PHENOTYPIC AND GENETIC ANALYSIS OF INDICES OF LIABILITY TO ADDICTION

by

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The inability to measure individual risk for SUD, particularly at a young age, hinders etiologic research and prevention. Previous research has developed an index of transmissible liability (TLI) that covers the entire range of liability phenotypes, does not rely on SUD symptoms, is derived from items drawn from psychological and psychopathological instruments, and can be applied in a young or otherwise asymptomatic population. TLI has high heritability and has been validated as a measure of transmissible risk for SUD in previous studies. This index, however, requires information obtained not only from the individuals but also from their parents and teachers. Developing SUD liability indices that do not involve those additional informants could augment the feasibility and efficiency of measurement. One of the goals of this study was to construct new indices based on a reduced number of questionnaire items used to derive the original TLI and determine their utility in measuring risk for SUD. Another purpose of this study was to investigate composition of phenotypic variance of the newly developed liability indices. Participants were self-selected twin pairs attending the 2006, 2007, and 2009 Twins Day Festivals in Twinsburg, OH, and participants in the CEDAR database from the University of Pittsburgh. Results of this research indicate that the ability of the newly developed liability indices to predict SUD is similar to that of the original TLI. Biometrical genetic analysis showed that the phenotypic variance of the new SUD liability indices is comprised of approximately

equal additive genetic and unique environmental components. This study has public health relevance as it developed new measurement techniques to identify individuals at high risk of developing SUD, which will be beneficial for prevention and intervention.

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PREFACE

I would like to thank the faculty of the Graduate School of Public Health, especially those within the Human Genetics Department for their guidance and support. Most importantly, to Betsy Gettig and Robin Grubs who provided me with the knowledge and support to prepare me for my future life as a genetic counselor, which I can't wait to begin!

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1.0 INTRODUCTION

An individual's risk to develop a substance use disorder (SUD) is a phenotype for a continuous latent complex trait termed liability. Liability (Falconer, 1965) includes the effects of all factors influencing the likelihood to develop a disorder. Phenotypic values that are above a particular point, the threshold, on the liability scale are likely to result in a clinical diagnosis of the particular substance use disorder. In the Diagnostic and Statistical Manual of Mental Disorders (DSM), there are hundreds of possible diagnostic combinations of symptoms corresponding to a SUD diagnosis. Thus, the disorder phenotype is extremely heterogeneous. The diagnostic classification collapses the continuous trait into two diverse phenotypic classes. While necessary for clinical psychiatric work, this method of phenotyping is not optimal for research purposes and cannot be used in primary prevention (Vanyukov et al., 2003a; Vanyukov et al., 2009).

In order to measure liability, members of the Center for Education and Drug Abuse Research (CEDAR) developed the transmissible liability index (TLI). The TLI was formed based on behavioral traits as indicators of future development of SUD, using high-risk/family design and item response theory (IRT) (Vanyukov et al., 2003a,b). The TLI has been validated as a measure of transmissible risk for SUD (Vanyukov et al., 2009; Kirisci et al., 2009). While the TLI quantifies transmissible SUD liability, transmissibility may be due to both genes and environment. Twin studies have shown it to be entirely due to its high heritability ($h^2 = 0.79$)

(Vanyukov et al., 2009; Hicks et al., in press). The TLI can quantitatively evaluate the risk for SUD and distinguish individuals that will later develop SUD from those who will not.

This study expands on the previous work with the TLI to investigate the utility of abbreviated liability indices for prediction of SUD. Subjects from the CEDAR database from the University of Pittsburgh and from the Twinsburg Twins Days festival were used in this study. The original TLI was formed based on 45 questionnaire items, and a subset of six items from this original 45 was chosen to form the abbreviated liability indices. This research evaluates predictive properties and estimates heritability of the new indices. This study was approved by the University of Pittsburgh IRB (PRIM #0410086 and Twinsburg #0606138). This work also describes the practical part of the thesis project, collecting and maintaining a twin registry for research purposes. The following specific aims were pursued in this study.

2.0 SPECIFIC AIMS

SPECIFIC AIM 1

To expand and maintain the Pittsburgh Registry of Infant Multiplets (PRIM) for future use by researchers.

SPECIFIC AIM 2

To evaluate the efficacy of abbreviated liability indices in predicting SUD development.

Hypothesis A: Predictive ability of abbreviated indices is lower than that of the TLI.

Hypothesis B: The IRT-derived index based on multicategory items is a better predictor of SUD than the index based on binary items, and the latter, in turn, is a better predictor than the index based on item summation.

SPECIFIC AIM 3

To estimate phenotypic variance components for the indices of liability to substance use disorders.

Hypothesis C: The abbreviated indices of liability have significant heritability.

Hypothesis D: Heritability estimated for the IRT-derived index is greater than that for the additive index.

3.0 BACKGROUND AND SIGNIFICANCE

3.1 LIABILITY TO SUBSTANCE USE DISORDERS

Substance abuse is a problem in our nation as statistics indicate millions of users each year, and addiction has some of the highest overall costs of any medical disorder when comorbidities are factored in (Kreek et al., 2005). Most individuals who use drugs with the potential for abuse can control this use and do not experience serious consequences; these individuals are classified as occasional drug users (Substance Abuse and Mental Health Services and Administration, 2005). It is those individuals that have the susceptibility to develop substance abuse problems that are the main focus of this research. It is estimated that 10-16% of outpatients seen in the medical setting have such problems, and as many as 40% of hospitalizations in the United States involve drug-related issues. Being able to measure individual risk for SUD development would then allow targeting those at high risk and developing interventions for these individuals. Addiction is a difficult disorder to treat, especially when diagnosed late into the condition, although remission can be achieved in up to 60% of patients. This variation is based on many factors, such as premorbid functioning, comorbid conditions, and the support systems available to a patient (Santora and Hutton, 2008; Hoffmann and Miller, 1992).

The liability to a complex disease such as SUD is comprised of both the individual's susceptibility to develop the disease and the environmental factors. The variation in liability results from both genetic and environmental factors; thus SUD liability is a polygenic or multifactorial trait. Phenotypic values that pass the threshold on the liability scale are likely to exhibit a disease phenotype and be considered affected (see Figure 1).

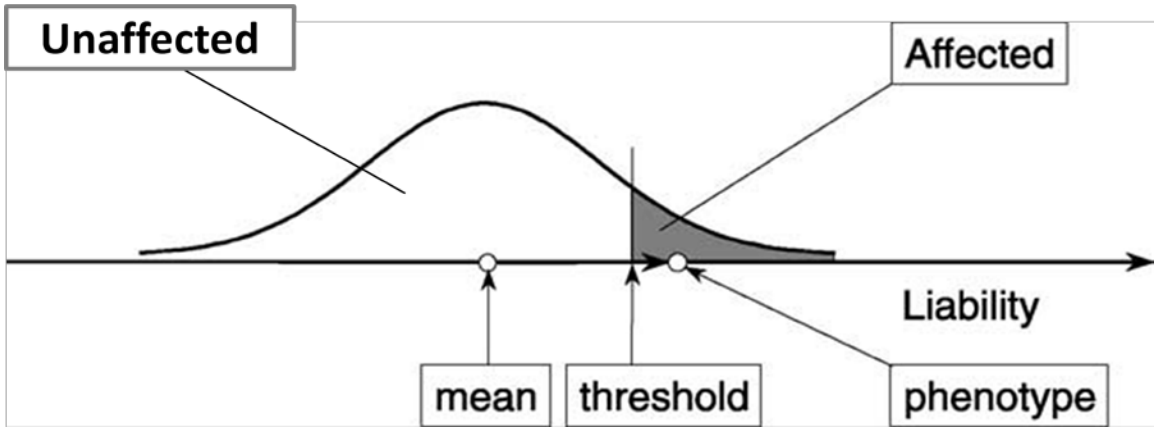

Figure 1. SUD Liability Distribution (Vanyukov et al., 2003a)

For liability to a behavioral disorder like SUD, where there is difficulty distinguishing between affected and unaffected, the threshold is defined by the constantly changing diagnostic criteria (Vanyukov et al., 2003).

There is a wide array of research that has been conducted on the transmissibility of liability to SUD and the genetic contributions to variance, as is the focus of this study. Many researchers have found that genetic and environmental influences are dependent on the specific substance used and severity of the disorder. In regard to alcoholism, twin studies have found heritability estimates reaching 60% on alcoholism among men, with 48-58% of the variation attributed to additive genetic factors and the rest due to non-shared environmental influence. Shared environmental factors had little influence on liability variation (Prescott and Kendler, 1999). A study by van den Bree et al. (1998) investigated the genetic influence for drug abuse

and dependence for any drug, which showed an additive genetic contribution of 0.79 for males and 0.47 for females. Liability to SUD was found to be largely non-specific to particular illicit drugs (Tsuang et al., 1998; Kendler et al., 2003), with a common factor accounting for the entire genetic variance. Substance-specific SUD diagnoses can be modeled as indicators of a single latent continuous trait (Kirisci et al., 2002), also supporting the concept of common liability. Measuring common liability to SUD would thus to a large degree account for the risk to specific drug use disorders.

An index of transmissible liability to SUD, specifically, has been developed by Center for Education and Drug Abuse Research (CEDAR) at the University of Pittsburgh (Vanyukov & Tarter, 2000; Vanyukov et al., 2003a; Vanyukov et al., 2009) based on the application of item response theory (IRT). IRT is a psychometric test theory that relates an individual's performance on a test item to a latent trait that is being measured. The relationship between performance on a test item and the latent trait is described by an item response function (IRF). Parameters of IRT allow for taking to account that different items have various difficulty and ability to discern values of the trait. IRT analysis is also able to provide testable models. Whereas face-value indicators of SUD liability (disorder symptoms) are not available in children, the high transmissibility (due to high heritability) of liability allows determining children's non-symptom characteristics that may be used as liability indicators. These characteristics should discriminate between children of affected and nonaffected parents, thus relating these indicators to parental and thereby children's transmissible liability. The potential indicator items were chosen from various psychological and psychiatric instruments, and analyzed using factor analysis and item response theory to derive the Transmissible Liability

Index (TLI). The TLI has been found to be psychometrically valid and has been shown to have significant heritability ($h^2 = 0.79$) using the twin method.

3.2 THE TWIN METHOD

The twin method is the main approach to the evaluation of the components of phenotypic variance, enabled due to the known differences between genetic correlations in the two categories of twins, monozygotic (identical, MZ) and dizygotic (fraternal, DZ). MZ twins occur from the fertilization of a single egg that later splits into two embryos, while DZ twins occur from the fertilization of two separate eggs. MZ twins share essentially 100% of their genetic material in common. DZ twins share, on average, only 50% of their segregating genes in common and are genetically related to one another as any full sibling pair. In DZ twins, however, age is controlled for, unlike with full siblings where age is a confounding factor.

Genetic effects at a single locus can be divided into additive and dominance genetic effect. The total amount of genetic influence on a trait's variation is then the sum of the additive and dominance effects of alleles at multiple loci and the variance due to the interaction of alleles at different loci (epistasis). An estimate of the contribution of additive genetic factors, A, to phenotypic variation of a trait can be calculated as twice the difference between the MZ and DZ twin correlations, $A = 2(r_{MZ} - r_{DZ})$. An estimate of the contribution of dominant genetic influences, D, to phenotypic variation of a trait can be obtained by subtracting four times the DZ correlation from twice the MZ correlation, $D = 2r_{MZ} - 4r_{DZ}$ (Posthuma et al., 2003). Thus the

total genetic variation of a trait involves both additive and non-additive genetic factors, the latter including both dominant and epistatic effects.

Environmental effects on variation of a trait are divided into two categories, shared and non-shared environmental influences. An estimate of the contribution of shared environmental influences, C, to the phenotypic variation of a trait is given by subtracting the MZ correlation from twice the DZ correlation, $C = 2r_{DZ} - r_{MZ.}$ Non-shared environmental influences, including measurement error that is always present, are indicated by MZ correlations less than 1. The contribution of non-shared environmental influences, E, can be calculated by subtracting the MZ correlation from unity correlation, $E = 1 - r_{MZ}$. These estimates of phenotypic variance components, however, depend on the accuracy of the MZ and DZ correlation estimates and the true causes of variation of a trait within the population.

The phenotypic variance of a particular trait, $V_{P,}$ is usually modeled as being composed of four components: V_A, the additive genetic component, V_D, the dominance genetic component, V_C, the shared environmental component, and V_E, the non-shared environmental component,

$$V_P = V_A + V_D + V_C + V_E$$

V_A accounts for the phenotypic variance attributable to the additive genetic effects of alleles at one locus, while V_D refers to the genetic variance at a single locus that is attributable to the dominance of one allele over another allele. V_C accounts for non-genetic factors that are shared within families making members more similar to each other, and V_E refers to the contribution of non-genetic factors that cause phenotypic differences between family members (Neale and Maes, 2004; Posthuma et al., 2003).

Heritability is a measure of the extent to which genetic variation influence phenotypic variation. Heritability is a proportion of the phenotypic variance attributed to genetic factors

divided by the total phenotypic variance of a trait (Neale and Maes, 2004). Broad sense heritability, H^2, is a measure of all combined genetic effects, V_G, on phenotypic variation and is denoted in the following manner:

$$H^2 = V_G / V_P \quad \text{or} \quad H^2 = (V_A + V_D) / V_P$$

Narrow sense heritability, h^2, is a measure of additive genetic effects on phenotypic variation, or $h^2 = V_A / V_P$ (Neale and Maes, 2004).

One of the main approaches to using twin data in estimation of phenotypic variance components is based on path analysis. Path analysis was first described by the geneticist Sewall Wright in 1921, and it has since been widely applied to genetics and behavioral sciences. This method allows representation of linear structural models in diagram form and thus derives predictions for variances and covariances of variables under the particular model. The path diagram is a useful tool to display causal and correlational relations, or the paths between variables. Another advantage of path analysis is that is goes beyond measuring the degree of association by the correlation coefficient, and instead, allows the researcher to make hypotheses about relationships between the variables that are quantified by path coefficients (Neale and Maes, 2004). The model's predictions are compared statistically with observed data, which tests the model. The expectations for variances and covariances of MZ and DZ twins may also be inferred from a path diagram (Posthuma et al., 2003).

In path diagrams (see Figure 2), squares represent observed (manifested or measured) variables, and circles represent latent (unmeasured) variables. Single-headed arrows are used to define causal (regression) relationships between variables, with the variable at the tail end of the arrow causing the variable at the head end of the arrow. Omission of a path from one variable to another implies that there is no direct causal influence of one variable on the next. Double-

headed arrows represent a covariance between two variables, which may occur through a common cause, their reciprocal causation, or both. Upper-case letters denote observed or latent variables, and lower-case letters represent the values of paths or two-way arrows, respectively termed path coefficients and correlation coefficients. .

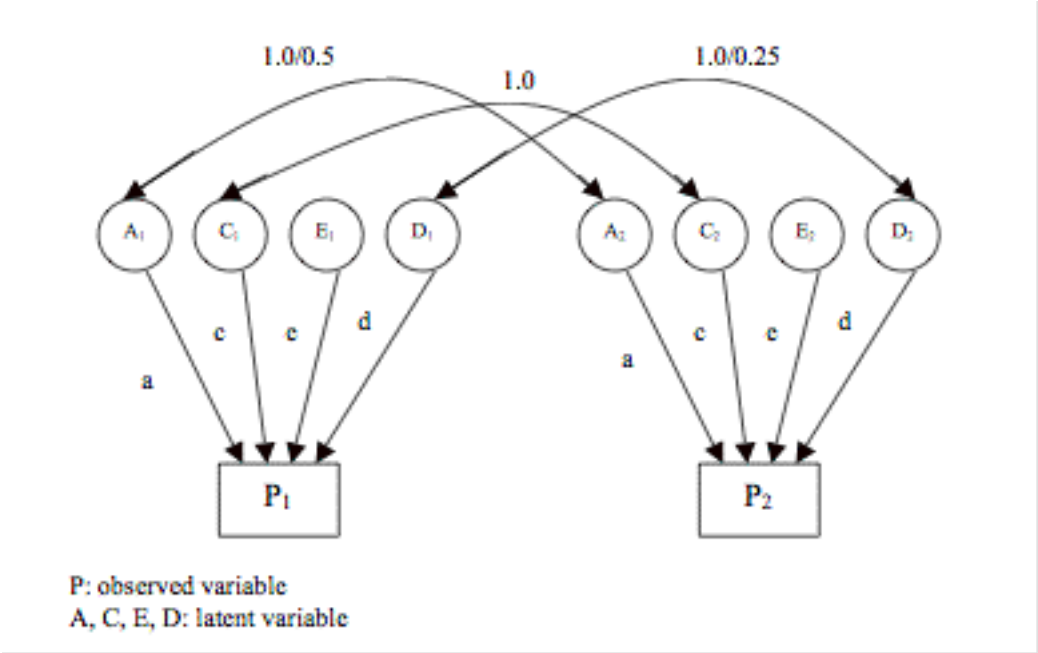

Figure 2. Twin Model Univariate Path Diagram

The correlation between any two variables in the diagram can be expressed as a sum of the compound paths that connect the two points (Figure 2). A compound path is a path along arrows that adheres to the following conditions: 1) no tracing forward and then back, 2) passing through each variable only once in each chain of paths, and 3) passing through only one two-way arrow in each chain of paths (Neale and Maes, 2004).

In path analysis, multivariate approaches allow all the relationships between variables to be examined at the same time with the underlying goal to find a model that best fits the data. When using the twin design, as in this study, it is important to recognize that dominance genetic

effects (D) and shared environmental effects (C) cannot be estimated simultaneously because they are confounded in twin data: shared environmental effects increase similarity between MZ and DZ correlations, and dominance effects decrease this similarity, and simultaneous modeling may result in negative variance component estimates (Neale and Maes, 2004).

This project contributes to the field behavior genetic and twin research behavior genetic research by maintaining and expanding the Pittsburgh Registry of Infant Multiplets. In its analytic part, this project develops abbreviated liability indices using a subset of items employed in the TLI, and tests the utility of these indices in measuring risk for SUD. This study also determines the variance composition of the abbreviated indices using the twin method.

4.0 METHODS

4.1 SAMPLE POPULATION

4.1.1 PITTSBURGH REGISTRY OF INFANT MULTIPLETS

The Pittsburgh Registry of Infant Multiplets was established in 1996. This registry work is conducted with approval from the University of Pittsburgh Institutional Review Board (IRB #0410086). The Pittsburgh Registry of Infant Multiplets (PRIM) is a computerized database which contains information on all multiple births occurring at Magee-Womens Hospital. The purpose of the registry is to serve as a resource of participants for interested researchers from the University of Pittsburgh who would like to conduct twin studies of human behavior and development. Enrollment in PRIM is voluntary, and is offered to all mothers of twins and other multiplets who are born at Magee-Womens Hospital of UPMC in Pittsburgh, Pennsylvania. In 2007, this hospital was ranked by *U.S. News & World Report* as one of the country's best hospitals, accommodating approximately 45% of deliveries that occur in Allegheny County (Magee-Womens Hospital, 2009).

The goal of the PRIM coordinator is to visit the postpartum units of Magee-Womens Hospital daily to invite all mothers of multiplets for participation. Participants are not excluded by race, gender, or age; however, mothers under age 18 years must have a guardian's consent. In

order to identify potential participants, the coordinator walks through three postpartum halls within Magee-Womens Hospital that mothers and babies are taken to after delivery. Identification and congratulatory tags are hung from every mother's postpartum unit door after delivery, which identify the birth of any twins or multiplets. Pinks tags represent female newborns and blue tags represent male newborns. A multiple birth will have the corresponding number of tags of appropriate color hung on the door. Once a multiple birth has been identified by the coordinator, the room number is recorded, and the coordinator approaches an available unit nurse to seek permission to speak to mothers. Only a unit nurse or other healthcare provider can give permission, which is usually by phone. If the mother agrees to meet, the PRIM coordinator directly speaks with the mother about the registry and what participation involves. If she chooses to participate, she signs informed consent and HIPAA forms. Additionally, several brief questions are asked about the multiplets and their parents, such as names, delivery date, birth weight, APGAR scores, parent's birthdates, race, and contact information.

For mothers that join PRIM, a monthly newsletter from the North Pittsburgh Mothers of Multiplets (NPMOMs) group is distributed. Those participants who enroll in PRIM are classified as "joined." Mothers who do not wish to speak with the coordinator or who decline participation after speaking with the coordinator are classified as "declined." Mothers who speak to the coordinator but are unsure of participation and wish to receive more information in the mail are classified as "pending." These mothers sign the HIPAA form in-room with the coordinator and then and an informational flyer, consent form, and questionnaire are sent in the mail for further consideration. Those mothers who are eligible for participation, but are unavailable to speak with the coordinator for any reason are classified as "missed." The most

common reason for this is having babies in the neonatal intensive care unit (NICU), where parents spend the majority of their time rather than in their postpartum room.

Mothers who join the registry also receive a welcome letter in the mail and copies of their signed consent and HIPAA forms. Additionally, the NPMOMs group gives fliers to send that invite all mothers to be part of their group. Information gathered from the in-room questions will then be entered into the Microsoft Access PRIM database, with each participant receiving a unique identifying number. For researchers interested in contacting participants from the registry for enrollment in their studies, a protocol must be submitted to Michael Vanyukov, PRIM Principal Investigator, for approval. Descriptions of a researcher's study are then mailed to all qualifying registry members on behalf of the researcher, and participation is completely voluntary. Those who participate and fulfill all requirements of a researcher's study will be compensated for their time.

One goal of the PRIM coordinator is to reduce the rate of missed, declined, and pending families. Another goal of the coordinator is to maintain communication via mail with enrolled families to reduce loss of future contact by updating addresses and alternate contact information. In the future, additional methods to maintain contact may be implemented. Updating the registry will keep enrolled participants current and available for possible future contact for research studies.

4.1.2 CEDAR SAMPLE

Participants

The Center for Education and Drug Abuse Research (CEDAR) is a NIDA-funded longitudinal family/high-risk study of drug addiction etiology (IRB #0107007). Participants are members of

families of adult men, the probands, who either have a DSM-III-R diagnosis of SUD related to illicit drug use or have no psychiatric disorder (SUD+ and SUD-, respectively). The men had at least one son between ages 10 to 12 years (index cases, IC), and a wife (mate) who is the biological mother of the IC. Recruitment for the study was done by newspaper or radio announcements, public service announcements, and substance abuse treatment programs (Vanyukov et al., 2009). The CEDAR sample had a total of 500 male IC participants with ages ranging from 10 to 12 years. Of these, 378 individuals were white, 106 were black, and 16 were identified as other races. Of the 500 participants, 127 developed SUD.

Instrumentation

The IC subjects are longitudinally tracked from age 10-12 until age 30. An initial evaluation is performed upon study entry, followed up at ages 12-14, 16, 19, and then annually until age 30. Evaluations over time assess a number of individual and environmental characteristics that are critical to understand SUD etiology, and having data from the transition from childhood to adolescence and from adolescence to adulthood will aid in this. The index cases, along with both parents, also answer questionnaire items over the years, which can be used to estimate liability to SUD. Items came from a variety of sources that are formulated to measure an individual's personality characteristics (antisocial behavior, impulsivity, anxiety). Sources include the Dysregulation Inventory, Disruptive Behavior Disorders Scale, and Dimensions of Temperament Survey, among others (Vanyukov et al., 2009). The data used in this thesis are from the index cases' initial evaluation upon study entry.

4.1.3 TWINSBURG TWIN STUDY

Participants

Participants for this research study were recruited at the 2006, 2007, and 2009 Twins Day Festival in Twinsburg, Ohio. The Twins Day Festival is an annual event for twins of all ages and their families from around the world. The festival is an opportunity for twins to see former friends, play games, participate in twin competitions (most alike twins, most dissimilar twins), see live entertainment, and interact in a carnival-like atmosphere. It also provides twins with the opportunity to participate in research studies, as researchers from around the world come to conduct various types of research studies using twins as the primary subjects.

Participants in the 2006 and 2007 studies were invited if they had registered with the Twins Day Festival, were between ages 9 and 18 years of age, and had at least one parent available to participate. The 2006 and 2007 Twinsburg data were combined for a total of 306 twin pairs participating in the Twinsburg study. Ages ranged from 9 years to 19 years, with an average age of 13.66 years (SD = 2.49). Parents were required to consent to the study and both children's assents were also obtained. Each family member completed anonymous paper-and-pencil questionnaires independently, which took about 30-40 minutes to complete. Additionally, family members provided saliva samples in a DNA collection container. Participants in the 2009 study were invited if they had registered with the Twins Day Festival and were between 14 and 30 years of age. Those twins under age 18 years were required to have a parent's consent to the study and then provide their own assent. The 2009 Twinsburg study had a total of 190 sets of twins participate. Ages ranged from 14 years to 30 years, which an average age of 19.8 years (SD = 4.47). It should be know that the 2006, 2007, and 2009 Twinsburg samples were merged

together for all analyses of Twinsburg data, so any mention of Twinsburg sample denotes the merged sample.

Instrumentation

Each twin was given a paper-and-pencil questionnaire, which they were required to take independently. These questionnaires took, on average, 20-30 minutes to complete. Each twin was then asked to provide a saliva sample in a DNA collection container. Following this, each subject took the Computer Adaptive Testing (CAT) version of the questionnaire on a laptop computer. In the CAT, the same items from the questionnaire were presented in a computer-based format, allowing for response patterns to lead to item skipping within the program to provide an abbreviated version of the questionnaire, but providing the same type of information. In total, each participant spent 30-45 minutes, on average, participating in this study.

It should be noted that the questionnaires distributed to 2006/2007 participants differed from those given to 2009 participants. The questionnaires were based on an age appropriate scale of questions. Additionally, those questionnaires completed by 2006/2007 participants were done by both twins and a parent; 2009 questionnaires were only completed by twins.

The objectives of the overall research protocol were to examine the heritability of behavioral regulation. A set of questions were used that were extracted from a battery of standard behavioral assessments that have been shown to predict SUD liability in a previous study. Additionally, DNA samples that were collected would be used to examine the contribution of specific candidate genes to heritability in future research.

Zygosity Determination

Each twin at Twinsburg was required to complete the "About Your Twin Questionnaire" (Appendix B) to determine zygosity. This concise questionnaire was developed by Nichols and Bilbro, 1966, and the parallel zygosity determination algorithm was developed by Eley and the collaborators for the Twins Early Development Study (TEDS) in London. In the 2006/2007 Twinsburg studies, a parent answered the questions about the twins. The original questionnaire was modified for the 2009 study so twins could answer the questions themselves, rather than a parent answering. The modified questionnaire consisted of 15 items. The original method has an accuracy of 94% for zygosity determination (Strassberg et al., 2002; Rowe, 1981). Two hundred sixty same-sex pairs participated in the 2006/2007 Twinsburg study, and 181 same-sex pairs participated in the 2009 study.

4.2 STATISTICAL ANALYSIS

4.2.1 DESCRIPTIVE STATISTICS

Descriptive statistics were obtained for the liability indices using SPSS 16.0 for Windows. T-tests were computed for comparison of whites and blacks in the CEDAR sample. All p-values are two-tailed.

4.2.2 DEVELOPMENT OF LIABILITY INDICES AND ITEM RESPONSE THEORY

The development of the transmissible liability index involved a complex process of several steps. It is known that liability is a quantitative and latent trait that is difficult to measure. There are numerous factors that contribute to variation in this trait, and their effect sizes are mostly unknown. Liability can usually be measured in affected individuals, but this is limiting because measurement can only be done when the population reaches the age of risk and begins using drugs. This hinders the ability to understand differences in cause and effect of SUD (Vanyukov et al., 2003a). To develop the original transmissible liability index (TLI), a family/high-risk method was used in conjunction with item response theory (IRT). As described above, the CEDAR sample was used to develop the original TLI. This method is able to index liability at an age when symptoms of a disorder are not yet manifested.

On average, the children of affected and nonaffected fathers differ in their SUD risk, forming high-average (HAR) and low-average risk (LAR) groups, respectively. Any differences between these groups are attributed to the differences in the paternal SUD liability, and due to its large component of heritability, to the differences in the children's personal SUD liability (Vanyukov et al., 2003a).

IRT is a psychometric test theory that relates how an individual responds to a test item to a latent trait that the test is measuring. Performance of an individual on an item depends on parameters characterizing items themselves and defining the item response function (IRF). This method takes into account that different items have different difficulty and ability to discriminate between values of the trait. Another benefit of IRT is that this analysis also provides testable models, unlike classical psychometric test theory. Additionally, data-fitting IRT models provide

trait estimates invariant of the subsets of items used and item parameters invariant of the sample used (Vanyukov et al., 2003a).

IRT analysis has allowed researchers to construct a set of psychological indicators of adult SUD liability from items encompassing standard psychological scales and psychiatric instruments based on their potential for measuring variables related to SUD. To begin the process of developing the transmissible liability index, constructs representing psychological characteristics (e.g., antisociality, activity) were identified, and items indicating these constructs were submitted to confirmatory factor analysis (CFA) to reduce the number of items and check for unidimensionality. The HAR and LAR groups constructs were then compared on these constructs. This comparison relates the constructs to paternal SUD liability and, due to its transmissibility, to the child's own SUD liability (Vanyukov et al., 2003a). Items that do not cross a certain factor loading threshold and the constructs that do not demonstrate significant group differences are excluded from the set. The items that are indicators of the constructs which showed HAR and LAR differences are next submitted for CFA to further weed out unrelated constructs and test for unidimensionality. After this step in the process, the data have been further reduced and an intermediate liability index has been formed. The final stage in the development of the TLI is IRT analysis for the derivation of an IRT-based index of transmissible liability (Vanyukov et al., 2003a).

In this study, the original CEDAR 45-items, indicators of TLI) were reduced to six item indices with multicategory (6MLI) responses and with binary (6BLI) data responses. These indices were initially derived by IRT analysis. As described above, a six-item index was also derived by summation of the same six binary items (6ALI) and psychometrically tested. The 6MLI and 6BLI were analyzed using IRT to obtain item parameters and scores for the CEDAR

sample. Parameters derived from IRT analysis were applied to the Twinsburg sample to generate 6BLI scores using the computer program Multilog (Thissen et al., 2003). The indication for this reduction in items and for rescaling of items for both CEDAR and Twinsburg samples is described above.

4.2.3 PSYCHOMETRIC ANALYSIS OF LIABILITY TO ADDICTION

To construct the new shorter liability indices, items which were common across the CEDAR, Twinsburg 2006/2007, and Twinsburg 2009 samples were chosen. Items chosen to formulate the new indices were those answered by the index cases, not by a teacher and parent. Clearly, reducing item sets so they are common across all samples is more economical, and choosing only items answered by index cases is best because data from parents and teachers are not always available. Chosen items were selected from a large set of items from various psychological and psychiatric instruments, which were originally selected in CEDAR because they had the ability to measure variables related to SUD. Items characterize an individual's behavior and personality (e.g., mood, attention, antisociality), which may affect the propensity to SUD (Vanyukov et al., 2003a)

The CEDAR sample is made of a group of father, mother, and son families who answered the original questionnaire items that were used to develop the TLI. The Twinsburg 2006/2007 sample encompasses a group of male and female twin participants who answered 11 of 45 questionnaire items. The remaining items were answered by a parent. The 2009 Twinsburg sample is formed using a group of male and female twin participants who answered all 45 questionnaire items themselves. These questions that the index cases only answered were

different between the samples, and those which were common between the three samples needed to be pulled out.

Because of this discrepancy between the three samples, a subset of common items that were answered by all index participants across the three samples was compiled. By careful examination of the questionnaires from CEDAR, Twinsburg 2006/2007, and Twinsburg 2009, equivalent or exactly the same items were identified for all assessments. In total, six items out of the original 45-item TLI set were found to be common across the questionnaires from these samples. These items were used to derive the item indices to be used in twin data analysis. Table 1 below lists these six items. Prior to performing IRT analysis (described below), a classical psychometric analysis was performed on the six item responses from CEDAR and Twinsburg which were given binary responses and were summed across for each participant. Binary responses were given by recoding questionnaire responses to positive and negative

Table 1. Items Selected for 6-item Index

Item Code	Question	Response Options
CD6	Did you often do things to annoy people like grabbing another child's hat?	yes (1) no (0)
CD10	Did you often do things to annoy people on purpose to get even?	yes (1) no (0)
CD34	Were things so bad that you were thinking a lot about death or that you would be better off dead?	yes (1) no (0)
CA5	Did you blurt out answers to questions before they had been completed or did you get in trouble because you would rush into things without thinking?	yes (1) no (0)
DT32	I move a great deal in my sleep.	usually false (0) more false than true (1) more true than false (2) usually true (3)
CA36	Did you skip classes or school without an excuse?	yes (1) no (0)

categories. For example, the item DT32 in Table 1 “I move a great deal in my sleep” has four possible responses of usually false (0), more false than true (1), more true than false (2), and

usually true (3). To put into binary form, responses 0 and 1 are denoted with 0, and responses 2 and 3 are denoted with 1.The other five items are already in binary form. After item DT32 was put into binary form, the individual responses to the six items were summed to obtain an additive index.

4.2.4 PREDICTIVE VALIDITY ANALYSIS

The relationship between the liability indices and the rate of disorder development was analyzed using survival analysis (Cox proportional hazard regression) in the CEDAR data.

4.2.5 CORRELATION ANALYSIS

Intraclass correlation (ICC) analysis was used to estimate intrapair correlations in twins. ICC gives an indication of how strongly the twins within a pair resemble one another. When using ICC analysis, the data are scaled using a pooled mean and standard deviation, which differs from Pearson correlation analysis, where each variable is scaled by its individual mean and standard deviation. ICC is most optimal when using twin data because with twin pairs there is essentially no meaningful way to order measurements among the twins and ICC provides a more natural measure of association (Neale and Maes, 2004).

4.2.6 STRUCTURAL EQUATION MODELING

The maximum-likelihood model fitting, when applied to variance-covariance matrices on an assumption of multivariate normaility, maximizes the fit between the model and the data.

Variance-covariance matrices are used when model-fitting for traits with continuous distribution because differences in variance between MZ and DZ twins may be observed when these differences would be overlooked using correlations. This model fitting also allows determination of confidence intervals and of standard errors of parameter estimates. Additionally, model fitting can not only test the fit of a particular model and estimate its parameter, but also allow a comparison in fit of alternative models (Neale and Maes, 2004; Posthuma et al., 2003).

MZ and DZ twin correlations are used to determine which general model, ACE or ADE, to fit to the data first, depending on whether rMZ is lower or larger than 2rDZ, respectively. As mentioned previously, non-additive genetic and common environment variance components cannot be estimated together in twin data. Nested models, which are models obtained by dropping one or more of these parameters, are then fitted, and the fit of all models compared to finally determine the best fitting model (Neale and Maes, 2004; Rijsdijk and Sham, 2002).

The χ^2 statistic is used to determine goodness of fit. A model with good fit is indicated by the absence of significant differences between expected and observed data, whereas a large χ^2 value and low p-value indicates a poor fit of the data to the particular model. Models with large χ^2 values and p-values less than 0.05 are rejected. The fit of a model can be changed by adding or removing parameters. This can be quantified by calculating the change in χ^2 as the difference between the chi-square of an initial model and a nested model, which itself is a χ^2. A nested model is one that uses a subset of parameters from the original general model. The number of degrees of freedom used when assessing improvements in the model's fit is equal to the difference in degrees of freedom in the initial model and the nested model. Comparisons of the goodness of fit using the same number of parameters can also be obtained from Akaike's

Information Criterion (AIC), which provides information about how economical the model is (Neale and Maes, 2004). Choosing a model with the smallest discrepancy between the true and approximating models is equivalent to choosing a model with the lowest AIC. AIC is defined as:

$$AIC_i = -2\log L_i + 2V_i$$

In this equation, L_i is the maximum likelihood for the candidate model and V_i is the free parameters. When comparing nested models to determine which is best fitting, the nested model with the p-value nearest 1 is chosen. If more than one model fits well, the goodness of fit is compared using AIC.

SEM was used to analyze the model fit for the index derived by IRT analysis of six items converted into binary responses and the index derived by summation of the same six binary items, and the best fitting model was chosen. Model-fitting analyses in this study were conducted using the Mx program (Neale et al., 2003). Variance-covariance matrices were used to test the models.

5.0 RESULTS

5.1 STANDARD STATISTICS

5.1.1 PITTSBURGH REGISTRY OF INFANT MULTIPLETS DATA

To date, 881 participants have been enrolled in the Pittsburgh Registry of Infant Multiplets. Twins enrolled range from newborn to age 14 years. Enrollment from August 2008 to March 2010 was 144 sets of multiplets including 31% male/male twin pairs, 28% female/female twin

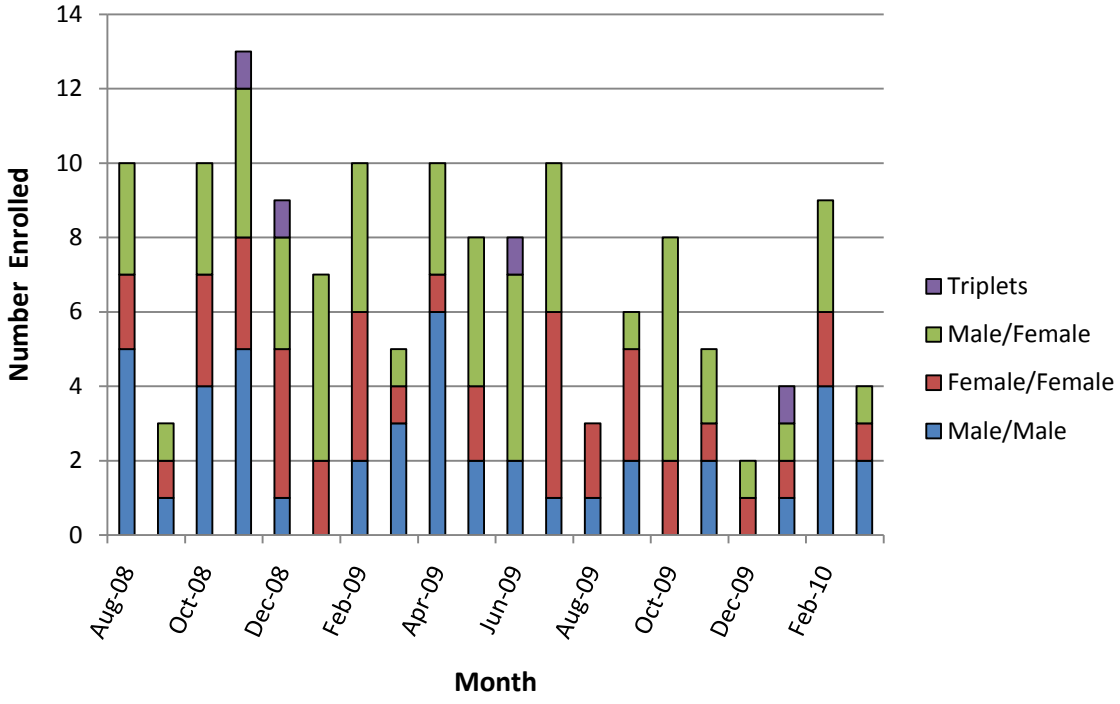

Figure 3. Monthly PRIM Enrollment by Multiplet Type

pairs, 38% male/female twin pairs, and 3% triplets. This is further broken down by month (See

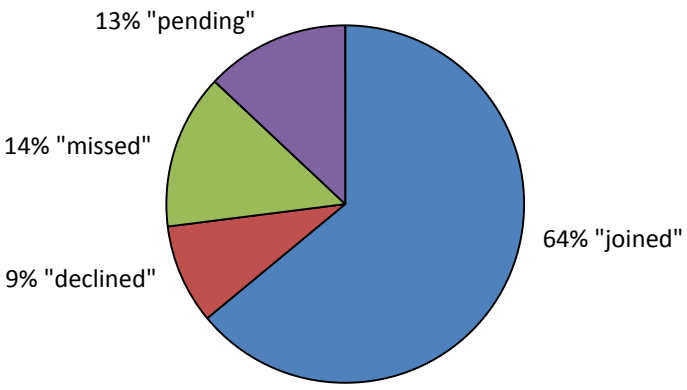

Figure 4. Enrollment Classification of Eligible Participants

Figure 3). Monthly distribution of twin births does not have any specific pattern based on seasonality, based on Spearman regression analysis (p=0.828). The rate of families classified as "declined," "missed," and "pending" in comparison to those who are "joined" can be seen in Figure 4.

5.1.2 CEDAR DATA

Of the 500 CEDAR participants, 127 developed SUD. The majority (120) developed cannabis use disorder, one individual develop cocaine use disorder, and five had opioid use disorder. Thirteen individuals had all three types of SUD. Race comparisons were made on the effectiveness of the various liability indices, and the standard statistics can be seen in Table 2 below. For all indices but TLI, the means for whites and blacks are very similar. Consistent with this, the t-test for this data shows that the TLI is the only index where significant differences between the races are seen (Table 3).

Table 2. Standard Statistics Comparing Race and Liability Indices

Liability Index	Race	N	Mean	SD	SE
TLI	W	378	-0.0773	1.002	0.055
	B	106	0.2539	0.948	0.092
6MLI	W	378	-0.3872	0.406	0.021
	B	106	-0.3724	0.435	0.042
6BLI	W	378	0.1557	0.616	0.032
	B	106	0.1376	0.618	0.06
6ALI	W	378	1.06	1.032	0.053
	B	106	1.02	1.078	0.105

Table 3. T-test Comparing Liability Indices Between Race Groups

Liability Index	Race	Mean	SD	t	df	p	Mean Difference
TLI	White	-0.0773	1.0015	-3.045	482	0.002	-0.3313
	Black	0.2539	0.9476				
6MLI	White	-0.3872	0.4065	-0.326	482	0.744	-0.0148
	Black	-0.3724	0.435				
6BLI	White	0.1557	0.6158	0.267	482	0.79	0.0181
	Black	0.1376	0.6183				
6ALI	White	1.06	1.032	0.366	482	0.714	0.042
	Black	1.02	1.078				

5.1.3 TWINSBURG DATA

2006/2007 DATA

Of the total 612 individuals who participated, 365 were female and 245 were male. One twin pair did not report gender. No significant difference was found in the age between the sexes (Females: N = 365, Mean = 13.75 years, SD = 2.46; Males: N = 245, Mean = 13.53 years, SD = 2.45; P = 0.283) (Moss, 2008).

2009 DATA

Of the total 380 individuals who participated, 275 were female and 105 were male. No significant difference was found in the age between the sexes (Females: N = 275, Mean = 19.88 years, SD = 4.38; Males: N = 105, Mean = 19.42 years, SD = 4.71; P = 0.509).

Additional demographic and general data that was collected from the 2009 participants showed that 13.7% of twin pairs also participated in the 2006 study, 5.8% of twin pairs also participated in the 2007 study, and 4.7% of twin pairs also participated in both the 2006 and 2007 studies. Twin participants came from all over the country, with most residing in Ohio and Pennsylvania presumably due to the location of the festival. Four twin pairs were from other countries, including Germany and Hungary. The majority (83.2%) of twins identified themselves as European-American, and 6.3% were African-American. The remainder of twins was various combinations of biracial ancestry.

Zygosity Determination

For the 2006 and 2007 Twinsburg studies, parents completed zygosity questionnaires for all same-sex twin pairs. 206 questionnaires were completed for both years combined. The zygosity and gender composition of all twin pairs from 2006 and 2007 can be seen in Table 4 (Moss, 2008).

Table 4. Zygosity and Gender Composition of 2006/2007 Twin Pairs

Zygosity	Female	Male	Female/Male	Total
MZ	119	84		203
DZ	39	18	25	82
Total	158	102	25	285

For the 2009 Twinsburg study, each same-sex twin pair completed the zygosity questionnaire. 183 zygosity questionnaires were completed by the 2009 participants. Two female twin pairs gave discrepant results, which were unable to be classified by zygosity. The zygosity and gender composition of all twin pairs from 2009 can be seen in Table 5.

Table 5. Zygosity and Gender Composition of 2009 Twin Pairs

	Female	Male	Female/Male	Total
MZ	119	44		163
DZ	13	5	7	25
Total	132	49	7	188

5.2 STATISTICAL ANALYSIS

5.2.1 DESCRIPTIVE STATISTICS

Participants from the 2006 and 2007 Twinsburg studies were combined, such that anyone who participated in both years was included only once under the data where they were older. Descriptive statistics comparing male and female twins in this sample for the index derived by summation of six binary items can be seen below in Table 6. Likewise, descriptive statistics for those male and female twins in the Twinsburg 2009 and those index males CEDAR samples are also included. Overall, summed scores were higher for the male twins than for the females twins in both Twinsburg samples. For the final twin analyses, the 2006, 2007, and 2009 Twinsburg samples were merged together to form one sample called “merged Twinsburg.” Below, descriptive statistics for the index derived by summation of six binary items can be seen comparing twin 1 and twin 2 in the Twinsburg 2006/2007 sample, Twinsburg 2009 sample, and

merged Twinsburg sample. The Twinsburg 2009 sample has, on average, lower mean scores than the Twinsburg 2006/2007 sample. In the Twinsburg samples comparing twin 1 and twin 2, the scores between the twins are almost identical. The merged Twinsburg mean scores are in between the Twinsburg 2006/2007 and Twinsburg 2009 scores.

Table 6. Descriptive statistics for the index derived by summation of six binary items for all samples

Sample	Participant	N	Mean	SD	Range
Twinsburg 2006/2007	Male twins	209	2.641	1.481	0 - 6
	Female twins	323	2.068	1.308	0 - 6
Twinsburg 2009	Male twins	103	1.617	1.279	0 - 5
	Female twins	277	1.319	1.212	0 - 5
Twinsburg 2006/2007	Twin 1	239	2.255	0.095	0 - 6
	Twin 2	239	2.289	0.095	0 - 6
Twinsburg 2009	Twin 1	190	1.495	0.093	0 - 5
	Twin 2	190	1.416	0.092	0 - 5
Merged Twinsburg	Twin 1	429	1.97	1.449	0 - 6
	Twin 2	429	1.93	1.46	0 - 6
CEDAR	Index males	500	1.047	1.032	0 - 5

Additionally, descriptive statistics were computed for the merged Twinsburg sample for the IRT-derived TLI comparing Twin 1 and Twin 2. These data can be seen below in Table 7. There was no difference for TLI between Twin 1 and Twin 2 from the merged Twinsburg sample.

Table 7. Descriptive statistics for merged Twinsburg sample IRT-derived TLI

Twin	N	Mean	SD	Range
Twin 1	456	0.592	0.731	0.35 - 2.42
Twin 2	456	0.592	0.747	0.35 - 2.42

5.2.2 SURVIVAL ANALYSIS

As seen in Table 8, all four liability indices are significant predictors of SUD in the entire sample as well as the white sample. Whereas the 6MLI and 6BLI hazard ratios are somewhat higher than that for the full TLI, the differences are not significant. The confidence intervals for all

Table 8. Liability indices Cox regression analysis results

Sample	N	Liability Index	B	SE	Wald	df	Hazard Ratio	95% CI	P
All	500	TLI	0.529	0.101	27.193	1	1.70	1.39 - 2.07	<0.001
		6MLI	0.862	0.163	27.825	1	2.37	1.72 - 3.26	<0.001
		6BLI	0.612	0.102	25.884	1	1.84	1.46 - 2.33	<0.001
		6ALI	0.356	0.071	24.993	1	1.43	1.24 - 1.64	<0.001
Whites	378	TLI	0.571	0.120	22.520	1	1.77	1.40 - 2.24	<0.001
		6MLI	0.991	1.890	27.391	1	2.70	1.86 - 3.91	<0.001
		6BLI	0.681	0.145	22.108	1	1.98	1.49 - 2.63	<0.001
		6ALI	0.404	0.088	21.235	1	1.50	1.26 - 1.78	<0.001
Blacks	106	TLI	0.234	0.221	1.119	1	1.26	0.82 - 1.95	0.290
		6MLI	0.402	0.371	1.175	1	1.49	0.72 - 3.09	0.278
		6BLI	0.264	0.258	1.049	1	1.30	0.79 - 2.16	0.306
		6ALI	0.170	0.144	1.381	1	1.19	0.89 - 1.57	0.240

indices overlap, with 6MLI being nominally the best predictor of SUD, and 6ALI, the worst. Consistent with a previous study (Vanyukov et al., 2009), TLI is not useful in predicting SUD for Blacks. Also consistent with the prior results, the other three indices were not predictive for this ethnic group (the N for Whites and Blacks does not add up to the total for entire sample because there are a small proportion of other races in the CEDAR sample).

TLI - full 45-item transmissible liability index; 6MLI - index derived by IRT analysis of six multicategory items; 6BLI - index derived by IRT analysis of the same six items, converted into binary; 6ALI - index derived by summation of the same six binary items

5.2.3 CORRELATION ANALYSIS

Intraclass correlations (ICC) between MZ and DZ twins from the Twinsburg sample were computed for both the index derived by IRT analysis of six items converted into binary (6BLI) and the index derived by summation of the same six binary items (6ALI). This analysis was used to give an indication of how strongly the indices resemble one another in MZ versus DZ twins. Values corrected and uncorrected for age are included for both indices.

Table 9. Intraclass Correlations between MZ and DZ twins for 6ALI and 6BLI

Liability Index	Zygosity	N	Intraclass Correlation	95% CI	P
6ALI uncorrected	MZ	338	0.56	0.48 - 0.63	<0.001
	DZ	64	0.31	0.07 - 0.52	0.006
6ALI corrected	MZ	338	0.52	0.44 - 0.59	<0.001
	DZ	64	0.26	0.01 - 0.47	0.020
6BLI uncorrected	MZ	338	0.53	0.45 - 0.60	<0.001
	DZ	64	0.19	-0.05 - 0.42	0.060
6BLI corrected	MZ	338	0.47	0.38 - 0.55	<0.001
	DZ	64	0.11	-0.14 - 0.35	0.186

6BLI - index derived by IRT analysis of six items, converted into binary; 6ALI - index derived by summation of the same six binary items

As seen in Table 9 consistent with this, all correlations for MZ twins are statistically significant. ICC for both indices for MZ twins are very similar, as well as 95% confidence intervals. The DZ correlations are significant only for 6ALI.

5.2.4 STRUCTURAL EQUATION MODELING

Values uncorrected for age were used in SEM analyses. Although the relationship between the indices and age is significant, it is weak ($\beta \approx -0.3$ for BLI and -0.2 for ALI) and, from assessing the respective scatterplots, nonlinear. It is thus possible that the gains from regressing out the

age effect would be offset by violating the regression analysis assumptions and introducing an additional error element in the calculations. This may explain the higher intra-pair correlations estimated for the uncorrected indices.

The standard ACE model was initially fitted to the 6ALI merged Twinsburg data, while the standard ADE model was fitted to the 6BLI data. The ACE model was selected for the 6ALI

Table 10. Univariate Model Fitting for 6ALI and 6BLI Indices

Model	χ^2	df	P	AIC	Δχ2	Δdf	P Δ	ΔAIC
6ALI								
ACE	0.043	3	0.990	-5.883				
AE	0.191	4	0.996	-7.809	0.074	1	0.786	-1.926
CE	6.640	4	0.156	-1.360	6.523	1	0.011	4.523
E	109.409	5	<0.001	99.409	109.366	2	0	105.366
6BLI								
ADE	1.053	3	0.788	-4.947				
AE	1.834	4	0.766	-6.166	0.781	1	0.377	-1.219
E	115.302	5	<0.001	105.302	114.249	2	0	110.249

because r_{MZ} (0.56) < $2r_{DZ}$ (0.62). The ADE model was selected for the 6BLI because r_{MZ} (0.53) > $2r_{DZ}$ (0.38). The goodness of fit of the ACE model for the 6ALI and ADE for the 6BLI can be seen based on the low χ^2 value and high P value.

Whereas the full ACE and ADE models provide good fit for respective indices, the AE nested model is best fitting overall for both indices. The CE model provides a significantly worse fit (P=0.01) for 6ALI than the full ACE model, whereas the AE model is well fitting and more parsimonious for both 6ALI and 6BLI. The E models fail as expected, incompatible with intrapair correlations. Thus, the data suggests that the heritability of the indices is due to an additive genetic component (A), accounting for approximately half of the variance in the indices. Nongenetic sources of twin similarity (C) do not appear to play any role. Unique environment

(E) contributes the rest of the variance. Overall, heritability is estimated at 56 and 53% for 6ALI and 6BLI, respectively.

Table 11. Univariate Model Fitting: Best Fitting Model

Index	Variance Component		Fit Index			
	a^2 (95% CI)	e^2 (95% CI)	χ^2	df	P	AIC
6ALI	0.56 (0.48-0.63)	0.44 (0.37-0.52)	0.191	4	0.996	-7.809
6BLI	0.53 (0.45-0.60)	0.47 (0.40-0.55)	1.834	4	0.766	-6.166

6.0 DISCUSSION

Understanding the factors that contribute to variation in the liability for SUD is an important concern in the public health field. Not being able to quantify the risk an individual has to develop SUD hinders etiologic research and prevents intervention strategies from being implemented. Characteristics an individual has, such as in areas of behavior, cognition, emotion, and adjustment, are factors that may influence an individual's risk to develop SUD (Kirisci et al., 2009). Knowing if an individual is more susceptible to SUD gives the opportunity to intervene and possibly assist in the prevention of developing the disease.

One of the specific aims of this study was to maintain and expand the Pittsburgh Registry of Infant Multiplets (PRIM). This registry serves as a source of participants that can participate in biometrical genetic research to better understand the genetic influence to SUD. As explained, the Pittsburgh Registry of Infant Multiplets has continued to grow since its start in 1996. Currently, there are more than 880 sets of twins or multiplets in the registry which are recruited at Magee-Womens Hospital in Pittsburgh, PA. The nursing staff at MWH is very helpful in assisting with recruitment by speaking with the mothers to obtain their permission for the registry recruiter to contact them about joining. Most mothers choose to join the PRIM and are often enthusiastic about receiving information about future research. Less often, mothers decline enrollment and there appears to be various reasons for this. Some feel that they will be too busy to participate, while others are not comfortable with or are uninterested in research participation.

A small subset of eligible mothers express interest in the registry and would like consents sent home, but these are rarely returned. Another subset of eligible mothers is missed during recruitment because they are unavailable to speak. Most often this is because they are in the NICU with their children and not on the post partum unit where recruitment takes place. From August 2008 to March 2010, no researchers contacted the PRIM coordinator about participants for research studies, but past research focused on behavioral genetics. The PRIM, like most registries, provides a large sample of participants for future research studies. Currently, participants in PRIM range from newborn to age 14 years, and continued maintenance and growth of the registry will allow for the use of a wide range of study subjects. Upholding PRIM in the future will make longitudinal research more feasible and accessible at the university.

This study expands upon the TLI development to construct and evaluate the efficacy of abbreviated liability indices as quantitative measurements in predicting SUD risk, in comparison with the TLI. In the CEDAR sample, the original TLI based on the 45-item questionnaire was used, in addition to the development of three new indices that have previously not been examined as indices for liability to SUD. Four indices were evaluated in the CEDAR sample in this study: original TLI, index derived by IRT analysis of six multicategory items (6MLI), index derived by IRT analysis of six binary items (6BLI), and index derived by summation of the same six binary items (6ALI). Survival analysis indicated that all four indices were significant predictors of liability to SUD in the entire CEDAR sample as well as the white subsample, but not in the black subsample. This result was consistent with the prior findings for TLI where it is unclear whether TLI scores have comparable validity across racial groups (Vanyukov et al, 2009; Hicks et al., in press).

It is expected that an IRT-derived index and its additive counterpart are highly correlated. Using an additive form of the TLI in the Minnesota Twin Family Study (MTFS), Hick et al. (in press) have recently confirmed predictive validity and high heritability of the index. The present study used similar methodology with six binary summed items as an index (6ALI). Its heritability, while lower than for TLI, did not substantially differ from that of 6BLI.

The index derived by IRT analysis of six binary items (6BLI) and the index derived by IRT analysis of six multicategory items (6MLI) were not worse predictors of SUD that the TLI. Thus, Hypothesis 2.1, which states that the predictive ability of abbreviated indices is lower than that of the TLI, was not supported. The 6MLI and 6BLI both proved to be at least as predictive as the TLI, and the 6ALI somewhat less predictive, but insignificantly as well.

The findings that the 6MLI and 6BLI are stronger predictors of SUD than the TLI, if true, would be unexpected and important for future research in behavioral genetics. Rather than using the full 45-item questionnaire set to estimate TLI, these abbreviated 6-item scales can possibly be used if their heritability estimates prove to be high. Because the shorter indices have lower heritability (h^2 = 0.51 and 0.53) than the full TLI (h^2 = 0.79), however, different subsets of items may need to be tested to optimize liability evaluation. Shorter questionnaires would have logistical advantages and provide the opportunity to enroll more participants in field research (e.g., Twinsburg). Further studies will need to replicate the method using shorter indices to determine its validity.

Estimation of the relative contributions of the genetic and environmental sources of variation each component to a trait in quantitative genetic studies is important for determining further research directions . One of the main foci of the present study was to evaluate the genetic and environmental contributions to the variance of the liability indices to SUD and find a model

that fits the data (Rijsdijk and Sham, 2002; Posthuma et al., 2003). The heritability estimates are lower than determined in previous studies using the full TLI for male and female twins (Vanyukov et al., 2009; Hicks, Iacono, and McGue, In Press). This result may be due to the usage of the abbreviated indices in model fitting unlike previous work which used the full TLI. Another reason may be the addition of an older sample from Twinsburg 2009, which was joined with the younger Twinsburg 2006/2007. The sample heterogeneity may have affected the measurement precision, resulting in a larger error than that attendant to the TLI measurement in the more homogeneous CEDAR sample. In the variance component analysis, this error would contribute to the unique environment component. Indeed, it is this variance component that determined the non-heritable variation in both abbreviated indices. Interestingly, whereas heritability of both indices is significant, it is virtually identical, suggesting that in this case IRT did not improve the genetic informativeness of the index. This may be related to the fact that the measurement error due to the shortness of the 6-item pool is not amenable to IRT. It is unlikely that the age heterogeneity substantially increased the measurement error, because the effect of age would tend to increase the shared environment component, as there is no significant difference between the zygosity types in the strength of the relationship and the age effect would thus contribute to the twins' nongenetic similarity. However, the best-fitting models (AE) for neither index include a shared environment component.

One of the limitations of this study was that genetic analyses by sex were not performed because the number of DZ twins is too few and correlations are not significant. Previous research shows that the TLI has high heritability in the male twin sample ($h^2 = 0.79$), but this did not hold true for the female twin sample (Vanyukov et al., 2009). However, for the summed index, heritability is virtually equal in males and females based on research by Hicks et al. (in

press) and high (h^2 = 0.76). In the future, the addition of more participants to the Twinsburg sample and PRIM may allow for the analysis of sex differences in the sample.

As Conway et al. (in press) show, a new measurement approach for addiction liability is needed, as existing methods do not give researchers the best information available. A prototype for such approach is provided by the TLI. The methods to be developed are likely to use the advantages of IRT analysis for measuring risk for SUD. This study was a step in the development of this methodology.

7.0 CONCLUSIONS

In this study, we evaluated the effectiveness of newly developed liability indices in predicting risk for SUD in comparison with the original transmissible liability index. We also investigated the contributions of genetic and environmental components to the variance of liability to SUD using the biometrical genetic approach. Results from this study include:

1. The Pittsburgh Registry of Infant Multiplets (PRIM) has continued to gain enrollment, with an increase of 17% over the past 19 months. PRIM continues to serve as a resource for researches at the University of Pittsburgh by being a source for participants and information for future longitudinal studies.

2. In the CEDAR sample, the original TLI was again found to be a significant predictor of risk to SUD. In addition, the shortened indices were found to predict SUD as well as the TLI does.

3. In the Twinsburg sample, the abbreviated liability indices were found to be moderately heritable, with variation explained by additive genetic effects (h^2 = 0.51 and 0.53) and unique environmental influences. Heritability for the indices is lower than that for the full TLI, and further biometrical genetic studies will need to replicate this to determine the validity and utility of new indices.

Expansion of the current study would be beneficial to explore the further utility of shortened liability indices in measuring risk for SUD. A larger sample, possibly recruiting more participants in Twinsburg or using PRIM subjects, could allow for analysis of sex and age heterogeneity. Additionally, important future research that can be expanded from this study would be the investigation of associations of candidate gene polymorphisms with liability to SUD to further understand of the mechanisms of variation in this complex trait.

7.1 PUBLIC HEALTH SIGNIFICANCE

There is no doubt that substance abuse is a problem in the Unites States, with millions of Americans affected by the disease each year. It is important to continue to find effective ways for targeted prevention and treatment. This study, examining liability indices highly predictive of SUD, contributes important information germane to this problem.

Based on research that indicates that heritability of SUD liability is high, there are likely specific genes contributing to variation in this trait. Future work will allow determining which genes account for heritability. This information and the understanding of inheritance patterns for this complex trait may open up an exciting new field of practice in the future.

APPENDIX A: LIABILITY INDEX

Over the past six months,	Not True	Somewhat or Sometimes True	Very True or Often True
1. ....have you destroyed things that belonged to others?	○	○	○
2. ....have you broken rules at school, work, or elsewhere?	○	○	○
3. ....have you been impulsive or acted without thinking?	○	○	○
4. ....have you been biting your fingernails?	○	○	○
5. ....have you picked your skin or other parts of your body?	○	○	○
6. ....have you stolen something?	○	○	○
7. ....have you had trouble finishing assignments?	○	○	○
8. ....have you had trouble concentrating or paying attention?	○	○	○
9. ....have you had trouble sitting still?	○	○	○
10. ....have you been pretty honest?	○	○	○
11. ....have you been getting into a lot of fights?	○	○	○
12. ....have you had a hot temper?	○	○	○
13. ....have you threatened to hurt people?	○	○	○
14. ....have you failed to pay your debts or meet other financial responsibilities?	○	○	○

	Never True	Occasionally True	Mostly True	Aways True
15. Do you interrupt people when they are speaking?	○	○	○	○

Within the past year, did you..	Yes	No
16. ....frequently do things without first thinking about the consequences?	○	○
17. ....steal things?	○	○
18. ....have a bad temper?	○	○
19. ....threaten to hurt people?	○	○
20. ....frequently do risky or dangerous things?	○	○
21. ....get into more fights than most people your age?	○	○
22. ....have trouble concentrating?	○	○
23. ....did people stare at you?	○	○
24. ....get tired very quickly when you exerted yourself?	○	○

	Yes	No
25. Did you get ever into trouble a lot for talking out of turn in school or talking without the teacher calling on you or for bothering people a lot?	○	○
26. Did you ever think about a specific plan to commit suicide?	○	○
27. Did you ever do things to annoy people a lot like grabbing other children's hats?	○	○
28. Did you ever frequently annoy people on purpose to get revenge?	○	○
29. Did you ever have difficulty staying in line in the supermarket or waiting for your turn while you were playing with other children?	○	○
30. Did you ever blurt out answers to questions before they had been completed or get into trouble for being impulsive?	○	○
31. Did you ever talk too much in such a way that your teachers, parents or friends complained about it?	○	○
32. Did you ever get into trouble because you would do things without thinking about them first, for example, running into the street without looking?	○	○

The following four questions is a list of common health problems. Read the list carefully and answer yes or no for each item. Do your best to read each item carefully and answer as honestly as you can

	Yes	No
33. Have you experienced bone pain?	○	○
34. Have you experienced joint pain?	○	○
35. Have you experienced muscle pain?	○	○
36. Have you had headaches?	○	○

	Yes	No
37. Have you ever run away from home?	○	○
38. Have you ever taken your parent's car without their permission?	○	○
39. Have you ever stolen something from a store or from someone else?	○	○
40. Have you ever been in a physical fight on school property?	○	○
41. Have you ever threatened someone with violence?	○	○
42. Have you ever had violent outbursts at school or been physically aggressive with others?	○	○
43. Have you ever stolen a car?	○	○
44. Have you ever resisted arrest?	○	○
45. Have you ever assaulted someone?	○	○
46. Have you ever assaulted someone with a deadly weapon?	○	○

	Yes	No
47. Have you been in a lot of physical fights (3 or more) or have you physically assaulted someone? **Do not answer "yes"** to this question if thefights or assaults were in response to self defense or a result of protecting someone else (e.g., spouse abuse or child abuse)	○	○

The following is a list of statements a person might use to describe her/his attitudes, opinions, interests, and other characteristics. Read the statement and decide whether it is true or false.

	True	False
48. My future looks very bright to me.	○	○
49. When faced with a decision, I usually take time to consider and weigh all aspects.	○	○
50. I often act without thinking.	○	○
51. I like to stop and think things over before I do them.	○	○
52. When I have to stand in line, I never try to get ahead of others.	○	○
53. It is very easy for me to see the bright side of things.	○	○
54. I admit that I sometimes take pleasure in hurting someone physically.	○	○
55. People consider me forceful.	○	○
56. I feel that life has handed me a raw deal.	○	○
57. I usually make up my mind through careful reasoning.	○	○
58. I know that people have purposely spread false rumors about me.	○	○
59. People rarely try to take advantage of me.	○	○
60. I worry about terrible things that might happen.	○	○
61. I have often been lied to.	○	○
62. I find it very easy to enjoy life.	○	○
63. Sometimes I just like to hit someone.	○	○
64. I always seem to have something pleasant to look forward to.	○	○
65. Some people oppose me for no good reason.	○	○

APPENDIX B: ZYGOSITY QUESTIONNAIRE

ABOUT YOUR TWIN QUESTIONNAIRE

For each question, please fill in completely only one of the circles

Are you and your twin of opposite sex? **YES** ◯ **NO** ◯

If YES, do not continue with the following questions,

	None	Only slight difference	Clear difference
1. Are there differences in the shade of you and your twin's hair color?	◯	◯	◯
2. Are there differences in the texture of you and your twin's hair (fine or coarse,straight or curly, etc?)	◯	◯	◯
3. Are there differences in the color of you and your twin's eyes?	◯	◯	◯
4. Are there differences in the shape you and yourr twin's ear lobes?	◯	◯	◯

5. Did you and your twin's teeth begin to come through at about the same time?
   - ◯ We had matching teeth on the same side come through within a few days of each other
   - ◯ We had matching teeth on opposite sides come through within a few days of each other
   - ◯ We had different teeth come through within a few days of each other
   - ◯ Our first teeth did not come through within a few days of each other
   - ◯ I don't know

6. Do you know of any physical differences between you and your twin that are not clear from looking at you (e.g. differences in internal organs?)

YES	NO	Don't Know
◯	◯	◯

7. If there are differences between you and your twin, are they due to problems at birth, an accident or illness?

YES	NO	Don't Know	There are no differences
◯	◯	◯	◯

	YES	NO
8. Do you know you and your twin ABO blood group and Rhesus (Rh) factors?	◯	◯

IF YES, are they:

Yours:	A ◯	B ◯	AB ◯	O ◯	Rh+ ◯	Rh- ◯
Your Twin:	A ◯	B ◯	AB ◯	O ◯	Rh+ ◯	Rh- ◯

9. As you and your twin have gotten older, has the likeness between you:

Remained the same	Become less	Become more
◯	◯	◯

10. When looking at a new photograph of you and your twin, can your family tell you apart (without looking at your clothes or using any other clues)?

◯ **Yes, easily** ◯ **Yes, but it is hard sometimes** ◯ **No, I often confuse them in photographs**

11. Do any of the following people ever mistake you and your twin for each other?

	Yes, often	Yes, sometimes	Rarely or never	
Your mother	◯	◯	◯	◯ No Contact with mother
Your father	◯	◯	◯	◯ No Contact with father
Brothers or sisters	◯	◯	◯	◯ No other siblings
Other relatives	◯	◯	◯	
Teachers	◯	◯	◯	◯ Not in School
Parents' close friends	◯	◯	◯	
Parents' casual friends	◯	◯	◯	
People meeting both of you for first time	◯	◯	◯	

12. If the you are ever mistaken for one another, does this ever occur when you are together?

Yes, often	Yes, sometimes	No, almost never	We are not mistaken for one another
◯	◯	◯	◯

13. Would you say you and your twin:

◯ Are as physically alike as "two peas in a pod" (virtually the same)
◯ Are as physically alike as brothers and sisters
◯ Do not look very much alike

14. Have you ever been told by a health professional (for example doctor, nurse, consultant) that you and your twin are identical (monozygotic) or non-identical (fraternal, dizygotic)?

Yes, identical	Yes, non-identical	NO
◯	◯	◯

15. Do you think that your are identical or non-identical?

Identical ◯ **Non-identical** ◯

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