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Merging Genetic and Environmental Effects in the Iowa Adoption Studies: Focus on Depression

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Background. *It is generally acknowledged that the vast majority of serious mental disorders have significant genetic contributions that manifest complex inheritance patterns. Despite this, few gene polymorphisms have been unambiguously identified as risk factors for behavioral illness and the mechanisms through which these select polymorphisms affect human behavior is completely unclear. One of the major reasons for this lack of progress is the phenomenon of gene-environment (GxE) interactions.*
Methods. *We review prior evidence of GxE interactions for major depressive disorder (MDD) in the Iowa Adoption Studies.*
Results. *The results demonstrate the usefulness of these cohorts to direct G effects for MDD.*
Conclusions. *We conclude that further use of the adoption paradigm will generate critical insight into the effects of candidate genes for a variety of complex human behavioral illnesses.*

Keywords Genetic, Environmental effects, Iowa adoption studies, Major depressive disorder

INTRODUCTION

Complex behavioral illnesses are conceptualized as resulting from pure genetic (G) and environmental (E) effects, as well as from interactions between environment and genetic (GxE) factors. A large number of studies seeking to identify specific G factors have been conducted with the vast majority of these studies having focused exclusively on pure G effects. These studies, in particular family and sib-pair studies, have demonstrated that the major psychiatric disorders, including schizophrenia, alcoholism, and bipolar effect disorder, have strong genetic (G) contributions to their overall etiology. Therefore, it was widely anticipated that the sequencing of the human genome coupled with the broad functional understanding provided by the "decade of the brain" would provide unparalleled insight into the role of genetic variation in moderating vulnerability to neuropsychiatric illness. However, despite the undeniable wealth of knowledge, behavioral scientists have identified only a small proportion of the genetic variation that predisposes humans to psychosis, depression and substance use syndromes.

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Recent findings by Caspi and colleagues with respect to the serotonin transporter promoter polymorphism (5HTTLPR) suggest that part of the reason prior studies of major depressive disorder (MDD) were unsuccessful is the strength of unanticipated strength of GxE interactions (1). Altered serotonergic neurotransmission is commonly implicated in the pathogenesis of MDD (2). However, despite several studies demonstrating the functionality of the 5HTTLPR in regulating serotonin transporter mRNA levels (3), studies of its role in MDD have been mixed. Hypothesizing that GxE effects were critical in moderating vulnerability, Caspi and colleagues examined the effects of the 5HTTLPR in a cohort of individuals whose childhood environments were well characterized. In keeping with prior findings by a number of groups, no pure G effects were observed. However, when lifetime history of stressful events is used as a co-variate, a significant effect of the 5HTTLPR on vulnerability to MDD was observed.

The surprising strength of GxE interactions suggests that the adoption paradigm is a good methodology through which to examine the etiology of neuropsychiatric illness and in particular, MDD (4). The adoption paradigm ideally uses an adoptee who is separated shortly after birth and raised in an adoptive home whose environment is subsequently well characterized. In this methodology, the biological parents represent

the heritable component while the adoptive home, and in particular the adoptive parents, represent the environment. By selecting for a given illness in the biological parents, the adoption paradigm can be effectively "loaded" to provide a robust cohort of informative probands.

The Iowa Adoption Studies are an excellent example of this strategy and are loaded for the genetic diatheses of substance use, antisocial personality disorder, and major depression (4). In this article, we will review prior evidence of GxE effects for MDD in the Iowa Adoption Studies and update our genetic information with respect to the *HOPA* locus for MDD in this population.

METHODS

The design and methodology of the Iowa Adoption Studies, including the sub-groups of individuals delineated in the current study have been described in detail previously (5,6). Briefly, these studies recruited adoptees and their adoptive parents from Iowa adoption agencies starting in 1975. Adoptees were separated from their biological parents shortly after birth and raised by their unrelated adoptive parents. These adoptees were solicited using a case and control method. Briefly, adoptees with high risk for psychopathology were contrasted with control adoptees who were matched to high risk individuals on the basis of age, sex, agency, and age of biologic mother.

Clinical variables, such as biological parent diagnosis and characterization of the adoptive parent used for the demonstration of GxE effect in the Iowa Adoptees (Table 1), were derived from review of biological parental records and interviews conducted between 1989 and 1991 using the Diagnostic Interview Schedule, Version III, Revised (7) and the Assessing Environment Scales (8) as described in Cadoret et al. (5). The presence of MDD for the *HOPA* studies was determined using DSM-IV criteria and information from the adoptees most recent interview using the Diagnostic Interview Schedule, Version IV (n = 5) or the Semi Structured Assessment for the Genetics of Alcoholism (n = 89) (9) as described in Philibert et al. (6). The genetic material for the present studies was obtained from a subset of subjects who consented to buccal swabbing. All Iowa Adoption Studies protocols have been approved by the IRB of The University of Iowa College of Medicine.

DNA was prepared from the buccal swabs using a Qiagen kit as previously described. Amplification of the *HOPA* exon 43 locus was conducted using our standard methods (10). The resulting PCR products were electrophoresed on polyacrylamide gels, imaged using silver staining then read by personnel blind to genotyping status.

Statistical Analyses of G, E, and GxE Effects: The full methods of statistical analysis used to estimate the contributions of G, E, and GxE effects using the psychiatric histories of 102 female adoptees have been described previously (5). In brief, tests of model fit using logistic linear regression examining alcoholic and sociopathic genetic diatheses were conducted in a stepwise fashion starting with Model 1: genetic factors or G as indicated by the presence or absence of a parental history of alcoholism or sociopathy only (referred to as Bioparent), continuing to Model 2 which examined G and environment (or E) effects (disturbed adoptive parental environment as assessed by the AES) and concluding with a Model 3 which includes G, E and GxE effects.

Genetic Analyses: Since *HOPA* is an X chromosome gene with evidence of differing effects in males and females (6), and our prior findings suggest more prominent effects with respect to MDD in these cohorts, we limited our analyses to females and used Fisher's exact test (11).

RESULTS

In the first analysis, we review prior evidence of whether GxE effects exist for MDD in the Iowa Adoptions studies. A summation of the results of the logistic linear regressions

Table 1 G, E, and GxE Effects in MDD in Female Iowa Adoptees

	Test of Entire Model Fit				Score Tests for G and E contributions					
Model Tested	Score	p	df	R^2(L)	G	df	E	df	GxE	df
Alcoholic Genetic Factors										
Model 1: BioParent (G) Only	0.02	0.90	1	<0.001	0.02	1				
Model 2: BioParent and Adoptive Parent (E) (no interaction)	7.39	0.02	2	0.076	0.35	1	7.4**	1		
Model 3: BioParent and Adoptive Parent with interaction (GxE)	15.15	0.001	3	0.175	7.58*	2	15.62***	2	7.47**	1
Antisocial Genetic Factors										
Model 1: BioParent (G) Only	0.46	0.50	1	0.005	0.46	1				
Model 2: BioParent and Adoptive Parent (E) (no interaction)	8.05	0.02	2	0.084	1.13	1	7.75**	1		
Model 3: BioParent and Adoptive Parent with interaction (GxE)	8.05	0.05	3	0.085	1.26	2	7.82**	2	0.11	1

Model 1, Additive effects of BioParent diagnostic status (G) only; Model 2, Additive effects of G and Adoptive Parent variable (E) as assessed by AES; Model 3, additive effects of G and E as well as the GxE interaction. Adapted from Cadoret et al., 1996. For the test of entire model fit, the R2(L) is the fraction of the negative log likelihood of a model that is accounted for when covariates are added to that model. For the score tests for G and E contributions, the approximate Chi square value is reported.

*p < 0.05; **p < 0.01, ***p < 0.001.

Table 2 Association of the $HOPA^{12bp}$ Polymorphism with MDD in Female Iowa Adoptees

Genotype	$HOPA^{12bp}$	$HOPA^{Wild}$
MDD		
Present	5	38
Absent	0	50

p = 0.0185.

conducted by Dr. Cadoret and colleagues in 1996 with respect to female adoptees is shown in Table 1. In this analysis, the presence or absence of a specific genetic factor (G) was determined by review of the biological parent (Bioparent) diagnoses, while the presence of a disturbed adoptive parent environment (E) was operationalized by analyzing the results of the AES. The resulting data was then analyzed by logistic linear regression. As the table illustrates, by itself a bioparent diagnosis of alcoholism or sociopathy does not predict whether the adoptee will be affected (Model 1). The addition of information on whether a supportive adoptive home environment is present to the model results in the model becoming significant in predicting outcome (Model 2). However, in the case of an alcoholic Bioparent diathesis but not an antisocial diathesis, a significant GxE interaction is observed that results in increased power to predict adoptee outcome (Model 3).

In the second set of analyses, we present a demonstration that pure G effects for MDD can be detected using the Iowa Adoption Studies. In this analysis, we examine the relationship between HOPA allele status, an allele in which we have preliminary evidence of association with a mild increase in relative risk for MDD (12). The results are shown in Table 2. In total, 94 females, including 68 from our previous study, were genotyped for the *HOPA* exon 43 polymorphism. Five females were heterozygous for the $HOPA^{12bp}$ polymorphism, one female was heterozygous for the $HOPA^{-6bp}$ polymorphism and 88 were homozygous for the $HOPA^{wild}$ sequence. Since it is not known whether the exceedingly rare $HOPA^{-6bp}$ polymorphism results in an altered gene product (13), and our prior studies have focused on the physiological, behavioral, and evolutionary characteristics of the $HOPA^{12bp}$ and $HOPA^{wild}$ polymorphisms, the data from the $HOPA^{-6bp}$ were excluded from our analyses.

Analysis of the clinical data from the remaining 93 $HOPA^{12bp}$ and $HOPA^{wild}$ demonstrated that 43 of these probands met lifetime criteria for MDD. Interestingly, all five $HOPA^{12bp}$ probands met lifetime criteria for MDD (Exact test; p = 0.0185).

DISCUSSION

In summary, we demonstrate that significant effects for GxE interactions for MDD exist in the Iowa Adoption Studies and that we can use the Adoption Studies to analyze these interactions and identify the behavioral effects of candidate loci in this population. However, before discussing these data, it is important to note that the numbers presented in this study are small, the adoption cohorts are not a general population sample and only a small portion of this data is newly derived.

The review of the prior work by Cadoret and colleagues is timely given recent findings by Caspi and colleagues and the COGA collaboration. The former group demonstrated that effects of the 5HTTLPR polymorphism were evident only when environmental interactions were noted (1). The current findings from the Iowa Adoption Studies mirror these findings by Caspi and associates and additionally suggest that GxE effects may be important only for loci that also predispose to alcoholism, but not sociopathy. Given the recent findings of the COGA collaboration, which has reported the presence of a major locus for alcoholism (14), using the adoption paradigm, we may be able to put these prior findings to direct testing.

The findings with respect to the *HOPA* locus demonstrate the utility of the adoption study when examining pure G effects. The chief limitation in using this adoptee population in these types of studies is that the power of any given association study is restricted by the fixed size of the adoptee population, as well as frequency and effect size of the risk allele. This becomes problematic if further samples are needed because, unlike association analyses conducted using general population samples, it is generally not feasible to collect additional samples from other adoptees. On the other hand, there are some benefits for the study of neuropsychiatric illness from the case and control design of the Iowa Adoption Studies. The intentional genetic loading of the adoptee population for neuropsychiatric illness should increase the frequency of risk alleles thus decreasing the number of individuals that need to be genotyped in order to obtain a significant effect. In fact, that is what is seen in these preliminary findings. The frequency of the $HOPA^{12bp}$ is less than 2% (~1 in 60 X chromosomes) in the Iowa population (13). Thus, normally, we would have to screen 150 individuals to identify 5 $HOPA^{12bp}$ probands. In this study, this was accomplished by screening less than two-thirds of that number. At the same time, as a result of that intentional loading, the results may not be directly generalizable to the rest of the population. Indeed, in our unpublished studies, the presence of the $HOPA^{12bp}$ allele is only a weak predictor for MDD. Yet, for putative risk alleles such as the $HOPA^{12bp}$ allele that have only been studied in severely ill populations, well characterized populations such as the Iowa Adoptee Study cohorts may present the first opportunity to form working hypotheses on the effect of the allele in the general population. In the next several years, we anticipate that we will collect and characterize an additional 200 female probands which will allow us to more exactly calculate the relative risk of the allele in the adoptee population and the interaction, if any, of the allele with the adoptive environment.

We believe that the ability of the Iowa Adoption Studies to examine the effects of evolutionarily conserved risk alleles for neuropsychiatric illness such as $HOPA^{12bp}$ (15)or the DRD4 7 repeat allele (16) may be particularly important. Traditionally, genetic studies of psychiatric illness have utilized sib pair or family studies in which the majority of individuals characterized

tend to be ill and the environments of these subjects tend not to be independent. Whereas these studies are well designed to identify vulnerability alleles, they are ill-suited to identify the beneficial effects of balanced polymorphisms (i.e., alleles which have both positive and negative effects). In the Iowa Adoption Studies, the fact that less than half the females carry a diagnosis of MDD virtually ensures that many of the individuals with these modest effect alleles for MDD will be unaffected. If GxE interactions are important determinants mediating the effects of these low risk alleles, studies utilizing behavioral and cognitive characterizations conducted by Dr. Cadoret and associates over the past 30 years may present opportunities to identify E and GxE effects which may not only exacerbate but also protect against the development of illness and thus, perhaps, generate insight into new ways of ameliorating vulnerability to illness.

In summary, the Iowa Adoption Studies are a landmark in the understanding of GxE effects in the development of substance use and potentially other neuropsychiatric disorders. We anticipate that advances in genomics will capitalize on the careful characterizations compiled by Dr. Remi Cadoret and colleagues and look forward to further contributions by him to the understanding of the development and perhaps prevention of behavioral illness.

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