of SITSS.

Other SITSS studies have examined specific genotypes as they relate to externalizing behavior in young children. We have shown that children with the DRD4-L allele displayed more aggression with their siblings or peers when exposed to low levels of parental sensitivity or high levels of peer aggression. They were also more likely to share less with their twins and evoke less sensitivity from their parents during family interactions (DiLalla et al., 2009b). Similarly, those children with the DRD4-L allele and lower parental sensitivity were more likely to display conduct problems (DiLalla et al., 2011). Moreover, children with the risk allele for the 5HTT gene were more likely to display conduct problems, regardless of the level of parental sensitivity (DiLalla et al., 2011).

In addition to twins, the SITSS sample also includes other sibling pairs and singleton children, allowing for comparisons with twins. At age 5, twins tended to be less prosocial, but no more aggressive, than singletons. Conversely, when the children were examined 5–10 years later, twins were not less prosocial but were more aggressive than singletons (DiLalla, 2006). We also hypothesized that MZ twins would be more inhibited with strange peers because they are used to being with children who are genetically and phenotypically similar to themselves. Instead, MZ and DZ twins did not differ, but DZ twins were more inhibited than non-twin siblings (DiLalla & Caraway, 2004). Thus, some twins may demonstrate deficits in social interaction when compared to non-twin or singleton children in both early and middle childhood.

Finally, the longitudinal nature of the SITSS sample provides an opportunity to examine genetic and environmental influences throughout childhood and adolescence. We examined parent-rated preschool rule-breaking/delinquent behaviors and self-reported delinquency at adolescence and found that delinquent behaviors were related to concurrent social problems at both ages, but preschool rule-breaking was not predictive of future delinquency in adolescence (DiLalla et al., 2009a). We also have demonstrated that chronic victimization from age 5 through late adolescence was related to adolescent physical health problems and conduct problems but not to reported emotional problems (Biebl et al., 2011).

Conclusion

Thus far, we have demonstrated genetic effects on a range of social behaviors from preschool through adolescence, as well as documenting important differences between twins and singletons. In addition to our new 6- to 10-year-old follow-up study, testing of 1- to 5-year-olds continues. These analyses will further our understanding of both genetic and environmental effects on children's social and cognitive development with their parents and peers.

Acknowledgments

We owe a debt of gratitude to Dr. Andrew Smolen, who has collaborated with us on all the molecular genetics and zygosity work. This project has been funded by monies to the first author from SIUC Special Research Project Grants, SIU School of Medicine CRC Grants, an SIU Center for Integrated Research in Cognitive and Neural Sciences grant, a grant from the SIU School of Medicine RSG, and the SIU School of Medicine's Department of Family and Community Medicine. Special thanks go to Bo Bishop, Lorrie Gehlbach, Brad Gehlbach, Paula Mullineaux, Kit Elam, Kristin Hoffman, Sara Biebl, Sarah Long, Rashida Barner, and Jaimi Mathias for devoted work to SITSS. Of course, very special thanks go to the families of SITSS.

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https://doi.org/10.1017/thg.2012.69 Published online by Cambridge University Press