
Minnesota Twin Family Study

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The Minnesota Twin Family Study is a longitudinal study of 11-year-old and 17-year-old twins and their parents designed to examine factors related to the etiology of substance abuse and related problems. At study intake, the twins and their parents participate in a day-long assessment in our laboratory that includes measures of endophenotypes (e.g., event-related potentials, EEG, autonomic nervous system reactivity, startle eye-blink), psychopathology, personality, cognitive ability, anthropometry, and environmental risk/protective factors. DNA derived from blood is also collected. A parallel longitudinal study of adolescent adoptive siblings, biologically related siblings, and their parents is also underway. Over 1500 twin families and 350 adoptive and biological sibling families have already entered the longitudinal phase of the study. This article provides an overview of study methods, highlights published findings, and describes procedures in place to foster collaboration with other investigators.

Originating over 10 years ago, the Minnesota Twin Family Study (MTFS) is an ongoing population-based, longitudinal investigation of same-sex twin children and their parents that examines the etiology of substance use disorders from a developmental, behavior genetic perspective. Much of the focus of the MTFS is on the notion that an inherited predisposition to develop these disorders is expressed in early adolescence through undersocialized or disinhibited behavior. We further hypothesize that this predisposition is manifest in psychophysiological markers derived from tasks that tap individual differences in inhibitory control. A comprehensive overview of the project along with a summary of early findings can be found in Iacono et al. (1999).

Twin Recruitment

Two age cohorts are included, preadolescent twins recruited at approximately age 11, when they are in grades 5 or 6, and late adolescent twins, recruited at approximately age 17, during their last year in secondary school. Because we rely on publicly available Minnesota birth certificates to identify twins, only those born in Minnesota were eligible for study inclusion. This statewide sample is broadly representative of the Minnesota population. About 60% of the sample come from the seven counties that compose the Minneapolis-St. Paul urban area. The remainder live in smaller cities, towns and rural areas throughout the state and in towns in neighboring states that border Minnesota. Reflecting the ethnic composition of the state at the time they were born, almost all the twins are Caucasian (over 95%), with the majority having German and Scandinavian ancestry.

At study intake, the twins, together with their parents, travel from around the state to spend a day in our university laboratory. The day is split between interviews with all four family members and time in the psychophysiology laboratory. This procedure, with some variations, is repeated every three to four years. Because the first twins were assessed in 1991, some of the twins who were 11 at study intake are now in their early 20s, and some of the 17-year-olds are in their late 20s.

As originally conceived, the MTFS was launched to study male twins. About three years later, a parallel study of female twins was initiated. Although twin families have been located using a consistent set of procedures since the inception of the MTFS, several different protocols have been used to recruit families for assessment. Initially, the recruitment pool consisted of all male and/or female twins born in Minnesota during selected birth years spanning 1971–1985. From this effort, we located a total of 1695 male and 1729 female twin pairs, over 90% of the twins born in the state. We have successfully assessed in person 1,383 of these families, with about 17% of located families refusing to participate in the study. The remaining families were either ineligible to participate (e.g., lived more than a day's drive from our labs), or not recruited for an in-person assessment. Comparisons of participants to nonparticipants indicated that MTFS families assessed in person are broadly representative of Minnesota families with adolescent twins (Iacono et al., 1999).

Because substance use disorders are less prevalent in females than males, we have continued to add a small random sample of 11-year-old female twins born in the birth years following 1984. To increase the representation of undersocialized and behaviorally disinhibited twins in the MTFS, in 2000, we began a high-risk recruitment strategy for 11-year-old twins. We selectively recruit twin families because at least one of the 11-year-old twins has symptoms of attention deficit hyperactivity (ADHD) or conduct disorder (CD). A random sample of unselected twin families is assessed along with the high-risk group. To date, these additional recruitment efforts have added 364 male and 435 female twins to the registry, with additional 33 male and 65 female twin pairs completing the assessment. By the time intake for this high risk MTFS

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component is completed in about five years, we expect approximately 2000 twin families will have completed our in-person assessment, with twins ranging in age from about 11 to 34.

These twin studies are paralleled by an adoption study initiated in 1998 that, although it does not include psychophysiological measurements, employs most of the same assessments used in the MTFs. This longitudinal study involves parents and a pair of adolescent children who fall in the same age range as the MTFs twins. Included will be 400 families with adopted children and 200 families with biological children. Besides providing an opportunity to test many of the same hypotheses relevant to the MTFs, this adoption study allows us to examine adoptee adjustment and how older siblings influence their younger siblings' use of alcohol and drugs.

Measures

MTFS intake and 3-year follow-up protocols are similar, adjusted to include age-appropriate self-report, psychiatric, and psychophysiological assessments. Interviews, diagnostic case conferences, and psychophysiological assessments are conducted by staff with no knowledge of the status of other family members. At study intake, all assessments are carried out in person, simultaneously in parallel laboratories. Follow-up assessments are also conducted in the laboratory or by phone, with the protocol emphasizing in-person laboratory assessments with the younger study participants. Parents and teachers are used as informants to collect diagnostic and adjustment data that complements that obtained by direct interview with the twins. There are over 50 interview, self-report, psychophysiological, and other informant assessments for each study subject, many of which are detailed in Iacono et al. (1999). Key assessments are described in Table 1.

Recent Findings

Older twins and parents have entered or passed through the age of risk for developing substance use disorders, so much of our published work has focused on the disorders and problem behaviors already expressed in these individuals. Because the data on male twins were available earlier than that for the females, these publications emphasize results for male youth.

Behavioral Undercontrol and Substance Abuse

Examining fathers of male twins, Elkins et al. (1997a) found that fathers with more severe and persistent antisocial behavior had a greater likelihood of developing a substance use disorder, and a greater likelihood of having antisocial sons. In a follow-up study (Holdcraft et al., 1998), we found that fathers with both alcoholism and antisocial personality were more likely than alcoholics with other forms of psychiatric comorbidity to abuse illicit substances. Childhood externalizing psychopathology is also related to substance abuse risk. Examining both 17-year-old boys and girls, we found that for both sexes, the odds that a youth with conduct disorder would be dependent on alcohol, nicotine, or cannabis were substantially elevated compared to youths without this diagnosis (Disney et al., 1999). In addition, conduct disordered children who developed adult symptoms of antisocial personality disorder between the ages of 15 and 17 were about three times more likely to develop a substance use disorder by age 17 than youths with conduct disorder who did not develop adult symptoms.

Focusing on the longitudinal data available from the 11-year-old boys, Taylor et al. (2002) found that children who engaged in delinquent behavior by age 11 were more likely than those who developed delinquency later and those who were nondelinquent to develop substance dependence symptoms over a 6-year period. In addition,

Table 1

Overview of Design and Measures Used in the Minnesota Twin Family Study

Participants	2059 male and 2164 female twin pairs located from birth certificates spanning 1971–1990 are in the registry; 699 male and 786 female twin families have completed in-person assessments. An additional 400 twin pairs born 1991–1995 are to be assessed when twins turn 11. Biological and step-parents of the twins are included in the assessment.
Longitudinal Design	At intake, all twins and their parents are seen in person for a daylong psychophysiological and interview assessment using a cohort-sequential design targeted to recruit twins when they turn either 11 or 17 years old. Approximately every three years, these assessments are repeated on the twins as well as on the parents of the younger twins when the twins turn 17.
Measures	<p>Psychophysiology: Event-related potentials from a visual oddball paradigm, resting EEG, smooth pursuit and antisaccade eye tracking, emotional modulation of eye blink startle, electrodermal habituation, autonomic reactivity in anticipation of aversive stimuli</p> <p>Anthropometry: Limb, trunk, and head measurements, resting heart rate, blood pressure</p> <p>Psychopathology: <i>DSM-III-R/IV</i> diagnoses of substance use disorders, externalizing disorders of childhood, antisocial personality disorder, major depression, anxiety and eating disorders (females only)</p> <p>Cognitive Ability: Wechsler IQ, spatial working memory, academic achievement, reading achievement</p> <p>Personality: Tellegen's Multidimensional Personality Questionnaire (MPQ), Piers-Harris Children's Self Concept Scale, State-Trait Anxiety Inventory, Socialization Scale from the California Psychological Inventory, self report measures of antisocial behavior and delinquency</p> <p>Environmental Risk and Protective Factors: Family Environment Scale, Parental Environment Questionnaire, Dyadic Adjustment Scale, religious practices and attitudes, peer group quality, life events, trauma, social adjustment</p> <p>Teacher ratings: Personality, peer group, externalizing behavior, academic progress</p> <p>DNA: Samples from blood have been obtained on approximately 3000 participants</p>

compared to late-onset and nondelinquent groups, early-onset delinquents had more adjustment difficulties that suggested problems with inhibitory control (Taylor et al., 2000c). For instance, they were more likely to have childhood externalizing disorders, be impulsive, score low on a personality measure of constraint (low scores are associated with risk taking, unconventionality, and a lack of restrained behavior), show a reduction in spontaneous autonomic (electrodermal) activity, and have more first- and second-degree adult relatives with antisocial behavior. Although samples were too small to warrant biometric analysis, comparison of twin concordance data suggested that early delinquency was more heritable than late-onset delinquency.

Because early onset drinking has repeatedly been demonstrated to be associated with adult alcoholism, we examined data from both age cohorts and sexes to investigate causal mechanisms that may underlie this association. We found that early-onset drinking was associated broadly with indicators of poor inhibitory control such as substance use disorder generally, antisocial behavior, and low constraint (McGue et al., 2001a). In pre-adolescents who had no experience with alcohol, measures of behavioral disinhibition such as oppositionality, hyperactivity/impulsivity, and inattentiveness collected at age 11 predicted drinking onset by age 14. Biometric analysis of the twin data indicated that early alcohol use was heritable, and genetic factors underlying symptoms of disinhibitory psychopathology were found to contribute to risk of early alcohol abuse, especially in boys (McGue et al., 2001b). These results suggest a common inherited vulnerability to disinhibitory behavior that includes early drinking and eventually leads to alcoholism. The findings do not support the alternative hypothesis that early drinking per se disrupts normal development, which in turn increases the likelihood of an alcoholic outcome. Legrand et al. (1999b) showed further that an inherited vulnerability may interact with environmental risk in the etiology of early onset male adolescent substance use. Positive peer influences and attachment to mother, school and church appeared to buffer the likelihood of initiating substance use from age 11 to 14 even in the presence of high familial risk defined as having a substance abusing parent. High environmental risk at age 11, as indicated by negative peer influences and poor attachment, combined with high familial risk, was associated with more substance use at age 14 than expected from a simple summation of relative environmental and familial risks.

A series of MTFs publications have explored the relationship between personality and substance abuse. Alcoholism was associated with low constraint in MTFs parents (McGue et al. 1997). Severe alcoholism in men, characterized by early onset drinking, antisocial behavior, relatives with problem drinking, and illicit drug abuse and dependence, was associated with especially low constraint scores (McGue et al. 1997). Illicit drug abuse or dependence alone, in the absence of alcoholism, was associated with low constraint (McGue et al. 1999).

To further elucidate the relationship between personality and the spectrum of disorders related to substance abuse, Krueger et al. (2001) examined the covariation

among symptoms of the mood, anxiety, substance dependence, and antisocial clinical disorders assessed in MTFs parents and found that a two-factor model describing internalizing (mood and anxiety disorders) and externalizing (substance and antisocial disorders) psychopathology dimensions best fit the data. Of special significance here, the personality dimension of constraint was found to correlate with the externalizing psychopathology dimension, suggesting that this personality factor may underlie the covariance among a spectrum of externalizing disorders. This possibility was further explored in the older twin cohort by Krueger et al. (2002) who proposed a hierarchical model consisting of a general externalizing factor reflecting the covariation among externalizing disorders (antisocial and drug dependence disorders) and constraint as well as specific factors that account for distinctions among the phenotypes within the spectrum. Biometric model fitting revealed that the majority of the variance in the externalizing factor was heritable ($h^2 = .81$), but both genetic and environmental factors accounted for distinctions among spectrum phenotypes.

In the aggregate, these findings converge on the conclusion that there exists an inherited predisposition for a spectrum of externalizing psychopathology that includes ADHD, CD, antisocial personality, alcoholism, and other substance use disorders. This liability is expressed as well in the personality dimension of low constraint and in behaviors reflecting disinhibition. When signs of this predisposition are expressed early, they predict the development of substance abuse later in life. The simultaneous presence of multiple indicators of this liability signal heightened risk for substance abuse. As the next section highlights, psychophysiological measures may reflect the presence of this liability.

Psychophysiological Endophenotypes

Much of our psychophysiological data analysis has focused on the 17-year-old male twins and their fathers. Several reports have examined the association between central and autonomic nervous system reactivity and substance use-related psychopathology.

When an infrequent stimulus of special significance (e.g., one requiring a response) is presented along with frequent stimuli of no particular meaning, the infrequent (or "odd") stimuli in the series produce a brain event-related potential called a P300 wave, so labeled because it has a latency equal to or exceeding 300 milliseconds. In a series of investigations, we have used a visual oddball paradigm to examine the relationship of P300 to a broad range of substance-use related phenotypes in children and adults, building on the well-replicated finding that reduced P300 is associated with genetic risk for alcoholism. Katsanis et al. (1997) reported that P300 amplitude was highly heritable ($h^2 = .79$), with the within-individual similarity in amplitude about equal to the similarity across members of an identical twin pair. Fathers of twins who are alcoholic, abuse illicit drugs, or have antisocial personality disorder have reduced P300 amplitude (Malone et al., 2001). When 17-year old boys were separated into groups corresponding to those with large, intermediate, or small amplitude P300

waves, the likelihood of a substance use disorder or an externalizing disorder of childhood (e.g., ADHD, CD) was inversely related to P300 amplitude such that the prevalence of these disorders was elevated in those with small P300 and diminished in those with large amplitude waves (Carlson et al., 1999). McGue et al. (2001a) extended this research by showing that P300 was associated with age at first drink, such that those 17-year olds who initiating drinking early had smaller P300s than those who began drinking after age 14. In Iacono et al. (2002), we found that boys with childhood externalizing disorders or nicotine, alcohol, or illicit drug use disorders had reduced P300; that boys whose fathers had substance use disorders had small P300; and that those developing a substance use disorder between the ages of 17 and 20 had reduced P300 at age 17. All of these findings are consistent with the hypothesis that small amplitude P300 may indicate genetic risk for a dimension of disinhibiting psychopathology that includes childhood externalizing, adult antisocial, and substance use disorders.

In Taylor et al. (1999) we relied on a subset of MTFs 17-year old males to examine autonomic nervous system functioning in anticipation of an aversive stimulus (a loud noise blast), the predictability of which varied. We hypothesized that individuals with dysfunctional inhibitory control would not be able to take advantage of stimulus predictability to mitigate the psychological impact of the stimulus, and thus demonstrate greater autonomic reactivity to the unpredictable than to the predictable noise blast. A modulation index was derived, with high scores indicating good modulation, characterized by relatively diminished response to the predictable aversive sound. As expected, symptoms of substance dependence varied with the ability to modulate reactivity, with those showing the highest modulation scores having the fewest symptoms of substance dependence. The intraclass correlation for modulation scores was higher in MZ (.42, from 42 pairs) than DZ (.29, from 23 pairs) twins, a finding consistent with a genetic effect on individual differences in modulation. In a subsequent report, we found that P300 and modulation scores were uncorrelated, but individuals who showed both small P300 and poor modulation had substantially elevated risk for substance use disorders compared to those with only one or neither of these psychophysiological deviations (Iacono et al., 2000). These findings suggest that reduced P300 and poor autonomic modulation have the potential to serve as endophenotypes, indicators for the genetic liability to develop a spectrum of disorders indicative of behavioral disinhibition and associated with substance abuse.

Other Findings

The richness of the MTFs data has provided opportunities to explore many other topics thematically related to our major aims. Eating disorders and internalizing psychopathology in adolescents also increase risk for poor adjustment and substance abuse (Marmorstein & Iacono, 2001; von Ranson et al., 2002). Biometric models derived from twin data have been used to examine genetic and environmental contributions to various psychopathologies, including the spectrum of childhood externalizing disorders

(Burt et al., 2001), ADHD (Sherman et al., 1997a, 1997b), and substance use disorders (McGue et al., 2000), as well as behaviors and traits related to psychopathology such as substance use (Han et al., 1999), eating behavior (Klump et al., 2000, 2001), state and trait anxiety (Legrand et al., 1999a), delinquency (Taylor et al., 2000a, 2000b), and parent-child relationships (Elkins et al., 1997b). Several reports illustrate the heritability and sensitivity to development of various candidate endophenotypes, including spontaneous EEG (McGuire et al., 1998), smooth pursuit eye tracking (Katsanis et al., 1998, 2000), antisaccade eye tracking (Malone & Iacono, in press), eye-blink startle (Carlson et al., 1997), P300 (Katsanis et al., 1996), and spatial working memory (Zald & Iacono, 1998).

Future Plans

Over the next several years, we hope to continue to explore core hypotheses, expanding our use of longitudinal data. Examining possible gene-environment interactions and identifying environmental factors that contribute to substance abuse outcomes will receive added attention. Gender differences and the possibility that different mechanisms influence the development of substance use disorders in males and females will also receive heightened emphasis. Because DNA is available on most study participants, we hope to examine candidate genes possibly associated with phenotypes we measure, as we did recently in a study that failed to confirm a relationship between dopamine receptor gene polymorphisms and personality traits related to novelty seeking (Burt et al., in press). Structural and functional magnetic resonance imaging of the brains of twins discordant for various disorders is also planned, as is the longitudinal study of brain structure and function in preadolescent twins as they pass through the age of risk for the development of substance-related problems.

Collaborative Opportunities

We have established a set of guidelines and a formal review mechanism to encourage collaboration with students and investigators interested in our data. The process involves submitting a brief (two page) proposal outlining hypotheses, presenting a research plan, and describing the resources needed or provided to accomplish the proposal aims. All such proposals require one of the principal investigators as a sponsor. Because ours is a longitudinal study, we have some flexibility to add new measures if they would assist us to better address study objectives. We have made collaborative arrangements with investigators at eight different universities. Most of these collaborations are with former students who have worked on the MTFs, but we welcome inquiries as well from nonaffiliated investigators. Individuals interested in possible collaborations should contact one of this paper's authors for additional information. We especially encourage collaborations with investigators who have skills and interests that complement ours, such as those with expertise in molecular genetics, brain imaging, or interests in topics that are pertinent but not central to our major aims.

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