TITLE

Familial Loading and Schizophrenia Risk Indicators

ABSTRACT

Schizophrenia is a severe mental illness, causing impairments in multiple psychosocial domains. Individuals with schizophrenia are often unable to maintain gainful employment to which private insurance is tied, and commonly must receive treatment in public-sector settings. For this reason, the mental health services at Grady Health System treat a disproportionately high number of individuals with schizophrenia compared to other local psychiatric services. The PI for this grant proposal is trained as a community psychiatrist and intends to focus his career on schizophrenia research in the public-sector setting. He is in his second year on faculty at Emory and is based full-time at Grady, where he sees both inpatients and outpatients with schizophrenia. This research project will extend and add to a study conducted by the PI over the past year, The ARIS Project (Associations among Risk Indicators in Schizophrenia). Five risk markers will be studied in patients with schizophrenia, their first- and second-degree relatives, and normal comparison participants: (1) impairments in olfactory identification (IOI), (2) impairments in working memory (IWM), (3) neurological soft signs (NSS), (4) minor physical anomalies (MPA), and (5) dermatoglyphic abnormalities (DA), which are subtle abnormalities of the fingerprint dermal ridge patterns. Like The ARIS Project, the project proposed herein will focus on these 5 risk markers, but it also will add two important and innovative facets to this line of research. First, patients and their relatives will be further classified based on a thorough assessment of family history, including an assessment of singleton (only one person in the family affected) versus multiplex (more than one person in the family affected) status. Thus, familial loading (or genetic load for the illness based on details of the family history) will be estimated for each patient and relative. Second, the project will also examine a specific genetic polymorphism that has received wide attention among schizophrenia researchers, but not in the context of these other risk indicators—the functional polymorphism in the catechol-O-methyltransferase (COMT) gene. This project will rely on data recently collected from 75 ARIS participants (requiring only a supplemental assessment from them) and will recruit an additional 125 participants for the full assessment. The project will seek to better understand the five risk indicators, especially in the context of familial loading, and will investigate associations between the risk indicators and the COMT polymorphism. The PI is interested in pursuing larger research projects on risk prediction and risk stratification for schizophrenia, and this project will collect important data for an R01 proposal. The PI's mentor for this research is Dr. Elaine Walker.

SPECIFIC AIMS AND HYPOTHESES

Aim #1: *To compare 5 subgroups on the 5 schizophrenia risk indicators of interest.* Similar to the research on MPA by Griffiths et al. (1998), 5 subgroups will be studied: (1) patients with schizophrenia from multiplex families (*Pt-Multi*), (2) relatives of patients from multiplex families (*Rel-Multi*), (3) patients with schizophrenia from singleton families (*Pt-Singl*), (4) relatives of patients from singleton families (*Rel-Singl*), and (5) demographically-comparable normal comparison subjects with no evidence of psychotic illness in first- or second-degree relatives (*Norm*). These samples will be drawn from a low-income, predominantly African American, urban population, which is an advantage of this study design because of the lack of research in this population, despite the prominent burden of disease within it.

- Hypothesis #1A: Three of the risk indicators (IOI, IWM, NSS) are predicted to be primarily markers of *familial / genetic* risk and will therefore show the following pattern:

Pt-Multi/Rel-Multi > Pt-Singl/Rel-Singl > Norm.

That is, multiplex family members (patients and unaffected relatives) will have greater impairments in olfactory identification and working memory, compared to singleton family members (patients and unaffected relatives), who will in turn have greater impairments than normal controls.

- Hypothesis #1B: Two of the risk indicators (MPA, DA) are predicted to be primarily markers of *developmental / environmental* insult and will therefore show the following pattern: *Pt-Singl > Pt-Multi > Rel-Multi/Rel-Singl > Norm.*
  That is, singleton patients will have more morphologic abnormalities than multiplex patients, and relatives of all patients will have fewer such abnormalities, though more than normal controls.

Aim #2: *To compare 5 subgroups on the prevalence of the COMT polymorphism, and to assess for associations between the polymorphism and the risk indicators.* Drawing on the work of Goldberg et al. (2003), the Val^{158}Met functional polymorphism affects prefrontal function, and the high-activity *Val* allele may be a genetic risk factor for schizophrenia. This project will genotype participants at this polymorphism, to compare the patients and family members from singleton and multiplex families. Again, this project will benefit from the sample characteristics because most past research on the COMT polymorphism has been conducted in Caucasian samples only.

- Hypothesis #2A: Patients and unaffected relatives from multiplex families will have a higher prevalence of the *Val* allele than singleton patients and unaffected relatives.
- Hypothesis #2B: *Val* homozygotes will have more prominent IOI, as well as more prominent IWM, compared to *Met* carriers.

Exploratory Aim: *To assess the utility of a multivariate prediction model in determining subgroup membership based on the 5 risk indicators and the Val^{158}Met genotype.* Discriminant function analysis or other modeling techniques will be used to determine the optimal set of predictors. Using the obtained discriminant functions, the sample can be reclassified to approximate the accuracy of prospective identification efforts based on the predictor variables (Carter et al., 2002). In such a model, specific interactions can be examined, including potential gene-environment interactions (e.g., COMT polymorphism **x** MPA, or familial load **x** DA).

BACKGROUND REVIEW

Schizophrenia is a major mental illness associated with prominent impairment in psychosocial functioning (in the domains of educational attainment, employment, relationships, etc.). Many patients with schizophrenia are treated in the public sector, and Grady Health System cares for a large number of patients with schizophrenia. For example, on the inpatient psychiatric units at Grady Memorial Hospital, 65% of patients have schizophrenia or a related illness such as schizophreniform disorder or schizoaffective disorder (Compton et al., 2005a; Compton et al., 2005b). Several outpatient services at Grady, including the adult outpatient mental health clinic (Florida Hall), a specialty program for homeless or noncompliant patients (Community Outreach Services), and the intensive day treatment programs (Psychosocial Rehabilitation and the FOCUS program at the Haverty building) focus predominantly on patients with severe and persistent mental illnesses such as schizophrenia. Continued research on this psychiatric disorder is needed, especially in terms of risk identification, which is a prevention-oriented topic that is the focus of the PI's research interests.

What are the main issues to be addressed by this proposed research project?

Some clinically unaffected relatives of patients with schizophrenia show neurobiological impairments similar to those seen in patients. An endophenotype, which is a concept similar to a

risk indicator, is a trait that reflects an underlying genetic liability for schizophrenia, and may thus identify unaffected relatives who are carriers of the genetic risk. Other markers may be indicators of environmental risk for the illness. Among the dozen or so putative risk indicators that have been studied in schizophrenia, *5 risk indicators that have been studied individually, but that have not been studied together within the same sample, will be studied in this project*: impairments in olfactory identification (IOI), impairments in working memory (IWM), neurological soft signs (NSS), minor physical anomalies (MPA), and dermatoglyphic abnormalities (DA). Although each of these risk indicators has received some research attention in first-degree relatives, very few studies have focused on the important issue of *familial loading using singleton and multiplex families*. Similar to studying monozygotic and dizygotic twins, considering the level of familial loading (rather than viewing all first-degree relatives as genetically equivalent without further distinction), may be helpful in elucidating the genetic and environmental components of the etiology of schizophrenia. It is expected that unaffected relatives from singleton families have a lower genetic loading than those from multiplex families (Faraone et al., 2000). This project also posits that singleton probands may have a higher prevalence of markers of abnormal fetal development than multiplex probands because they are more likely to have been affected by an environmental (intrauterine) insult in the absence of obvious genetic loading. This study will expand the literature by carefully assessing the familial loading of relatives. In addition to these 5 risk indicators, *the Val^{158}Met functional polymorphism of the catechol-O-methyltransferase gene, which has been studied in relation to schizophrenia and cognitive functions, will also be studied*, as described further in a later section.

What is The ARIS Project, and how is this proposal related to it?

The proposed project will extend and expand upon a research project that is nearing completion, The ARIS Project (Associations among Risk Indicators in Schizophrenia). The PI received the "Young Minds in Psychiatry" award from the American Psychiatric Institute for Research and Education (APIRE) / AstraZeneca in July 2004. This 1-year, $45,000 grant allowed the PI to begin pursuing research in the area of risk markers in schizophrenia among patients who receive services at Grady Health System. The ARIS Project focuses on investigating the correlations between the 5 specific risk markers listed above. The ARIS Project has been very successful, especially in terms of providing the PI with experience related to participant recruitment and measurement of the 5 risk indicators of interest. Recruitment has improved tremendously from the beginning of the project in August 2004 to the present, and now the project is filling all of its available assessment slots with participants. Data collection for The ARIS Project will end on July 01, 2005. Some preliminary findings of The ARIS Project were presented at the "Evening of Excellence" sponsored by AstraZeneca at the annual meeting of the American Psychiatric Association on Tuesday, May 24, 2005. A mini-print of this poster is attached in **Appendix A**. Analyses of the ARIS dataset will begin in July 2005, and four papers are currently planned, under the mentorship of Dr. Elaine Walker:

1. Correlations between verbal memory domains and olfactory identification,
2. Correlations between neurological soft signs and minor physical anomalies,
3. An examination of dermatoglyphic indices in patients, relatives, and controls, and
4. Overall inter-correlations, factor analysis, and discriminant function analysis using the 5 schizophrenia risk indicators.

What exactly is a schizophrenia risk indicator?

A risk indicator (also termed vulnerability marker) is a trait that is not a recognized symptom of the disease, but that reflects an underlying genetic liability, and may thus identify

unaffected relatives who are carriers of the genetic risk. For brevity, and because there has been very little research on the other risk indicators in the context of familial loading, only IWM will be briefly reviewed herein, as an example of the risk indicator concept. Although IWM, which is likely related to a disruption in the dorsolateral prefrontal cortex, has been found in healthy relatives of patients, only two publications have documented the effect of familial loading (Faraone et al., 2000; Tuulio-Henriksson et al., 2003). This research suggests that in relatives with only one affected first-degree relative, some memory deficits are less pronounced than in members of multiplex families, suggesting an effect of schizophrenia-related familial loading on these functions (Faraone et al., 2000). Similarly, healthy siblings from multiply-affected families performed worse than healthy siblings from singleton families on immediate visual memory and components of visuospatial working memory (Tuulio-Henriksson et al., 2003). All other studies on working memory in family members, except the few studies on monozygotic and dizygotic twins (Cannon et al., 2000), have considered relatives as a single group without classifying them based on familial loading. Coupled with other endophenotypes, working memory may be useful in the development of a multivariate high-risk phenotype (Conklin et al., 2000). While IWM may serve as an endophenotype and may be more prevalent among relatives in multiplex families, in singleton cases, congenital factors may interfere with development, placing individuals at elevated risk for "sporadic" schizophrenia. Thus, in the absence of genetic history, developmental insults may be especially necessary for expression of the illness.

The other four risk indicators of interest, IOI, NSS, MPA, and DA, have also been shown to be elevated in patients with schizophrenia compared to their first-degree relatives, and that first-degree relatives may have elevations in these risk markers compared to normal controls (references for these respective risk indicators among patients with schizophrenia and their relatives include: Kopala et al., 2001; Ismail et al., 1998; Egan et al., 2001; Avila et al., 2003). Again, although that body of research will not be reviewed herein, these risk indicators have been studied in isolation of one another, without consideration of potential correlations and interactions. The ARIS Project has begun to examine these potential correlations. This proposal seeks to extend that research by examining not only the strength of genetic loading based on family history, but also a genetic polymorphism that is of increasing interest to the field.

What is the COMT Val^{158}Met functional polymorphism?

There is accumulating evidence linking a functional polymorphism of the catechol-O-methyltransferase (COMT) gene (mapped to chromosome 22qll) with risk for schizophrenia. COMT is an enzyme involved in extracellular dopamine metabolism. A common functional polymorphism has been described at codon 158 (Val^{158}Met) in the gene coding for COMT, with the low-activity *Met* allele leading to a three- to fourfold reduction in enzyme activity compared to the high activity *Val* allele. The consequences of this are shown below:

Val allele	***Met allele***
High enzyme activity	Low enzyme activity
↳ Greater metabolism of synaptic dopamine	↳ Lower metabolism of synaptic dopamine
↳ Lower synaptic dopamine levels	↳ Higher synaptic dopamine levels
↳ Poorer prefrontal function	↳ Enhanced prefrontal function
↳ Poorer neurocognitive performance in patients, relatives, and controls	↳ Better neurocognitive performance in patients, relatives, and controls

In one study, among patients with schizophrenia (n=26), the *Val* allele was associated with slower reaction time, whereas the *Met* allele was associated with better cognitive stability (Nolan et al., 2004). The *Met* allele has been associated with better performance on executive cognitive

functions compared to the *Val* allele (Rosa et al., 2004). To date, no study has examined the COMT Val^{158}Met polymorphism as a risk indicator in the context of other established risk indicators. In this project, this will be accomplished, and in the context of familial loading.

How will the 5 markers, familial loading, and Val^{158}Met genotype be combined in this project?

Regarding **Aim #1** (comparing 5 subgroups on the 5 schizophrenia risk indicators), hypotheses 1A/1B consider IOI, IWM, and NSS to be markers of genetic risk (endophenotypic) while MPA and DA are markers of non-genetic/environmental/intrauterine risk. These hypotheses are novel because there is virtually no prior research on these risk markers in the context of familial loading. The notion that some risk indicators may be mostly environmentally-determined is consistent with some prior research. For example, Wood et al. (2005) reported that left hippocampal volumes were significantly smaller in a group of adolescents/young adults at very high risk for developing psychosis *without* a family history compared to a similar group *with* a family history of psychosis. (They discussed that subjects with a family history may be more likely to show neuropsychological deficits such as in working memory and olfaction, whereas other individuals might reach a similar degree of risk through environmental influences, such as intrauterine insults.) Regarding **Aim #2** (comparing 5 subgroups on the prevalence of the COMT polymorphism, and assessing for associations between the polymorphism and the risk indicators), a second set of hypotheses (2A/2B) will study the inter-correlations between the Val^{158}Met polymorphism and the risk markers. It is hypothesized that the *Val* allele will be associated with IWM (which would be consistent with prior research), and that the *Val* allele also will be associated with IOI (there have been no studies of this association to date). The **Exploratory Aim** (for which there are no *a priori* hypotheses) will assess all 6 variables, as well as familial loading, in a multivariate prediction model. Such modeling can assess potential gene-environment interactions, and will determine the optimal set of makers and interactions for distinguishing participants. A goal of research on risk factors and markers is to not only better understand the etiology of this complex disorder, but also to advance the possibility of risk prediction in the future. If a battery of simple, non-invasive tests could successfully stratify individuals based on level of risk, then future preventive efforts could target individuals at the highest risk for developing the disorder. This line of research is particularly relevant in light of the sample—more research is clearly needed among urban African American samples, especially those seeking treatment in public-sector settings.

METHODOLOGY

Setting and Sample

This project will be conducted at the Grady Health System, and will focus on low-income, predominantly African American, urban patients with schizophrenia and their family members. The target sample size will be *n*=75 patients, *n*=75 relatives, and *n*=50 controls, for an overall sample size of *n*=200. All relatives included in the study will be free of lifetime or current Axis I disorders, eliminating confounding effects of psychiatric symptoms or medications. More than a third of the sample will consist of participants who completed an ARIS assessment over the last year. By July 01, 2005 (the last day of data collection for The ARIS Project), this will include *n*=38 patients, *n*=27 first-degree relatives, and *n*=36 controls (overall *n*=101), all recruited from Grady Health System. Basic demographic characteristics of this sample include: 57.3% female, 89.2% African American, 49.3% single/never married, 25.7% with less than 12 years of education, and 65.3% unemployed (based on *n*=75).

It is expected that 75% of the original ARIS sample will be available and willing to participate in the supplemental assessment. That is, 28 patients are expected to return (47

additional patients will be needed); 20 relatives are expected to return (55 additional relatives will be needed); and 27 controls are expected to return (48 additional controls will be needed). Thus, the expected number of ***supplemental assessments*** is 75, and the expected number of ***full assessments*** is 125. These numbers are thought to be entirely feasible given the experience of The ARIS Project in terms of working out an effective recruitment strategy (the number of ARIS assessments increased from 20 in the initial 4 months of the project, to 64 in the last 4 months).

In order to continue building this dataset, new participants will be recruited for the full assessment using the same recruitment methods that have been successful for The ARIS Project. Namely, controls will be invited to participate from the waiting room of Medical Clinic I and Medical Clinic II at Grady Memorial Hospital. (See the letter of support from Dr. Clyde Watkins.) Patient and relative recruitment will include two facets. First, recruitment will continue as in The ARIS Project by receiving referrals from clinicians at (1) Florida Hall (the adult outpatient mental health center), (2) Psychosocial Rehabilitation and the FOCUS program at the Haverty building, and (3) the 13A adult inpatient psychiatric unit at Grady. Inpatients will be assessed only once they have been stabilized clinically and are ready for discharge to outpatient care. Both of the outpatient sites are located 2 blocks from the hospital. The PI has a very good working relationship with the clinicians at these three facilities. (See letters of support from Drs. Jennifer Wootten, Karen Hochman, and Emile Risby.) Second, patients returning for the supplemental assessment will be invited to refer additional relatives to the project (The ARIS Project assessed only one relative per patient, whereas this project allows for more than one relative per patient). The overall sample size of n=200 is adequate for the hypotheses to be tested. Please see **Appendix B** for detailed power/sample size considerations.

Exclusion criteria (which are the same as in The ARIS Project) will include: inability to speak English, known mental retardation or dementia, history of alcohol or drug dependence in remission for less than 90 days, and history of neurological disease or medical conditions that could impact the measurement of the constructs being assessed. Specifically, medical exclusions include: current or past neurological diseases, history of serious head injury (causing unconsciousness, coma, or hospitalization), current active allergic rhinitis or upper respiratory infections that could interfere with smell ability, and physical disabilities that could interfere with the neurological examination (e.g., amputation, inability to walk, muscle weakness).

Procedures

Participants who have already completed The ARIS Project assessment will be administered the ***supplemental assessment*** only, which includes the Family Interview for Genetic Studies (FIGS) and collection of the saliva sample for genotyping. The total administration time will be one hour (see **Appendix C**), and participants will be reimbursed $45 for their time, effort, travel, and parking expenses. These past participants will be contacted via telephone and invited to participate in the supplemental assessment. The PI has discussed the protocol for calling past participants with the Emory University IRB Assistant Director, Patti Tuohy. Given that ARIS participants signed written informed consent documents to participate in the project, calling to invite them to return for a supplemental assessment will not be viewed by the IRB as a "cold-call." The IRB will approve this plan, and they simply request that the PI submit a brief telephone script as part of the IRB protocol for review.

Participants who have not yet completed The ARIS Project assessment will be administered the ***full assessment***, consisting of all measures and rating scales as conducted over the past year. Tests will be administered to participants in a fixed order. Additionally, the FIGS

will be completed and saliva samples will be collected. The total administration time will be 3.5 hours (see **Appendix C**). Full-assessment participants will receive a reimbursement of $65.

Saliva samples will be collected using the DNA Genotek OraGene self-collection kit (which Dr. Walker's lab is using). COMT genotyping will be performed at a collaborating genetics lab here at Emory (the lab of Dr. Joe Cubells in conjunction with the Center for Medical Genomics, a core facility of the Department of Human Genetics, which is run by Dr. Mark Bousky). Specifically, genotyping will be conducted as described by Egan et al. (2001), using polymerase chain reaction (PCR) amplification and digestion with *Nla*III. Genotyping will be repeated in 20 subjects to assess the reliability of the findings.

Measures

All measures to be used in this project have been used in prior schizophrenia research, have been used at least some in African American samples, and have documented good psychometric properties. The assessment packet for The ARIS Project is included as **Appendix D**. The FIGS for the supplemental assessment is in **Appendix E**. A brochure on the OraGene DNA kit is provided in **Appendix F**. Each measure is described briefly below:

- *A Demographics Sheet* will record basic sociodemographic data including: gender, race, marital status, educational attainment, employment, monthly income, and history of arrest.
- *Structured Clinical Interview for DSM-IV Axis I Disorders (SCID):* The SCID (First et al., 1998) is a semi-structured interview conducted by trained interviewers aimed at establishing the presence of disorders according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV). The SCID has high concordance with clinical diagnoses and is the gold-standard format for accurately establishing DSM-IV diagnoses in research settings. The SCID psychotic disorders and mood disorders modules will be used to verify and categorize schizophrenia-spectrum diagnoses. The substance use disorders module will be used also.
- *The University of Pennsylvania Smell Identification Test (UPSIT):* The UPSIT measures olfactory identification ability (Doty et al., 1984) and has been used to detect olfactory deficits in individuals with schizophrenia. It is a 40-item scratch-and-sniff smell test consisting of four multiple-choice options for each item. The 40 items are contained within four booklets, with each booklet consisting of 10 items each. For each item, a microencapsulated patch contains the odor and is scratched with a pencil (included in each packet) to release the fragrance. The number of correct items is summed to determine an overall score, with higher scores indicating better olfactory identification ability. Test-retest reliability has been established (r=.92) and criterion-related validity (r=.79) also has been demonstrated through correlations with a traditional odor detection task (Doty et al., 1984). (Not included in **Appendix D** due to cost.)
- *Subtests of the Wechsler Memory Scales – Third Edition (WMS-III):* The WMS-III is a battery of learning, memory, and working memory measures (The Psychological Corporation, 1997). The overall scales are comprised of 11 subtests, including 6 primary subtests and 5 optional subtests (Lichtenberger et al., 2002). For the current project, only subtests of verbal working memory and auditory immediate memory will be used. The WMS-III has been standardized with 1,250 participants (The Psychological Corporation, 1997), and is widely used in research.
- *Minor Physical Anomalies* will be assessed using a revised and extended version of the Waldrop scale for assessing MPA (Ismail et al., 1998). Items rated with this structured rating scale have been used previously in many prior schizophrenia studies.
- *Neurological Evaluation Scale (NES)*: The NES is a 26-item focused, simple neurological examination that assesses coordination, stereognosis, graphesthesia, rapid alternating movements, primitive reflexes, etc. (Buchanon and Heinrichs, 1989). Subscales have been

derived by extensive factor analyses, and include domains such as sensory integration, motor coordination, and sequencing of complex motor tasks.

◙ *Dermatoglyphics* will be assessed using the colorless ink method of dermatoglyphic analysis (Weinstein et al., 1999) used by Dr. Walker's research group and The ARIS Project. Dermatoglyphic patterns to be studied include: finger patterns, ridge counts, and asymmetries.

◙ *Positive and Negative Syndrome Scale (PANSS):* The PANSS is the most commonly used research measure for assessing the symptoms of schizophrenia (Kay et al., 1987). Seven items measure positive symptoms, 7 measure negative symptoms, and 16 measure general psychopathology symptoms. The PANSS is scored by the clinician-researcher after an interview with the patient. Reliability and validity have been determined by extensive prior research.

◙ *Schizotypal Personality Questionnaire (SPQ):* The SPQ is a self-administered "yes-no" questionnaire assessing schizotypal personality features (Raine, 1991). Norms, reliability, and validity are well established. The SPQ has been used extensively in past research with both non-psychiatric populations and first-degree family members of patients with schizophrenia. Cognitive-perceptual (similar to positive symptoms), interpersonal (similar to negative symptoms), and disorganized subscales can be calculated. Due to the low level of functional literacy in the population served at Grady, the SPQ will be read to individuals with any difficulty reading. However, the SPQ is administered to relatives and controls only (not to patients) and experience to date in The ARIS Project reveals that nearly all relative and control participants are able to read and respond appropriately to this self-administered questionnaire.

◙ *Family Interview for Genetic Studies (FIGS):* The FIGS is a guide for gathering diagnostic information about relatives in the pedigree being studied (Maxwell, 1992). FIGS data are collected from everyone interviewed about all known members of the extended family. A pedigree (including at a minimum the proband, his/her parents, grandparents, siblings, aunts, uncles, offspring, and spouse) will be drawn as part of the interview. The second step of the FIGS is to ask the General Screening Questions. The third step is to complete a Face Sheet and Symptom Checklists with selected relatives. As each informant completes the FIGS, the body of data about a single relative increases. Pedigrees will be assessed in detail, including an examination of unilineal *v*. bilineal inheritance in the multiplex families. Parents of multiplex patients will be further classified as presumed obligate carriers *v*. presumed non-carriers (e.g., in a patient who has a maternal grandfather with schizophrenia, the father will be considered a presumed non-carrier, whereas the mother will be considered a presumed carrier). A familial loading score will also be calculated, as previously described (Verdoux et al., 1996; Suvisaari et al., 1998). A detailed examination of the pedigree will allow for exploration of a potential confound that has not been consistently considered in past research: patients classified as having a family history may have more first- and second-degree relatives than those classified as not having a family history—if this were the case, there might be equivalent degrees of genetic risk in both groups, but the risk is only apparent in the group with a family history because of their larger family structure (Wood et al., 2005). The PI and his mentors appreciate the complexities of obtaining an accurate and complete family history in the population of patients with schizophrenia treated at Grady, due to the common fragmentation of families. Nonetheless, this project will aim to obtain the most comprehensive family psychiatric history possible.

Data Analysis

Analyses will be facilitated by the fact that the PI has an M.P.H. degree (2001–2003), and has thus taken courses in research methods, as well as 8 credits of biostatistics and 7 credits of epidemiologic methods. Additionally, because of his K23 award, the PI has a biostatistics

consultant (Paul Weiss, M.S., Rollins School of Public Health of Emory University, Department of Biostatistics) who will be available for consultation and statistical analyses. All hypothesis tests will be two-tailed with a significance criterion of $p<0.05$.

Hypothesis #1A: Analysis of variance (ANOVA) will test differences in mean scores of IOI, IWM, and NSS between the 3 groups. Post-hoc comparisons will be used as indicated based on the results of the overall *F*-test. Covariates (such as age and gender) will be included using analysis of covariance (ANCOVA) as indicated.

Hypothesis #1B: ANOVA and ANCOVA will test differences in minor physical anomalies and dermatoglyphic indices between the 4 groups.

Additional analyses will assess differences in measures between groups using linear mixed effects models. To control for intrafamilial correlation, family will be included as the random effect. Belonging to a multiplex or singleton family, number of siblings in the family, and gender will be included as fixed effects. The significance of the fixed effects will be determined using a Wald-type test of fixed effect parameter estimates.

Hypothesis #2A: Chi-square tests will be used to assess whether or not patients and unaffected relatives from multiplex families have a higher prevalence of the *Val* allele than patients and unaffected relatives from singleton families.

Hypothesis #2B: ANCOVA with age and gender as covariates and genotype and subject group as two between-subject factors will be carried out to examine the effects of COMT genotype on IWM and IOI.

Exploratory Aim: Discriminant function analysis will be used to determine the optimal set of predictors. Using the obtained discriminant functions, the sample can be reclassified to approximate the accuracy of prospective identification efforts based on the predictor variables. In such a model, specific interactions can be examined, including potential gene-environment interactions. Alternatively, based on the data, multivariable logistic regression can be used to model group membership (patient *v*. relative) using familial loading, the 5 risk indicators, the COMT polymorphism, and interaction terms. The best prediction model, based on specific combinations of risk indicators and/or interactions, will be determined. Further distinction between patients based on symptom levels (as measured by the PANSS) and between relatives based on self-reported schizotypy (measured by the SPQ) will be possible.

SIGNIFICANCE AND INNOVATION

This project will test several important and innovative hypotheses in the area of schizophrenia risk indicators, while focusing on low-income, predominantly African American, urban participants from a public-sector setting. The study design will use an underutilized approach (familial loading) to further clarify the genetic *v*. environmental nature of several risk markers. There has been no previous research combining this diverse set of putative genetic and environmental risk indicators in the context of familial loading. Eventually, these risk markers may prove useful, especially when used in combination, for improved prediction of the risk for schizophrenia, with an ultimate aim toward developing indicated preventive interventions.

FUTURE PLANS

The proposed research will add significantly to the expanding literature in this area and will provide important training and mentoring in the PI's development as a young schizophrenia researcher with a particular interest in African Americans and the public-sector setting. This grant will be a vital step in the PI's career development toward an independent investigator based full-time at Grady Health System. The project proposed herein will take advantage of the successes of The ARIS Project and will extend the scope and sample size of the project so that

the PI can continue to generate research findings of interest to the schizophrenia research community. This expansion of The ARIS Project will poise the PI to become a highly competitive candidate for an R01 grant from the National Institute of Mental Health (NIMH), as well as a candidate for independent investigator grants from foundations such as the National Alliance for Research on Schizophrenia and Depression and the Stanley Medical Research Institute. An EMCF grant will assist the PI in a successful transition from a young investigator to an independent research scientist, by providing additional early faculty support. In 2006, the PI will begin preparing an NIMH R01 grant application, which also will focus on risk indicators in schizophrenia. Because his K23 award does not address risk indicators (it is focused on determinants of treatment delay in the early course of schizophrenia), the PI needs to continue gathering data to support an R01 application. While The ARIS Project has been extremely successful in getting this area of research started, an EMCF grant will provide additional essential data and publications to enhance the likelihood of an R01 being funded. Furthermore, this line of research is likely to be applicable and beneficial someday to patients and family members in the Grady Health System, because the data is collected in this setting, under relatively naturalistic and real-life public-sector conditions.

MENTORING PLAN

As part of the PI's NIMH K23 award, an early career mentoring plan already has been developed. The PI meets regularly for detailed career development mentoring with his primary mentor, Nadine J. Kaslow, Ph.D. She has extensive experience with conducting research at Grady Health System, and is an internationally renowned family psychologist. These attributes will be especially helpful during mentoring on issues related to the conduct of research with socially disadvantaged, urban, African Americans; and engaging family members in clinical research. The PI will continue to meet with Dr. Kaslow at least monthly for 2 hours for focused mentoring.

The PI will also meet with Elaine F. Walker, Ph.D., his secondary sponsor on the K23 award, and primary mentor for The ARIS Project and the research project proposed herein. Dr. Walker is the Samuel Candler Dobbs Professor of Psychology and Neuroscience, and is an internationally-recognized schizophrenia researcher. She has extensive research experience in the area of neurological soft signs, minor physical anomalies, dermatoglyphics, etc., and she and the PI meet monthly to discuss research progress and future plans (in addition to frequent e-mail contact). She will also review and collaborate on all manuscripts prepared from this dataset. Additionally, since this project will be the PI's first experience with genetics research, the PI will collaborate with, and receive mentoring from Joseph Cubells, M.D., Ph.D. (Associate Professor, Department of Human Genetics).

In the initial stages of planning for this particular research proposal, the PI has had the unusual opportunity to get mentoring on the details of the research plan due to his participation in the Research Colloquium for Junior Investigators (all day on Sunday, May 22, 2005), which was held during the annual conference of the American Psychiatric Association. This research proposal was the focus of the PI's mentoring during this exceptional day of guidance and advice from world experts in psychiatric research. For the colloquium, the PI presented a poster outlining this research proposal (included in **Appendix G**), and received feedback, advice, and suggestions from several researchers familiar with this content area. The proposal has been modified and improved based on feedback from Drs. Kaslow, Walker, Cubells, and mentors at the Research Colloquium.