
Colorado Twin Registry

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The Colorado Twin Registry (CTR) is a population-based registry housed at the Institute for Behavioral Genetics, University of Colorado. Recruited subjects' birth years date from 1968. Four samples comprise the CTR: the Community Twin Sample, Infant Twin Sample, Longitudinal Twin Sample, and the Early Reading Development Sample. Criteria for enrollment, recruitment strategies, demographic information and zygosity assignment are explained for each sample. In addition, 8 studies in which CTR twins have participated are highlighted. These include studies of early cognition, early reading ability, executive cognitive function, and vulnerability to substance abuse and antisocial behavior. Goals, measures, and brief results are provided for each study. The development of the CTR is an ongoing and evolving process, and it has proved to be a valuable resource, relatively representative of the population from which it was drawn.

The Colorado Twin Registry (CTR) is a population-based registry housed at the Institute for Behavioral Genetics (IBG), University of Colorado. Twins born within the state between 1968 and 2004 (recruitment is ongoing for twins born subsequently) were recruited into the registry with the assistance of the Colorado Department of Health, Division of Vital Statistics (CDH). Supplemental recruitment into the registry has also occurred with the assistance of participating school districts within Colorado. Prior to October 2000, recruitment was facilitated by access to Division of Motor Vehicle (DMV) address data banks. In some cases, twins who migrated into Colorado were also enrolled into the CTR.

Four samples comprise the CTR: the Community Twin Sample (CTS), Infant Twin Sample (ITS), Longitudinal Twin Sample (LTS), and the Early Reading Development Sample (ERDS). Of these, only the CTS is based on twin pairs from the entire state of Colorado. An additional twin sample participates in the Colorado Learning Disabilities Research Center (CLDRC) study. Although these twins were selected using specific criteria related to learning disabilities, about 30% of the CLDRC twins are also members of the CTR and participate in its studies. From these samples, participants for a number of specific studies have been recruited. Section one describes the four samples; the following section describes the studies.

CTR Samples

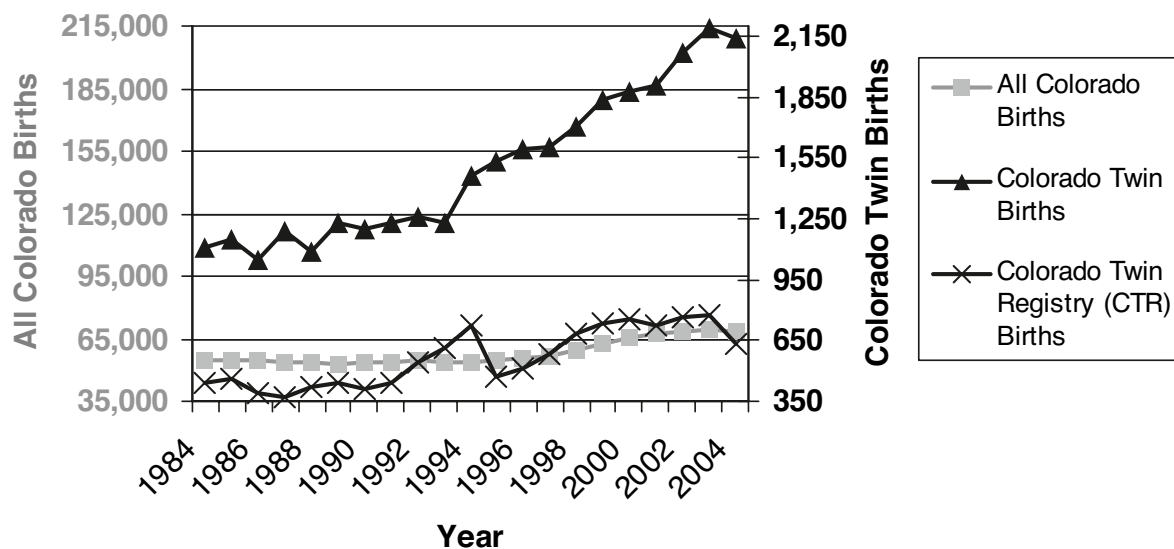
Community Twin Sample

Participation in the CTS is open to all twins born in the state of Colorado as well as additional twins who attended Colorado primary or secondary schools. In 1984 the CDH began mailing inquiry letters on behalf of IBG to parents of twins born from 1982 forward. More than a decade later, in 1997 and 1998, this process was extended to twins born from 1968 to 1981. Letters were not mailed to parents for whom CDH matched twin death records. Birth record data including names and contact information were made available to IBG staff for all respondents who returned a postage-paid reply card authorizing the CDH to release that data. The CDH sent a second mailing to nonrespondents and attempted to collect new contact information but these procedures may not have been systematic and thorough in all years. In 1999, the Health Department revised this procedure and initiated a 'negative consent' process, that is, data were released for twin births unless the parents returned a card specifically prohibiting the CDH from doing so. Though nonresponse was no longer an issue, the CDH did not release information if the inquiry letter was returned undeliverable and instead attempted to update contact information and post a second inquiry. In the CTS, the time between birth and recruitment varied considerably, ranging from 30 years later for older twins to as little as 3 months for twins who were recruited during the enrollment period for the Twin Infant Project (TIP) described below.

In 1993, IBG began working with the Colorado Department of Education to supplement the CTR. Although supported by the State Superintendent of Education, school districts and their schools independently chose whether or not to participate. Districts or schools initially provided IBG with lists of putative twins to whom enrollment inquiries were subsequently mailed. In other cases the district or school mailed the letters on behalf of IBG and then provided contact information from interested respondents. Periodic updates of school mailing occurred through

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**Figure 1****Colorado births.**

Note: There is a relatively modest increase from about 54,000 births per year in 1984 to about 68,000 in 2004 compared to a near doubling of twin births across the same period (all births are scaled on the left in 10,000s, twin births on the right in 100s). CTR enrollment represents about one third of the available twin births, with a slight drop-off in later years when there were no projects enrolling new subjects from the registry.

2003 with 170 of 176 districts eventually assisting our efforts at some level. The majority of subjects who enrolled via school mailings were either already in our registry or had previously been targeted by the CDH but had not responded (typically due to incorrect contact information). However, schools provided contacts for an additional 1134 pairs, representing both in-migration and families for whom the school information was insufficient for matching to birth records.

For both types of recruitment, subsequent to receiving contact information, IBG mailed a twin registry form to the parents and twins. Information on this form was basic, consisting primarily of a demographic survey, and designed to facilitate further contact with the twins as studies became available. During the years when enrollment into specific studies (detailed below) was underway, repeated mailings and phone calls were made to families who met criteria for those studies, resulting in higher levels of enrollment at those times.

Zygosity was determined for the CDH twins from parent ratings on a zygosity questionnaire based on Nichols and Bilbro (1966). For twins enrolled through the school system, zygosity was tentatively assigned based on two questions: how frequently are the twins mistaken for each other by people who know them, and are they 'as alike as two peas in a pod?' For twins subsequently seen in-person DNA has been collected and zygosity confirmed by twin concordance among a minimum of 11 highly informative short tandem repeat (STR) polymorphisms.

Figures 1 through 5 provide information on the number of births and parents' age, education, and

race/ethnicity for all Colorado births, twin births, and those enrolled into the CTR via CDH for the years 1984 to 2004. The figures are not inclusive for all years of enrollment because CDH records prior to 1975 are not available electronically and demographic data are not systematically available for CTR enrollees prior to 1984. These figures portray statewide and sample statistics only for twins who entered the CTR through CDH mailings as there is no way to ascertain the number or characteristics of the population from which the school enrollment was drawn.

As shown in Figure 2, twin parents are somewhat older than the Colorado population of all births. This difference is small in the earlier years but increased across the 21-year period. However, parental age in the CTR sample is about one-quarter standard deviation greater than parental age in the overall twin population from which it was drawn.

Figure 3 demonstrates that in terms of parents' education, in the earlier years, twin families are not substantially different from the Colorado population of all births, and our enrolled twin families are only slightly elevated in educational status than the population from which they were drawn. However, in later years, as the number of twin births and parental age increased, educational attainment for our sample increased as well.

Figure 4 summarizes the distribution for five categories of race/ethnicity summed across the 21-year period. The state population of Colorado is predominantly White/non-Hispanic with twin births slightly more so and the CTR sample yet more so. Changes across time for the two largest groups are shown in

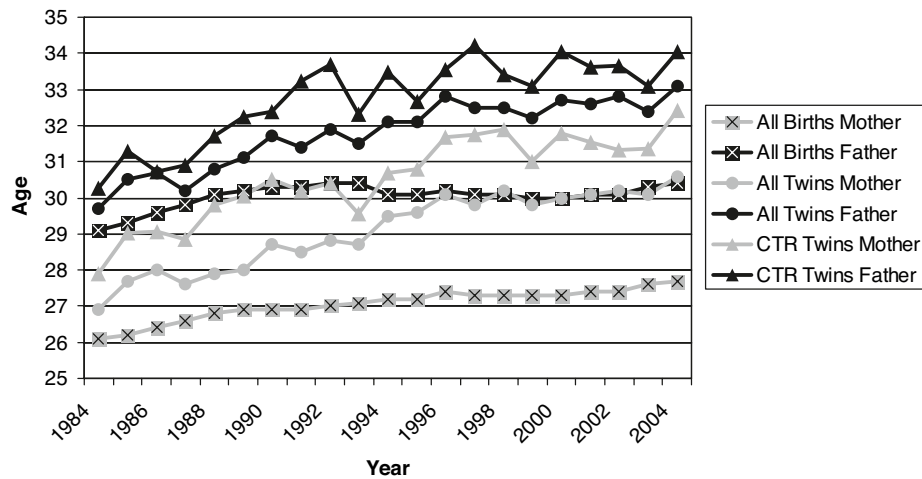


Figure 2

Mean ages for Colorado parents.

Note: Variability is similar for all three groups of parents but increases over time along with the increase in parental age, with standard deviations ranging from a low of 4.3 in 1985 (CTR mothers) to a high of 7.3 in 2003 (all fathers).

Figure 5 which demonstrates that Hispanic births have increased markedly since about 1994 and that up until that time the CTR was only slightly less diverse than the population from which it was drawn.

As described below, several of the samples drawn from the CTR include only subjects who live within driving distance of IBG. Therefore comparisons between the birth statistics for the entire state and these Front Range counties were conducted and the patterns for these demographic characteristics were the same for both groups. Specific details and tables for these characteristics may be found at ibgwww.colorado.edu/ctr.html

Infant Twin Sample

Recruitment for the ITS followed the same pattern as CDH recruitment into the CTS. Unlike the CTS, however, only same-sex twins born between 1984 and 1990 who were interested in participating in the TIP (described below) were enrolled into this sample. Additional criteria included married parents who resided within a 2-hour drive (~ 200 km) of IBG, birthweights of at least 2000 grams for both twins, gestational age of at least 34 weeks, and no evidence of abnormality. Fewer than half of the twin births met these criteria and further reduction resulted from the necessity of responding to inquiries in time to commence testing at 7 months

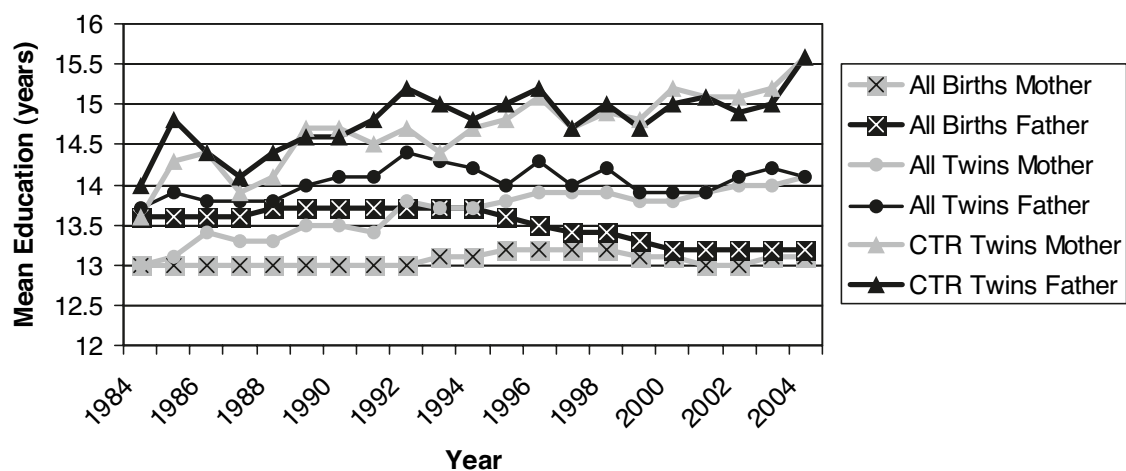


Figure 3

Mean education for Colorado parents.

Note: Prior to 1989, the Colorado Health Department capped their records of educational attainment at 17 years (representing less than 5% of the records). During these years, parents with more than 17 years of education were recorded as 17. Beginning in 1989 this cap was removed, slightly skewing mean education towards a lower value prior to 1989. Variability is similar for both genders and has remained relatively stable though increasing slightly over time, with standard deviations ranging from a low of 2.0 in 1989 (CTR twin mothers) to a high of 3.5 in 2004 (all twin fathers).

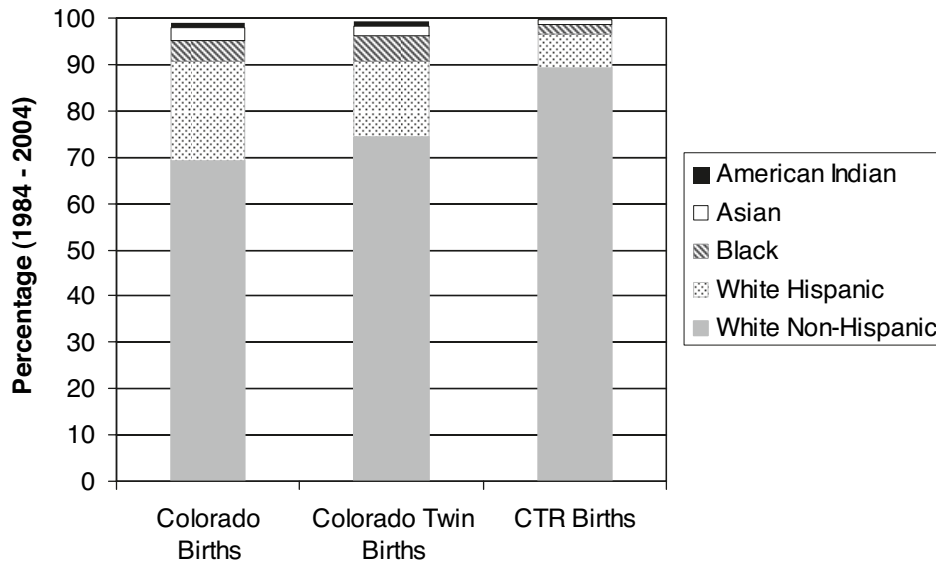


Figure 4

Maternal ethnicity for Colorado births.

Note: CDH reports ethnicity using these categories and for mothers only. These data reflect the sums of births across this time period.

during the first half of the project and by 5 months for the second half. Nonetheless, the 386 enrolled twin families (191 male–male twin pairs, 195 female–female twin pairs) appeared to be representative of Colorado births, though with somewhat more non-Hispanic White parents (about 90% as compared to about 80% for all births).

Zygoty was determined using tester ratings of a modified version of the Nichols and Bilbro questionnaire (1966) with a requirement that subjects complete at least two test sessions and receive 100% agreement from these four ratings.

Longitudinal Twin Sample

The LTS overlaps a great deal with the ITS. When the LTS was initiated in 1986, an attempt was made to recruit a separate sample of twins from CDH mailings. Despite using slightly broader criteria — married parents were not required, the driving distance was expanded to a 3-hour range (~ 300 km), the twins’ weight could be as low as 1000 grams (though 96% weighed 1700 grams or more), and the testing series that began at 14 months — there were not enough twin families available to meet the recruitment needs for both the LTS and ITS indepen-

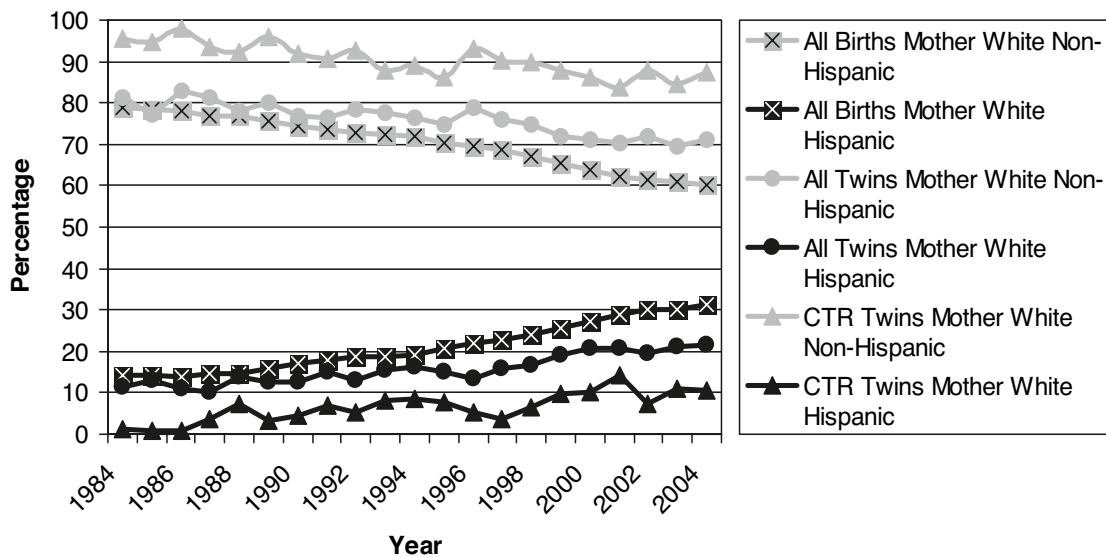
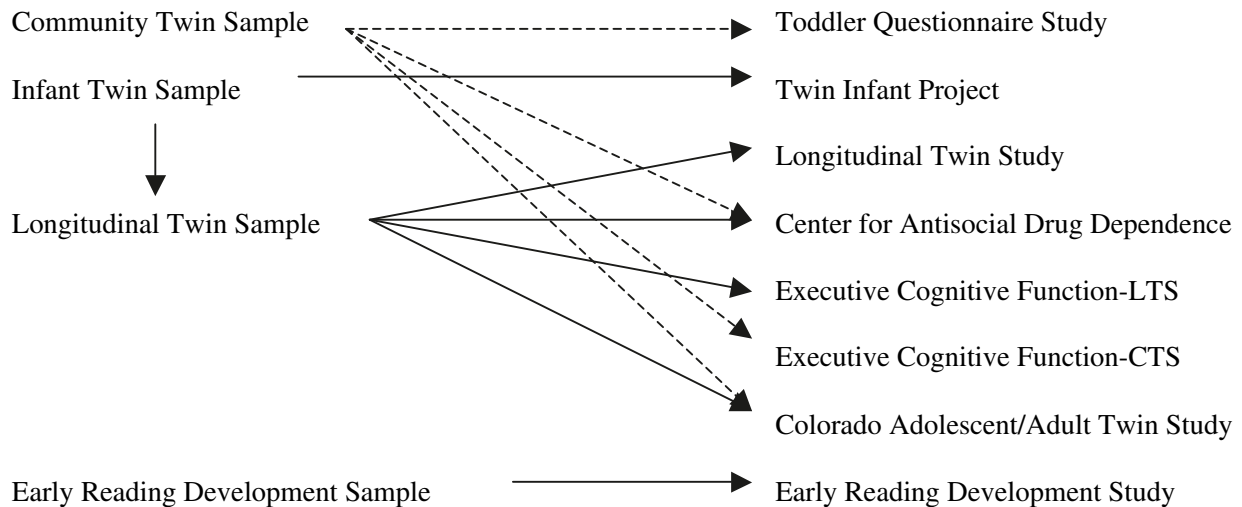


Figure 5

Percentage of White Non-Hispanic and White Hispanic Colorado mothers.

**Figure 6**

Links among samples and studies.

dently. Therefore, members of the ITS born from 1986 through its conclusion in 1990 were invited to participate in the LTS. A total of 244 infant twin families elected to do so. An additional 180 twins were enrolled independently into the LTS.

A separate group of 59 twin pairs from the ITS born prior to the LTS 14-month testing series were tested on protocols used by the Colorado Adoption Project (CAP) at IBG. The CAP protocols overlapped in some domains, notably cognition and personality; thus, data from these 108 subjects have been included with some LTS analyses at ages 1 to 3 years. At ages 4 and 7 years, all of the protocols of this separate ITS/CAP group were a subset of the longer protocols used in LTS. From age 9 and older, the protocols were identical which resulted in a merging of these ITS/CAP twins into the LTS sample. Thus the number of families who have participated in LTS is 483 (240 male–male twin pairs, 243 female–female twin pairs). Attrition has been low in this sample; at age 14, the age through which the entire cohort has passed, 412 families remain active. Since two thirds of the LTS originated in the ITS, it is not surprising that the demographics are quite similar, with the exception of more unmarried parents (approximately 8%).

Zygosity status of the LTS twins was established using a modified version of the Nichols and Bilbro questionnaire (1966). Assignment was initially based on having 85% agreement from a minimum of four raters. Subsequently these assignments were confirmed using 11 STR markers for the 92% of the LTS sample from whom a buccal cell sample had been obtained.

Early Reading Development Sample

In 2000, parents of 4-year-old same-sex twins with no known abnormalities who lived within a 2-hour driving radius of Boulder were invited to join the ERDS. Most of these twins had previously enrolled in the CTR, but

to fulfill recruitment needs, some qualifying families who had not returned the demographic questionnaire to IBG were enrolled directly. Recruitment continued through 2005 when 489 families (243 male–male twin pairs, 246 female–female twin pairs) had been enrolled. Zygosity in the ERDS has been determined from genotyping 11 independent STR markers.

From Samples to Studies

As shown in Figure 6, participants in each of the CTR samples may have participated in one or more projects. Also, some of them have participated, or will participate, in the CLDRC, a nonregistry study described elsewhere (DeFries et al., 1997).

Studies

The following descriptions include information about measures used, general goals of each study, and examples of study results.

Toddler Questionnaire Study

This was the first, and in a sense the feasibility, study for the CTR. In 1986 questionnaires were mailed to mothers of young CTS subjects born from 1981 to 1985. Mothers of twins born in 1984 and 1985 who did not qualify for or did not respond to the ITS were invited to participate. Thus, this sample included opposite-sex twins, those who lived throughout the state, and low birthweight twins. Over 300 twin families, representing about 20% of the twin births during this time period, participated. The questionnaire packets included items regarding demographics, health history, temperament scales and the Child Behavioral Checklist (CBCL; Achenbach et al., 1987) for ages 2–3. In 1992, a second packet containing similar items but using some items from the CBCL/4–16 as well as the CBCL/2–3 was mailed to participating families. A

third mailing in 1997 asked the mother, the teacher, and twins of age 11 and above to complete the behavior questionnaires (CBCL/4-18, TRF, and YSR, respectively; Achenbach, 1991a, 1991b, 1991c).

In an analysis conducted on the first wave of questionnaires, Cyphers et al. (1990) found high estimates of heritability (44% to 65%) for eight of nine temperament scales. In a separate study by Schmitz et al. (1995), shared environmental influences on a variety of problem behaviors reported by parents on the CBCL/2-3 were found to be more important in early childhood than in middle childhood. Conversely, genetic influences increased in importance from early to middle childhood.

Twin Infant Project

The TIP, for which the ITS was recruited, developed and utilized the midtwin–midparent design as an alternative to traditional longitudinal twin study design (DiLalla et al., 1990). This experimental design used a regression of the average twin (midtwin) IQ score on average parental (midparent) IQ as the best estimate of the infants' mature IQ. A battery of measures given to infants at ages 5, 7, 8 and 9 months included the Fagan Test of Infant Intelligence (Fagan & Shepard, 1986), the Visual Expectation Paradigm (Canfield & Haith, 1991), an auditory discrimination task, items from the Bayley Scales of Infant Development (Bayley, 1969), Modified Infant Behavior Record (M-IBR; Bayley, 1969) and Sequenced Inventory of Communicative Development (Hedrick et al., 1975). Research on infant predictors of adult IQ (Benson et al., 1993) found correlations between specific cognitive processes in infancy and later intellectual development. Specifically, visual processing speed, attention, and recognition memory were shown to be reliable predictors of parental IQ.

Longitudinal Twin Study

The LTS, a continuation of the original MacArthur Longitudinal Twin Study, is an ongoing, prospective study designed to investigate the genetic and environmental influences on individual differences in psychological development. Information on cognition, temperament, social competence, family relations, and behavioral problems has been collected almost yearly since the participants were 14 months old. Additional observations within the home environment were collected from the twins' parents, while teachers rated the twins in the school environment, often using highly similar questionnaires to those completed by parents. Recruitment, measures, and analyses through early childhood are described in *Infancy to Early Childhood* (Emde & Hewitt, 2001).

A primary focus of the LTS has been the investigation of sources of continuity and change over both short-term and longer-term intervals. In a study of infant cognitive development, Cherny et al. (2001) found that the observed continuity in general cognitive development from 14 to 36 months can be attributed

to both genetic and shared environmental influences. New genetic variation appeared at 24 and 36 months, but not at 20 months, indicating that general cognitive ability undergoes developmental changes from 14 to 36 months. A subsequent combined LTS and CAP study of cognitive development from ages 1 to 12 years (Bishop et al., 2003) found that during transitions to adolescence, genetic factors no longer contribute to change, just to continuity. Recently, Haberstick et al. (2005) conducted a study of internalizing and externalizing problem behavior based on teacher observations of twins at ages 7, 8, 9, 10, 11 and 12. Findings implicated genetic influences on stability of both externalizing and internalizing problem behavior over all 6 age points. Both heritable and environmental factors contributed to changes in problem behaviors at school.

Center for Antisocial Drug Dependence

The components of this center include studies of samples selected for substance problems as well as community samples of adoptees and twins. The goal of the twin component of the Center for Antisocial Drug Dependence (CADD) is to use a twin study to understand how genes and environmental influences contribute to vulnerability to comorbid drug abuse and antisocial behavior. All of the participants of the LTS described above were invited to participate at approximately age 12. In addition to those from LTS, 1002 twin pairs were enrolled from the CTS. The selection criteria for twins in the CTS included: being between 12 and 18 years of age and having no known abnormalities that would prevent participation, including an inability to provide meaningful consent. Of special note is that unlike other in-person studies at IBG, opposite-sex twin pairs and a sibling in the same age range were included in the CADD. Appropriate subjects in CTS were contacted and continuously enrolled until the target of 1000 pairs was reached. Both the LTS and CTS samples are currently enrolled in a 5-year follow-up to the original study.

Similar to the LTS, zygosity status for the CTS members of the CADD was determined by responses on a modified Nichols and Bilbro (1966) questionnaire and laboratory-based analyses of 11 independent STR markers. As is common in twin studies, monozygotic (MZ) pairs and females are overrepresented with 46% MZ, 26.5% same-sex dizygotic (DZ), and 27.5% opposite-sex DZ (334 male–male pairs, 393 female–female pairs, 275 male–female pairs).

An early paper from the CADD study examined the risk factors that precede problematic substance use. Young et al. (2000) identified a heritable 'behavioral disinhibition' phenotype that may underlie the comorbidity between substance experimentation, novelty seeking, and symptom counts for conduct disorder (CD) and attention-deficit/hyperactivity disorder (ADHD). More recently, Rhee et al. (2003) found moderate to substantial genetic influences and

modest to moderate shared environmental influences on substance initiation, use, and problem use. Problem use was more heritable than initiation. The significance of environmental influences shared only by twin pairs on tobacco initiation, alcohol use, and any drug use suggested the influences of peers, accessibility of substances, and sibling interaction.

Executive Cognitive Function (ECF) — LTS

The ECF-LTS was designed to examine the genetic and environmental contributions to executive cognitive functioning. The potential participation of all members of the LTS sample (325 pairs had participated through May 31, 2006; participation of an additional 82 pairs is anticipated) gives this study a unique longitudinal perspective on general and specific cognitive abilities. The focus of this study is on the three best-supported components of executive function: shifting of mental sets (shifting), updating information in working memory (updating), and inhibition of predominant responses (inhibition). Recently, Friedman et al. (2006) examined the relations of fluid intelligence, crystallized intelligence, and the Wechsler Adult Intelligence Scale IQ to inhibiting, shifting, and updating. Of these three executive functions, only updating was highly correlated with measures of IQ. The finding that these three ECF components are differentially related to intelligence measures suggests that current measures of IQ do not equally assess a wide range of executive control abilities.

Executive Cognitive Function — CTS

Similar, but not identical, measures of ECF have also been concurrently collected in the ECF-CTS during the 5-year CADD follow-up. The principal difference from the LTS-based study is that this study was designed to investigate the relationship between ECF components and the development of behavioral disorders such as CD, ADHD, and comorbid substance-use problems. Therefore, 300 twin pairs were selected from the CTS CADD cohort based on their responses to the first CADD assessment, with at least one member of the pair reported having symptoms of one or more of these disorders. A small number of control subjects (e.g., those in which both members of the twin pair report no symptoms) will also be tested.

Preliminary analyses show strong evidence for the heritability of primary ECF constructs. Future research plans include the identification of ECF deficits that may partially explain the overlap between childhood disruptive disorders, and others that may help discriminate among them.

Colorado Adolescent/Adult Twin Study

A primary goal of the Colorado Adolescent/Adult Twin Study (CATS) was to develop an instrument that predicted experimental and regular use of alcohol and drugs in a community sample. A secondary goal was to assess the feasibility of recruiting a sample from older birth records. Subjects were members of the CTS across the birth years 1968 to 1990; recently members

of the LTS have been invited to participate as well (through June 15, 2006: 315 male–male, 519 female–female, 187 male–female).

Adolescent and adult subjects were mailed a questionnaire booklet regarding demographics, health history, personality, interests, and a modified version of the Drug Use Screening Inventory (DUSI; Tarter, 1990). Siewert et al. (2004) developed six subscales of the DUSI that characterize the common behavioral problems of adolescence: conduct problems, hyperactivity, low self-esteem, neuroticism, social withdrawal, and school problems. In a related study (Siewert et al., 2003) these six problem behaviors were divided into two categories: internalizing or externalizing behaviors. Analysis showed that there were unique genetic influences on each of the two categories, suggesting distinct patterns of relationship to risk for substance use, abuse, and dependence.

Early Reading Development Study

The ERDS, a collaborative effort between researchers in Australia, Norway and the United States, is the first international longitudinal twin study of the genetic and environmental influences on pre-reading and early reading-related skills. Thus the Colorado sample is one of three samples utilized in this project. Preliminary studies of preschool cognitive measures implicate genetic contributions to phonological awareness and several measures of learning and memory (Byrne et al., 2002). In contrast, however, negligible additive genetic and sizable shared environmental contributions have been implicated for vocabulary, grammar, and morphology. A follow-up investigation of these same twins after their initial schooling suggests that preschool predictors that appear environmentally influenced become more genetically influenced after formal schooling begins (Byrne et al., 2005).

Discussion

As summarized in Table 1, the studies using the CTR samples encompass a broad range of domains. Further, most of these studies involve data collection across multiple time points resulting in rich and interrelated data sets.

Development of the CTR is an ongoing and evolving process. Contact with potential participants initially occurred through the CDH which has modified its policies across time. CDH records have only been utilized back to 1968, but could be extended to older twins if interest and consequent resources were available. School records were utilized for 8 years beginning in the mid-1990s but this was discontinued due to both having enrolled a sufficient pool for the CADD and lack of resources.

Colorado state privacy regulations eliminating our ability to use DMV records to locate subjects subsequent to 2000 has made maintaining contact with subjects more difficult. For our in-person projects, this difficulty has been overcome through the use of other

Table 1

Major Data Collection Domains for Each CTR Study

Domain	TQS	TIP	LTS	CADD	ECF-LTS	ECF-CTS	CATS	ERDS
Demographics	X	X	X	X			X	X
Health history	X	X	X	X	X	X	X	X
Cognition		X	X	X	X	X		X
Achievement (reading)			X		X	X		X
Temperament/personality	X	X	X	X			X	
Behavioral problems	X		X	X			X	X
Psychopathology				X				
Substance use			X	X			X	
Family environment			X	X			X	
Interests and activities			X	X			X	
Social competence and peer relations			X	X			X	

Note: In studies that use samples who have also participated in other projects, analyses may cross domains. For example, both ECF projects were designed to utilize data from the LTS and/or CADD studies.

TQS = Toddler Questionnaire Study, TIP = Twin Infant Project, LTS = Longitudinal Twin Study, CADD = Center for Antisocial Drug Dependence, ECF = Executive Cognitive Function, CTS = Community Twin Sample, CATS = Colorado Adolescent/Adult Twin Study, ERDS = Early Reading Development Study.

sources, such as credit bureaus, but the cost of these sources is prohibitive to an unfunded resource such as the registry.

In the early years, enrollment may have been biased by the demands placed on parents such as being able to read the CDH inquiry, return the consent to contact, and in the case of in-person studies, schedule and keep appointments, resulting in a slightly more organized higher functioning sample than the population norm. Ease of contact using the new CDH negative consent procedure along with exclusively home visits for the ERDS may make the recent sample more representative.

However, across the entire period of enrollment, few families have completely refused participation. Most twin families in Colorado who have not enrolled in the CTR were not located due to high levels of mobility and privacy concerns from state agencies. Further, most families who were invited to participate in the in-person studies enrolled, and scheduling conflicts were the primary reason some families did not. Thus, despite intermittent difficulties, the CTR has proved a valuable resource, relatively representative of the population from which it was drawn.

Collaborations with other investigators either using extant data or collecting new information are a possibility, dependent on resources and institutional review board approval. As an example, we recently collected questionnaire data on a modified version of Horney's personality inventory for a researcher at a different institution. Additionally, at IBG, we have benefited by being able to combine data from our twin studies with data from an adoption study and other selected samples. Integrated data sets are an important aspect of our intended future directions, allowing for a more detailed analysis of processes affecting behavior.

Acknowledgments

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