Genetic Methods and Life Course Development

Bethesda, Maryland
February 11–12, 2008

WORKSHOP SUMMARY

National Institute on Aging
Behavioral and Social Research Program

For Administrative Use
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List of Acronyms and Abbreviations

5-HIAA	5-hydroxyindoleacetic acid
5-HT	serotonin
5-HTT	serotonin transporter
ACTH	adrenocorticotropic hormone
AD	Alzheimer's disease
BMI	body mass index
BSR	Behavioral and Social Research Program
COMT	catechol-O-methyl transferase
CSF	cerebrospinal fluid
CRP	c-reactive protein
DβH	dopamine beta-hydroxylase precursor
dbGaP	database of Genotype and Phenotype
DCS	Dual Change Score
FFS	Fragile Families Study
FOA	Funding Opportunity Announcement
GR	glucocorticoid receptor
GWAS	genome-wide association studies
HDAC	histone deacetylase
HPA	hypothalamic-pituitary-adrenal
HRS	Health and Retirement Study
IQ	intelligence quotient
LG	licking and grooming
LGCM	Latent Growth Curve Model
MAOA	monoamine oxidase A
MAOB	monoamine oxidase B
MDR	Multifactor Dimensionality Reduction
MMSE	Mini Mental State Examination
NAS	National Academies of Science
NF-κB	nuclear factor kappa B
NIA	National Institute on Aging
NICHD	Eunice Kennedy Shriver National Institute of Child Health and Human Development

NIH	National Institutes of Health
NIMH	National Institute of Mental Health
PA	program announcement
PSID	Panel Study of Income Dynamics
RFA	request for applications
SES	socioeconomic status
SNP	single nucleotide polymorphism
WLS	Wisconsin Longitudinal Study

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EXECUTIVE SUMMARY

The National Institute on Aging (NIA) Behavioral and Social Research Program (BSR) has had a longstanding interest in factors influencing social behaviors across the lifespan, and it now supports more than 20 longitudinal studies, some nationally representative and some more local or subgroup specific. Because genetic methods can deepen the level of analysis in many of these studies, the BSR has begun to explore ways to better merge or integrate genetic analyses into social and behavioral research. Since 2002, this effort has included a series of workshops focused on genetics, behavior, and aging; environment and genetically informative studies on aging; the genetics of behavior in social environments; the social neuroscience of aging; and the refinement of economic phenotypes for genetic analyses. Many of these workshops, which focused on the common thread of gene-environment interactions and influences on behavior, have yielded special journal issues.

At the same time several interesting research directions have emerged. One set of studies suggests that early-life experiences have a long-term effect on aging and the lifespan in general, whereas another set of studies suggests that late-life events are more important. The tension between these two lines of thought suggests a need for genetic methods to assess heterogeneity, to more precisely estimate social and behavioral measures, and to address the large numbers of false positives that are likely when correlating genetics and behavior in large-scale studies. Indeed, findings from genetic studies have suggested mechanisms that account for continuity or discontinuity in social and emotional competence across the lifespan. The BSR, which has long supported large-scale studies such as the Health and Retirement Study, the English Longitudinal Study of Ageing, and the Wisconsin Longitudinal Study, hopes that genetic information will facilitate the disentanglement of causal pathways across the life course.

On February 11–12, 2008, the NIA held a workshop to determine how integration of lifespan development and genetics can clarify developmental mechanisms promoting selected domains of social and emotional competence in aging. Researchers representing the fields of economics, molecular biology, epigenetic science, and behavioral research presented their work and engaged in discussions focused on stability and change, the interplay between gene and environment, and gene expression and epigenetic mechanisms. They identified questions that could be answered best by the integration of genetic and lifespan data, explored existing lifespan studies that could be used or enhanced to address these questions, and discussed strategies and opportunities for integrating relevant genetic methods with existing lifespan studies. Discussions from this workshop will be incorporated into an overall plan, which will be reviewed in September 2008 by the National Advisory Council on Aging and used to guide development of funding opportunity announcements.

Emerging Discussion Themes

- *Methods of collecting and storing DNA.* Adding blood or tissue collection to existing studies might not be feasible because of the degree of research participant contact required. Workshop participants discussed contractual agreements in which investigators send samples to central facilities that extract DNA and store the remainder of the sample.
- *The need for databases that store information on associations among genes, social behaviors, and environments.* One participant discussed NIH's database of Genotype and Phenotype (dbGaP), which could prove useful in examining genetic influences over the life course.
- *The evolutionary advantage of polymorphisms that influence behavior.* Although many of the polymorphisms discussed at the workshop appear to be detrimental, they could prove adaptive.
- *Candidate gene studies versus genome-wide association studies.* Although studies supported by the BSR thus far have focused on candidate genes, the effects of these genes appear to be small, and heterogeneity hinders the ability to determine how much variation they account for. More exploration is needed to determine what these genes do in the general population, and further study of existing single nucleotide polymorphism (SNP) chips might yet prove to be valuable. However, studies also should move beyond candidate genes.
- *Moving beyond a focus on static gene-environment interactions associated with early- or late-life events toward a focus on the life trajectory.* The influence of some interactions might be continuous, whereas others are plastic.
- *The need to establish rigorously the heritability of personality traits, such as conscientiousness.* Personality traits normally refer to a collection of behaviors and outcomes, without accounting for causal pathways that might give rise to phenotypic differences and the context and health processes associated with these pathways.
- *The need to standardize behavioral phenotypes measured across studies, although methods of measurement might differ, and to further refine these phenotypes.*
- *The need for investment across different types of study designs, including twin and sibling studies.* For example, studies of monozygotic twins could prove useful in exploring epigenetic differences, whereas sibling or dizygotic twin studies could link various genetic differences while controlling for family environment.

Suggested Next Steps

- **Workshops focused on outcomes or risk factors of interest to the BSR.** These workshops would bring together participants who use different approaches or study different aspects of an outcome or risk factor. Which to focus on—outcome or risk factor—was a point of debate. Focusing on a risk factor could aid the development of biological models of the pathways between genetics and behavior. Focusing on an outcome, on the other hand, would guide investigators in recommending which gene modules to focus on.
- **Other workshops.** The BSR will hold a workshop on the interactions between interventions and genetics as well as a workshop at the Association for Psychological Science for psychologists entering aging research. Suggested topics for additional workshops included

statistical methodologies used by different disciplines to address the same research problem, new strategies for data analysis, and existing investment in research on genetics and behavior across the life course.

- **Encouraging efforts to learn languages used across disciplines.** Mini-courses, primers, and the book *Molecular Biology Made Simple and Fun* were suggested as resources to help investigators learn terminology in disciplines other than their own.
- **New project support.** A request for applications could encourage investigators to think about methods of collecting genomic data for behavioral studies.
- **Special journal issues.** Participants from this workshop and the fall 2007 workshop on Refining Economic Phenotypes for Genetic Analyses could submit papers for a special issue.

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INTRODUCTION

On February 11–12, 2008, the National Institute on Aging (NIA) Behavioral and Social Research Program (BSR) convened a workshop to explore areas where the integration of lifespan development and genetics can clarify developmental mechanisms promoting domains of social and emotional competence in aging. The workshop brought together researchers representing the fields of economics, molecular biology, epigenetic science, and behavioral research. These participants presented their work and engaged in discussions focused on stability and change, the interplay between gene and environment, and gene expression and epigenetic mechanisms. They identified questions that could be answered best by the integration of genetic and lifespan data, explored existing lifespan studies that could be used or enhanced to address these questions, and discussed strategies and opportunities for integrating relevant genetic methods with existing lifespan studies. The workshop agenda and participant roster are included as Appendices 1 and 2.

The BSR has had a longstanding interest in factors influencing social behaviors and how these factors are maintained or changed throughout the lifespan. Its foray into genetics began with the support or partial support of a series of twin studies, including the Swedish Twin Study, the Danish Twin Study, and the Minnesota Twin Study, and it now supports more than 20 longitudinal studies, some of which are nationally representative. Although the genetics research portfolio is currently a relatively small portion of the BSR research portfolio, the leadership at the BSR recognizes that genetic levels of inquiry can be applied to and integrated into behavioral and social research supported by the Program. The BSR has held a series of workshops over the past few years that build on this approach:

- The Workshop on Genetics, Behavior, and Aging, held in March 2002, focused on major human studies in behavioral genetics and explored questions and methods in such areas as genetic influence on depression, effects of genetics and environment on stability and change in cognitive performance, biobehavioral measures, and influence of mortality on models of change. Papers derived from this workshop were published in a special issue of *Behavioral Genetics* in March 2003.
- The Environmental Workshop for Genetically Informative Studies on Aging was held in February 2003. This workshop explored gene-environment dynamics and brought together people knowledgeable about key epidemiological studies and major findings related to environmental influences on age-related phenotypes. Discussions focused on interactions and correlations between genetics and environment and identified a robust literature on social effects. Papers from this workshop were published in a special issue of the *Journals of Gerontology Series B: Psychological Sciences and Social Sciences* in March 2005.

- A special symposium was held at the meeting of the Behavioral Genetics Association in 2006. This symposium focused on genetics of behavior in social environments. Papers from this symposium were published in a special issue of *Twin Research and Human Genetics* in 2007.

As investigators began to focus more on molecular inquiry, attention shifted toward genetic influences on social behavior and on gene-environment interactions. In this vein, the BSR held two more workshops. The Workshop on the Social Neuroscience of Aging (February 2007) explored the genetic basis of social behavior and ways to incorporate that basis in lifespan development approaches. The second, the Workshop on Refining Economic Phenotypes for Genetic Analyses (September 2007), explored ways to apply methods that already are available to a broad range of concepts of interest to the BSR including sociability, social competence, cognitive disability, and economic activity. The BSR also held a workshop on data-sharing among genetic studies in August 2006 to develop recommendations for data-sharing plans that facilitate collaboration across the entire research community. Specifically, the workshop examined how the BSR could aid in merging data already collected with plans for new collections.[1]

Biodemography is also of interest to the BSR, seeded by panels convened by the National Academies of Science (NAS). The BSR also has supported several large-scale surveys, including the Health and Retirement Study (HRS), the English Longitudinal Study of Ageing, and the Wisconsin Longitudinal Study (WLS), and some of these studies have collected DNA. As suggested by one NAS panel, genetic data should be collected in such population surveys to assess heterogeneity and to develop more precise estimates of social and behavioral factors. Genetic data also could aid in identifying the large numbers of false positives associated with large-scale studies. In addition, some studies have suggested the importance of early-life environmental factors in aging, whereas other studies contend that late-life factors are more important. It is hoped that genetic information will help researchers untangle causal pathways when assessing them over the entire life course. On a broader scale, many studies supported by the NIA/BSR are part of a family of studies, making them potentially amenable to the pooling of data and standardized connection of some areas of phenotypic evidence.

The results of this and other workshops will be incorporated in the BSR's plans, which will be reviewed at the September 2008 meeting of the National Advisory Council on Aging. The BSR intends for funding opportunity announcements (FOAs) to arise from this workshop, but it also encourages researchers to submit applications with good ideas without waiting for the publication of a FOA.

[1] Meeting reports are available on the NIA/BSR Web site: http://www.nia.nih.gov/ResearchInformation/ExtramuralPrograms/BehavioralAndSocialResearch/ConferencesAndWorkshops.htm

OVERVIEW

Gene-Environment Interaction in Problematic and Successful Aging

Terrie E. Moffitt, Ph.D., Duke University and King's College London

The term "gene-environment interaction" is often used to describe a myriad of different interactions. However, it is only one of four types of gene-environment interplay,[2] and they must be distinguished to facilitate further discussions.

- Heritability-environment interaction—the interaction between environmental context and heritability coefficient, which is obtained from studies of twins, adoptees, and other relatives. An example can be found in a study by Johnson and Krueger where the variation of health for low-income individuals was influenced more strongly by genetics, but that of high-income individuals was influenced more strongly by environment.[3] No genes were measured in this study, but the heritability coefficient differed among subgroups.
- The gene-environment correlation. Traditionally, investigators assumed that measures believed to be "environmental" would influence phenotypic outcomes only in limited ways. The newer view proposes that genetic endowment can influence the kinds of environments to which an individual is exposed. Thus, measures of environment are partially suffused with genetic variation,[4] and the direction of causal associations can be ambiguous.
- Epigenetic programming, or the regulation of gene expression mediated primarily through changes in DNA methylation and alterations in chromatin structure. As discussed in other presentations in this workshop, studies using animal models have yielded a large amount of persuasive evidence that environment can alter gene expression.
- Gene-environment interaction. As noted by Moffitt and colleagues, "A gene-environment interaction (denoted as GxE) occurs when the effect of exposure to an environmental pathogen on health is conditional upon a person's genotype, that is differences in a person's DNA sequence (or conversely, when environmental experience moderates genes' effects on health)."[5]

Moffitt provided many examples of a gene-environment interaction, including studies showing that severely maltreated boys were more likely to go on to develop conduct disorder if they had a polymorphism that conferred higher levels of monoamine oxidase A (MAOA).[6] Another study showed that a functional polymorphism in the serotonin transporter (5-HTT) promoter region moderated the influence of early stressful life events on later depression;[7] the prevalence of

[2] Rutter M, et al. Continuities and discontinuities in psychopathology between childhood and adult life. *J Child Psychol Psychiatry*. 2006;47:276-295.
[3] Johnson W, Krueger RF. Genetic effects on physical health: lower at high income levels. *Behav Genet*. 2005;35:579-590.
[4] Plomin R. The Emanuel Miller Memorial Lecture 1993. Genetic research and identification of environmental influences. *J Child Psychol Psychiatry*. 1994;35:817-834.
[5] Moffitt TE, et al. Strategy for investigating interactions between measured genes and measured environments. *Arch Gen Psychiatry*. 2005;62:473-481.
[6] Caspi A, et al. Role of genotype in the cycle of violence in maltreated children. *Science*. 2002;297:851-854.
[7] Caspi A, et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science*. 2003;301:291-293.

schizophrenia later in life was higher for adolescents who were heavy cannabis users and carried two copies of the valine allele of the gene encoding catechol-O-methyltransferase (COMT-Val/Val) but not for adolescents carrying two methionine alleles (COMT-Met/Met).[8] Babies who carried the CC allele of the FADS2 gene and were breastfed went on to exhibit slightly higher intelligence quotient (IQ) scores than those who were not breastfed.[9] Several investigators have attempted to replicate the findings from the 2002 MAOA and 2003 5HTT studies (the 2005 COMT study and the 2007 FADS2 study are too recent for replications to emerge, though some are under way). Overall, to date many more replications have succeeded than failed, and meta-analyses indicate that the original findings are holding up. However, Moffitt cautioned that there may be a "file drawer" publication bias in which those who have failed to replicate the findings are more hesitant to publish.

Caspi and Moffitt described three conceptual models:[10]

- The gene-association model, in which a gene is directly associated with a disorder and assumed to be the cause of it. This model works well for single-gene disorders but not for complex human behavior.
- The gene-environmental interaction model, which assumes that an environmental risk factor is the primary cause of a disorder and that genotype affects vulnerability or resilience to that factor. Research conducted under this model is primarily epidemiologic and observational in nature.
- A new model proposed by Caspi and Moffitt, which suggests that gene-environment interactions stimulate work at a neuroscience level. Research following this model can uncover mechanisms of action, and parameters can be experimentally controlled in a laboratory setting.

Examples of this third type of research abound. Carriers of the short allele of the 5-HTT gene exhibit more amygdala activity in response to fearful faces than do carriers of the long allele;[11,12] girls homozygous for the short 5-HTT allele react more strongly to stress challenges and take more time to recover, as evidenced by cortisol levels;[13] and rhesus macaques that carry a short 5-HTT allele and are raised by their peers exhibit the largest increases in adrenocorticotropic

[8] Caspi A, et al. Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. *Biol Psychiatry*. 2005;57:1117-1127.
[9] Caspi A, et al. Moderation of breastfeeding effects on the IQ by genetic variation in fatty acid metabolism. *Proc Natl Acad Sci U S A.* 2007;104:18860-18865.
[10] Caspi A, Moffitt TE. Gene-environment interactions in psychiatry: joining forces with neuroscience. *Nat Rev Neurosci*. 2006;7:583-590.
[11] Hariri AR, et al. Serotonin transporter genetic variation and the response of the human amygdala. *Science*. 2002;297:400-403.
[12] Hariri AR, et al. A susceptibility gene for affective disorders and the response of the human amygdala. *Arch Gen Psychiatry*. 2005;62:146-152.
[13] Gotlib IH, et al. HPA Axis reactivity: A mechanism underlying the associations among 5-HTTLPR, stress, and depression. *Biol Psychiatry*. 2007. Epub ahead of print.

hormone (ACTH) in response to stressful experiences in later life.[14] Studies in rodent models and human neuoroimaging studies also have been done, including studies of MAOA and COMT.

Longitudinal cohort studies also are valuable for exploring gene-environment interactions in the context of tracking early-life exposures and midlife phenotypes. As pointed out by Manolio,[15] longitudinal cohort studies can provide equal representation of both ill and healthy individuals; unbiased, premorbid exposure information; precise data on the timing of exposure and disease course; and a view of multiple disease outcomes. Because these cohorts represent more than one phenotype, longitudinal cohort studies can allow investigators to study comorbidities, and they can explore other health and behavioral outcomes to identify components of positive aging. Moffitt and colleagues have several ongoing studies to track gene-environment interactions across the life course. The Dunedin New Zealand Cohort, for example, enrolled a birth cohort from 1,037 families in 1972 and has followed them periodically with medical, psychosocial, and psychiatric assessments. The next assessment will take place in 2009. Other cohorts include the following:

- Christchurch New Zealand Cohort (N=1,200, age 32)
- E-risk Twin Cohort (N=2,200, age 12)
- British Mothers Cohort (N=1,100, age 30–50)
- Murcia Spanish Twin Cohort (N=850 women, age 50–70)
- Dunedin Cohort Parents (N=1,900, age 50–80)

DNA will be on hand by the end of 2008 for all projects.

To determine whether early-life experiences interact with genes to lay the foundation for age-related diseases or successful aging, Moffitt and colleagues are tracking several potential predictors in physical health, mental health, personality, economic behaviors, neuropsychological health, metabolic syndrome, telomere shortening, and inflammation. For example, they examined associations between childhood maltreatment (maternal rejection, harsh discipline, caregiver changes, physical and sexual abuse) between the ages of 3 and 11 years in the Dunedin cohort and inflammatory outcomes at age 32. Individuals who experienced maltreatment before age 11 exhibited more inflammation, as measured by levels of C-reactive protein (CRP), fibrinogen, and white blood cells.[16] High levels of CRP have been associated with risk for cardiovascular disease. Studies are under way to determine possible links to genotype and to determine whether candidate genes modulate the influence of early-life experience on midlife traits known to predict age-related diseases or successful aging.

[14] Barr C, et al. Rearing condition and rh5-HTTLPR interact to influence limbic-hypothalamic-pituitary-adrenal axis response to stress in infant macaques. *Biological Psychiatry*. 2004;55(7):733-738.
[15] Manolio TA. Genes, environment and the value of prospective cohort studies. *Nat Rev Genet*. 2006;7:812-820.
[16] Danese A, et al. Childhood maltreatment predicts adult inflammation in a life-course study. *Proc Natl Acad Sci U S A*. 2007;104:1319-1324. Epub 2007 Jan 17.

Personality Differences in Animal Models I

Stephen Suomi, Ph.D., Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)

Rhesus macaques live in troops that are organized around several female-led families. They spend the first month of life in constant contact with their mothers, forming a strong and enduring bond. As they get older, these young monkeys interact with other individuals of the same age, spending several hours each day engaged in play, which helps them learn and develop behaviors needed throughout life. At puberty, females stay in the troop in which they were born, but males leave the troop and join all-male gangs. This is the most dangerous time of the male's life, with a mortality rate of 40 to 50 percent. Eventually the males work their way into a new troop. They might stay in that new troop for the rest of their lives or move again. The birth of a new infant is a major event in which the entire family buffers the new mother and infant from stress. Thus, grandmothers and great-grandmothers continue to play an important role. Each troop has several dominance hierarchies, and the status differences in these hierarchies can serve as proxies for the socioeconomic differences observed in humans. On average, rhesus macaques live into their 20s, but in the Poolesville (Maryland) colony, they can live into their 30s. These monkeys can show effects of old age, including immune disorders, cancers, cardiovascular disease, and dementias.

Twenty percent of rhesus macaques in two field sites and at the Poolesville colony become fearful in mildly challenging or stressful situations, and they exhibit arousal of many biological systems, including the hypothalamic-pituitary-adrenal (HPA) axis. Similar to human children, these monkeys are at risk for developing anxiety or depressive disorders. Another 5 to 10 percent are excessively impulsive and aggressive and elicit aggressive behaviors from other monkeys. These monkeys display deficits in serotonin (5-HT) metabolism; for example, low levels of 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid (CSF). There is a large amount of individual variation in the timing of male emigration and the strategies employed by males to join a new troop. Fearful and anxious males postpone emigration, whereas impulsive and aggressive males are driven out of the troop early. The fearful and anxious males are thus more likely to be physically large and heavy when they finally leave and, ultimately, might be more likely to survive.

In studies by Suomi and colleagues, rhesus macaques taken from their mothers at birth and raised in peer groups become hyperattached to one another. When they do start to play, they never exhibit the complexity seen in the play of mother-reared infants because they must serve both as attachment objects and playmates. In the long term, these monkeys are more fearful and produce high levels of cortisol, they are much more aggressive and impulsive, and they drink to excess in alcohol studies. Like humans, rhesus macaques have functional polymorphisms in the 5-HTT gene, and similar gene-environment interactions are seen. Peer-reared monkeys carrying the short allele exhibit deficits in CSF 5-HIAA, high levels of aggression and impulsivity, and high levels of alcohol consumption, compared with peer-reared monkeys carrying the long allele. This difference disappears in mother-reared monkeys. Thus, good mothering might buffer carriers of the short allele from increased biological and behavioral risks. The gene-environment interaction can be interpreted in two ways. On the one hand, individuals carrying the long allele are protected from adverse early-life experiences. On the other hand, good early environments can protect carriers of genotypes that place them at risk for abnormal behaviors. These interpretations

are not mutually exclusive, and they could vary with different genes, but their implications differ with respect to future interventions and protections.

As discussed in other presentations, environmental factors could also regulate gene expression through epigenetic mechanisms. This hypothesis raises some questions:

- When does this regulation occur?
- Are individuals set on trajectories that are impervious to subsequent changes, or can gene-environment interactions take place at later time points?
- Are there events in the environment that can alter expression of specific genes to the same or similar degree as do events in early life?

Discussion

In response to questions from workshop participants, Suomi noted that a range of maternal care has been observed in the rhesus population, with some mothers highly involved in rearing their infants and other mothers engaged in abuse. Monkeys raised by abusive mothers display higher levels of cortisol, lower levels of 5-HIAA, and more fear and aggression, similar to peer-raised monkeys. Cross-fostering studies have shown that rearing behaviors are passed from generation to generation through nongenetic mechanisms.

Suomi also noted that the rhesus macaque is the second most successful (that is, populous and adaptable) primate after humans, and they exhibit allelic variation similar to that seen in humans. Other primate species, such as chimpanzee and great ape, on the other hand, are homozygous for every candidate gene that has been studied, and the vast majority of these species are endangered. Genetic diversity might be one mechanism of success, perhaps allowing individuals or groups to move into and adapt to new habitats.

Suomi and others emphasized that the two interpretations discussed—a genetic cause versus an environmental one—focus on the same data and differ only by the actions taken in response to them. As more precise data are obtained and as the mechanisms underlying gene-environment interactions become clearer, it might become apparent that both types of causation are at work. The implications of alternative explanations are considerable from a therapeutic or interventional standpoint.

As pointed out by Moffitt, investigators should strive to learn more about the biology of processes linking genes with sensitivities to environment. For example, in the FADS2 work, she and colleagues had a lot of nutrigenomic information and could make predictions about gene-environment interactions based on biology. Learning the underlying biology should be part of hypothesis-generating research. In addition, how measurements are made is important. Laboratory experiments can better control positive and negative aspects of the environment. Thus, research on gene-environment interactions should complement large, observational cohort studies with experimental models and involve both epidemiology and genomics.

STABILITY AND CHANGE

Childhood Health and Adult Socioeconomic Status Outcomes

James P. Smith, Ph.D., RAND Corporation

Data from the Panel Study of Income Dynamics (PSID) suggest a relationship between the likelihood of an adult having a severe health condition and a set of background characteristics, such as whether the parents had the same condition, household income during childhood, and the respondents' health status during childhood (up to age 16 years). The data also suggest an increasing separation between good health and bad as respondents grow older. In an extended model, excellent or good health in childhood appears to add one-third of a year in completed schooling as an adult. The impact of childhood health is much smaller and statistically non-significant within sibling comparisons. These outcomes have been examined repeatedly in the literature.

In addition, PSID data suggest that the effects of childhood health expand over the adult years; for example, influencing one's ability to experience skill growth, translated into increased family income, as an adult. Using the HRS measures of childhood health, Smith has found that common childhood diseases, such as measles, mumps, and chicken pox, have little impact on economic prospects. However, conditions such as asthma, chronic ear problems, depression, allergies, and speech impairment appear to affect economic prospects. The impact of rare childhood conditions, such as hypertension or type 1 diabetes, cannot be measured precisely because too few respondents had these conditions.

Because family effects also appear to matter, the addition of genetics to a study such as PSID would provide a way to disentangle the relative importance of gene-environment interactions. It would be useful to study genes related to highly prevalent diseases; it may not be as useful to study genes related to rare conditions simply because of the limited amount of data. The study of genetic influences related to the moderately prevalent childhood conditions, however, are more promising. Genes thought to be associated with personality or behavioral economic factors, such as impulsiveness or time preference, may not be good candidates for deriving instruments to aid in estimating causal effects because few if any equations could exclude them. Thus, at this stage of evolving knowledge regarding childhood health and adult economic incomes, whole genome analysis might prove a better strategy as opposed to preselecting specific genes. In addition, because of the importance of gene-environment interactions in any of these effects, additional information on environment should be included to avoid misinterpretation.

Wisconsin Longitudinal Study

Robert Hauser, Ph.D., University of Wisconsin-Madison

The WLS began in 1957 with a survey of Wisconsin high school graduates, and it follows this cohort from age 18 years to age 65 years. In addition to survey data, the WLS has administrative record data and information about many domains of respondents' and family members' lives, including positive and stressful events. This collection includes nonsurvey information such as IQ scores, class ranks, social participation in high school (assessed from yearbooks), and parents' adjusted gross income. The WLS also has access to the National Death Index. Methodological features of the WLS include interview by random replicates, bracketing amounts with random

anchors, selection of children with developmental disabilities or adolescents with severe onset of mental illness for a supplemental interview and survey, cognitive measurements over the life course, health vignettes, recorded interviews, and Internet resources.

In 1964 a short mail survey of parents was conducted to assess education, occupation, military service, and marital status. In 1975 WLS graduates participated in phone interviews, and in 1977 a randomly selected sample of siblings also participated in phone interviews. Phone interviews of the graduates and siblings were conducted again in 1993 and 1994, and in 2004 and 2005 graduates and siblings participated again in phone and mail surveys. Response rates at each of these stages were 80 percent or higher. In 2004 and 2005 WLS also surveyed spouses of the original graduates, with a 74-percent response rate. The sibling sample also has been expanded, and spouse data for siblings will be available soon. Although the response rate in this study is high and the sample large, the sample of Wisconsin high school graduates is almost all White and therefore not representative of the U.S. population.

The WLS data have been used to conduct behavioral genetic studies on education, comparing the education of biological and adopted children with respect to parental characteristics,[17] and to conduct studies on psychological well-being after parenthood, using a sibling-based design.[18] Other WLS-based studies include the following:

- Examinations of sibling resemblances in ability, educational attainment, and occupational success;
- A study of depression, which includes lifetime depression history and two measurements taken a decade apart;
- Two measures of personality, based on constructs from The Big Five (extroversion, agreeableness, conscientiousness, neuroticism, openness), taken a decade apart; and
- A study of cognition and decisionmaking assessing participants' expectations, aspirations, and plans when completing high school. This study, done in collaboration with David Laibson and Dan Benjamin, also will be used to validate studies of biomedical data from Iceland.

The WLS also offers biomarker data and information on several body mass index (BMI) measures, facial characteristics, DNA collected from graduates using the Oragene saliva kit, and experience with Medicare Part D. Home interviews and performance tests will be conducted in 2010, when graduates are about 71 years old, and a study on children of WLS respondents is under consideration. DNA has been collected from 4,500 graduates, and collection from siblings is under way. Initial assays will assess apoE, which is associated with Alzheimer's disease (AD); 5-HTT long and short alleles; and DRD2 and DRD4, which have been associated with impulsivity. Assays of BRCA1 and BRCA2, which have been associated with breast cancer, and GABBA, which has been associated with alcoholism, also are under consideration.

[17] Plug E, Vijverberg W. Schooling, family background, and adoption: Is it nature or is it nurture? *Journal of Political Economy*. 2003;111:611-641.

[18] Pudrovska T. Psychological implications of motherhood and fatherhood in midlife. CDE Working Paper No. 2007-02, Center for Demography and Ecology, University of Wisconsin-Madison, WI, 2007. Available at: http://www.ssc.wisc.edu/cde/cdewp/2007-02.pdf.

Collaborations with Laibson and Benjamin also include assays of 384 single nucleotide polymorphisms (SNPs), all related to cognition and decisionmaking, on a custom chip.

Information also is available on the 20 percent of individuals who did not respond to the WLS. Beginning in 1993, Hauser and colleagues observed a substantial difference in response based on grade point average and cognitive ability in adolescence; the lower IQ strata tend to be missing among WLS respondents. With respect to DNA collection, individuals in poor health are far less likely to provide DNA by mail than those in good health.

The Economics, Technology, and Neuroscience of Human Capability Formation

James J. Heckman, Ph.D., University of Chicago

Increasing evidence indicates a link between early-life events and adult health. The "fetal programming" literature demonstrates that the environment *in utero* affects adult health;[19] Robert Fogel has found a relationship between early nutrition and later health;[20] and David Barker has demonstrated the predictive power of environmental insults *in utero* and during infancy for the onset of adult coronary disease, stroke, diabetes, and hypertension.[21] In addition, birth weight, fetal and maternal nutrition, and growth by age can predict later adult health. Yet work in health economics often focuses exclusively on adult decisionmaking without accounting for the life cycle or developmental perspective. There is, however, an emerging literature in economics that demonstrates the importance of early environmental conditions on the evolution of adolescent and adult cognitive and noncognitive skills.[22] Like the fetal programming literature, this literature documents critical and sensitive periods in the development of human capabilities; unlike the fetal programming literature, it also considers environmental influences on development over the entire life cycle of the child and on into adulthood.

Ability matters, and abilities are multiple in nature, developing at different rates across the life cycle. Gaps between individuals and socioeconomic groups, in both cognitive and noncognitive abilities, open up at early ages, grow wider as individuals get older, and ultimately play a role in gaps in health status. In addition, the traditional question of nature versus nurture is obsolete. The modern literature on epigenetic expression and gene-environment interactions teaches that the sharp distinction between acquired skills and ability featured in the early literature on human capital is not tenable.[23] The additive nature and nurture models still used in many studies of

[19] Gluckman PD, Hanson MA, Eds. *Developmental Origins of Health and Disease*. Cambridge, UK: Cambridge University Press; 2006.

[20] Fogel RW. Secular trends in physiological capital: implications for equity in health care. *Perspect Biol Med*. 2003;46 (3 Suppl):S24-38.

Fogel RW, Costa DL. A theory of technophysio evolution, with some implications for forecasting population, health care costs, and pension costs. *Demography*. 1997;34:49-66.

[21] Barker DJ. In utero programming of chronic disease. *Clin Sci (Lond)*. 1998;95:115-128.

[22] Cuhna F, Heckman JJ. *The Technology of Skill Formation*. Institute for the Study of Labor Discussion Paper Series (IZA DP No. 2550), 2007.

Knudsen EI, et al. Economic, neurobiological, and behavioral perspectives on building America's future workforce. *Proc Natl Acad Sci U S A*. 2006;103;10155-10162.

[23] Gluckman PD, Hanson MA. Metabolic disease: evolutionary, developmental and transgenerational influences. *Nestle Nutr Workshop Ser Pediatr Program*. 2005;55:17-27.

Pray LA. Epigenetics: Genome, meet your environment. *The Scientist*. 2004;18:14-20.

Rutter M. Implications of resilience concepts for scientific understanding. *Ann N Y Acad Sci*. 2006;1094:1-12.

heritability and family influence mischaracterize gene-environment interactions. Abilities are produced, and gene expression is governed by environmental conditions. Thus, measured abilities arise from environmental influences, including *in utero* experiences, and genetic components.

The literature includes evidence, from both human studies and animal models, of critical and sensitive periods of development. For example, IQ scores appear to become stable by age 10, suggesting a sensitive period for their formation at ages younger than 10 years.[24] Remedial interventions targeted toward disadvantaged adolescents thus tend to be less effective and have lower economic return than similar investments in young, disadvantaged children. Later intervention is possible, but it is more costly than early remediation to achieve a particular level of adult performance. It is not surprising, then, that the empirical literature shows high economic returns for remedial investments in young, disadvantaged children. However, if these early investments are not followed by later investment, the effects of that early investment are lessened.

The health behavior literature has focused mostly on cognitive skills. However, socioemotional or noncognitive skills, such as perseverance, motivation, time preference, risk aversion, self-esteem, self-control, and preference for leisure, are equally important. They foster cognitive skills and promote healthy behaviors. Emotionally nurturing environments produce more capable learners, perseverance and motivation appear to be important factors in explaining compliance with medical protocols, and greater cognitive and noncognitive skills appear to reduce participation in smoking.[25]

A model proposed by Heckman and colleagues assumes that at each age, individuals possess a vector of capabilities that include pure cognitive abilities, noncognitive abilities, and health stocks. Health stocks include propensities for mortality and morbidity, including infant mortality. The model of capability formation proposes that all capabilities are produced by investment, environment, and genetics and that these capabilities are used with different weights in different tasks in the labor market and social life. Moreover, the model includes a developmental approach in which inputs or investments at each developmental stage produce outputs at the next stage, with each stage corresponding to a period in the life cycle of a child. It assumes that altruistic parents invest in their children. The model also assumes that investment is fully controlled by the parent, when in reality the child gains more control over investment as he or she matures.

By accounting for parental investment, Heckman's model explains the intergenerational transmission of health, personality, and cognition. It also allows investment behaviors to be triggered by initial conditions; that is, parents will react to their own resources, the environment, and the manifestation of genetic material at the earliest stages of child development. Thus, the model of capability formation provides a mechanism for understanding how skills evolve, how resilience plays a role, and how later investment can be effective in compensating for negative aspects of the early environment. This model could thus provide a means to explore how

[24] Schuerger JM, Witt AC. The temporal stability of individual tested intelligence. *J Clin Psychol*. 1989;45:294-302.
[25] Heckman JJ, et al. The effects of cognitive and noncognitive abilities on labor market outcomes and social behavior. *J Labor Econ*. 2006;24:411-482.

investments and other variables modify gene expression and its influence on behaviors. At present, however, no single gene has been tested.

The Bing Longitudinal Study

Yuichi Shoda, Ph.D., University of Washington

The Bing Longitudinal Study, which began in 1968, is a longitudinal study assessing delayed gratification in children in the Stanford University Preschool. The children were presented with a treat and told that if they could wait until the study staff came back to the room, they could have the larger of two piles. If the children could not wait, however, they could ring a bell, the staff person would return, and the children would receive the smaller pile. Study staff recorded the children and assessed how long they could delay and what they did while they waited. The first wave of the study, which ran from 1968 to 1974, enrolled 700 children who were 4 to 5 years old and collected measures of delayed gratification. The first wave of followup took place in 1984, and additional information was collected on measures of social adjustment, Scholastic Aptitude Test scores, and parents' ratings of their children's adjustment. The study was not initially designed to be longitudinal, so the sample size dropped from 700 to 300. However, the study team has maintained contact with the 300 subjects since 1984. In a second followup in 1993, additional measures, including of hostility, marijuana use, BMI, and marital status, were added. Current data collection includes cognitive measures as well as measures of brain activity. Each of these waves also has measures of self-control.

This study became a longitudinal study because the 1984 data revealed a surprising predictability; children who waited longer when they were 4 or 5 years old were more academically, socially, and coping competent.[26] The children who waited also were more attentive, could concentrate more, thought ahead, were more self-reliant, and could better handle stress. Data from a later wave also revealed that the ability to wait protected against rejection sensitivity. For those able to delay gratification, their self-worth did not appear to be affected by anxious expectations of rejection, whereas the low delayers' self-worth was reduced. To determine whether these effects were specific to this population, the study team conducted a second study in the Bronx in a primarily Latino population. In this study, the ability to delay was a buffering factor against bullying; high rejection sensitivity led to more bullying among children who were low delayers.

A lot of work has been done to assess the mechanisms underlying these behaviors. In one study, participants were assigned to look directly at the reward or to be presented with a hidden reward. When rewards were not exposed, most children waited. When both rewards were exposed, however, very few children waited. Thus, blocking unwanted stimuli improved the ability to delay. In another set of experiments, when children were asked to think about fun activities, bad things, or the rewards while they waited, children who thought about fun things were able to wait. Yet another set of experiments controlled elements of working memory. In the cool condition, children were asked to imagine marshmallows as cotton balls, whereas in the hot condition children were asked what the marshmallows would taste like. The cool condition improved the ability to delay.

[26] Mischel W, et al. The nature of adolescent competencies predicted by preschool delay of gratification. J Pers Soc Psychol. 1988;54:687-696.

These experiments show that attentional and cognitive strategies are important, and they suggest that individual differences in the availability of strategies and ability to execute them are related to delay and self-regulation activities. In a set of experiments with children aged 4 to 5 years, investigators assessed the children's gaze direction as they waited (high temptation, such as the reward or the bell, versus low temptation, which was elsewhere). In a later wave, these same children, who were now young adults, performed a go/no-go task testing their ability to stop the "go" key when a stop stimulus appeared. Children who had a high-temptation focus took a longer time as adults to perform the go/no-go task without errors. Now participants have laptops on which they do tasks tapping into each stage of self-regulation. Data from this set of experiments will be used to continue following trajectories based on high- or low-temptation focus.

Modeling Genetic and Environmental Influences on Stability and Change

Deborah Finkel, Ph.D., Indiana University Southeast

Several longitudinal models have been developed to model genetic and environmental influences on stability and change. For example, the Cholesky Model over Time assesses a trait over time or age. Genetic and environmental impacts are assessed at discrete points in time, allowing one to determine the time at which new factors or variance come into play. Thus, the model can be used to look at particular change points in the life course, such as transition from young to old, transition from old to older, retirement, or menopause. The Sweden-based OCTO-Twin study has used the Cholesky Model over Time to look at extroversion and neuroticism in 351 pairs of twins aged 80 years or older. No new source of genetic variation appears, but new environmental variance appears at times 2 and 3.[27] This variance could include changes in the physical environment, living arrangements, and support.

The Latent Growth Curve Model (LGCM) assesses the rate of change in a particular trait. A LGCM incorporating two linear slope parameters allows investigators to estimate or focus on inflection points, or the times at which the rate of decline changes. One can then assess genetic and environmental influences on that inflection point. With respect to life course, the LGCM allows one to determine the age at which rates of decline or improvement changes and whether genetic and environmental influences change at that age. The Change Point version of the LGCM can be used to assess individual change points for each subject, which in turn allows one to determine a mean change point and assess genetic and environmental influences on individual differences in change point.[28]

The LGCM can include quadratic models, assessing linear and quadratic parameters of change. Such a strategy has been used to determine genetic and environmental influences on rates of decline, as well as the heritability of change, in verbal, spatial, memory, and processing speed tasks.[29] For example, changes in verbal factors are mediated entirely by environmental factors. On the other hand, a similar strategy has been used to determine changes in genetic and

[27] Read S, et al. Stability and change in genetic and environmental components of personality in old age. *Personality and Individual Differences*. 2006;30:259-285.

[28] Ripatti S, et al. Twin change point models of cognitive aging. Paper presented at the *Annual Meeting of the Gerontological Society of America, Dallas, TXy* 2006 (Nov).

[29] Finkel D, et al. Surprising lack of sex differences in normal cognitive aging in twins. *Int J Aging Hum Dev*. 2006;62:335-357.

environmental variance, and thus changes in heritability, in general intelligence over age. In this study, genetic variation in intelligence decreases, and environmental variance increases, in late adulthood.[30]

Specific genes and environments can also be added to the LGCM. The presence or absence of a measured gene or environment can be entered as a covariate, and differences and trajectories can be assessed. Reynolds and colleagues used such a strategy to assess relationships between apoE and working memory.[31] In this study, they found that apoE4 homozygotes showed a different trajectory over time compared with heterozygotes.

Another model is the Dual Change Score (DCS) Model, which defines change with age based on both constant change and proportional change related to previous scores. Such a model can provide an estimate of nonlinear change over time, of acceleration or deceleration over time, or of stability. The bivariate version of the DCS model can assess how performance on X affects subsequent change in Y and vice versa. For example, the Bivariate DCS has been used to show the following:

- An increase in the relative size of the lateral ventricle is a leading indicator of lower scores on memory, but the reverse is not true.[32]
- Prior scores on socialization influence subsequent changes in perceptual speed, but the opposite does not hold.[33]
- Processing speed is a leading indicator of changes in spatial and memory ability but not verbal ability.[34]
- A portion of the genetic influences on change in spatial ability is acting through dynamic coupling with speed. The effect is unidirectional: genetic influences on spatial ability do not impact genetic influences on changes in processing speed.[35]

Thus, longitudinal models can assess genetic and environmental influences on stability and change if these models use twin, adoption, or family data; measured genes; measured environments; or some combination of these factors.

Discussion

Workshop participants first discussed technical aspects of the cohorts and methods presented during this session. Finkel clarified that the models she presented looked at lead-lag relationships

[30] Finkel D, Pedersen NL. Processing speed and longitudinal trajectories of change for cognitive abilities. The Swedish Twin/Adoption Study of Aging. *Aging Neuropsychol Cognition*. 2004;11:325-345.
[31] Reynolds C, et al. Longitudinal memory performance during normal aging: Twin association models of APOE and other Alzheimer candidate genes. *Behav Genet*. 2006; 36(2):185-194.
[32] McArdle J, et al. Structural modeling of dynamic changes in memory and brain structure using longitudinal data from the normative aging study. *J Gerontol B Psychol Sci Soc Sci*. 2004;59:P294-304.
[33] Lövdén M, et al. Social participation attenuates decline in perceptual speed in old and very old age. *Psychol Aging*. 2005;20:423-434.
[34] Finkel D, et al. Age changes in processing speed as a leading indicator of cognitive aging. *Psychol Aging*. 2007;22:558-568.
[35] Finkel D, et al. Genetic variance in processing speed drives variation in cognitive aging. *Developmental Psychology*. In press.

among genetic and environmental factors but did not establish true causality. Shoda clarified that the situational environment in the Bing Longitudinal Study was standardized during the first wave, when participants were 4 to 5 years old, and that investigators are now working to standardize data collected in the current wave. Participants also asked Hauser about controls for reliability with mailed samples. Some pointed out that in their experience, respondents did not follow instructions and that samples often included material that investigators did not want. Hauser acknowledged the value of selecting a subset of respondents and performing other measurements to get a sense of how reliable the samples are. Participants noted that saliva samples are not as useful for cell lines and epigenetic studies, and they suggested that WLS also collect blood from respondents.

The rest of the discussion focused on the value of candidate gene studies versus that of genome-wide association studies (GWAS). Focusing simply on particular genotypes can be expensive and is therefore not feasible for large studies. On the other hand, individual variations in a few genes do not account for behavior, and large datasets and large-scale analyses might therefore be useful to determine all the genetics of a particular behavior. A recently published study suggested that thousands of samples would be needed to find an effect size of 0.3. A case-control candidate gene study for schizophrenia enrolled 2,000 cases and 2,000 controls, and each gene was fairly well saturated, but only 5 percent of these genes were significant at 95 percent confidence, and only half that percentage was significant at 99 percent confidence. Thus, ways to add genetics to existing larger studies should be considered. That is not to say that candidate genes are not good approaches; at some point such approaches are necessary. However, one workshop participant suggested that there is no good arsenal of candidate genes yet.

Simply adding known small effects together and combining all known SNPs is not enough to understand the large heritability estimates observed in twin studies. A sample size approaching that of the entire human population would be needed just to examine a two-way interaction between SNPs. Some participants discussed the use of biology to narrow down a search or exploration and thus reduce the number of interactions under study. Even then, one cannot look only at genetic variation. Analyses of proteomics, epistatic relationships, and other sources of variation are needed. Studies of genetics in the life course also could benefit from the assembly of multidisciplinary teams that include members in such fields as molecular biology, statistics, and methodology.

Yet, smaller studies are still needed. Many smaller samples have been better characterized, and what is known about genetic influences, for example in inflammation, has been obtained from small studies. Small studies can provide information to guide design and interpretation of much larger studies. Individuals with a certain phenotype can be identified and the various alleles in that population examined. However, testing potential interactions statistically is not possible in a small group, and it is not clear, given the small effect sizes, whether new interactions would be identified.

One participant referred to a recent paper that suggested that if an effect size was 1.2 for known markers for myocardial infarction or schizophrenia, more than 400 markers would be needed to predict risk. This suggestion calls into question the potential usefulness for genetic testing in the health care setting. The paper concluded that studying family history would be more valuable; thus, study sample sizes could be manipulated by using family history as a variable.

Workshop participants noted the lack of visibility of studies that fail to replicate previous findings. They suggested the establishment of a registry for such studies. They also acknowledged, however, that replication studies are expensive and that typically there is less funding for them.

GENE-ENVIRONMENT INTERPLAY

Challenges in Building Models of Gene-Environment Interplay

Matthew McGue, Ph.D., University of Minnesota and Southern Denmark University

McGue provided a brief overview of longitudinal cohort studies, including the following:

- The Longitudinal Study of Aging Danish Twins, a cohort of 4,731 twins aged 70 to 102 years, followed biannually up to 6 times;
- The Danish 1905 Cohort, which includes everyone born in Denmark in 1905 and assessed through age 100 years;
- The Study of Middle-Aged Danish Twins, a cohort of 4,314 twins aged 46 to 68 years, assessed once; and
- The Minnesota Twin Family Study, a cohort of approximately 2,000 twins and 1,800 parents. Twins are 11 to 29 years old and followed every 3 or 4 years.

The Danish studies have been interested primarily in four outcome measures: (1) Strength, as measured by an 11-item self-report of physical activities; (2) depression, as measured by a 17-item depression symptom interview; (3) neurology, as measured by the Mini Mental State Examination (MMSE); and (4) cognition, as measured by a composite of 5 brief cognitive tests. On average, individuals aged 70 years and older exhibit significant changes in each of these domains. The most modest change occurs in depressive symptoms, but cognition, neurology, and physical strength all undergo an accelerating decline with age. There are many individual differences in these changes such that a long period of time might be marked not only by significant changes on average but also by a reassortment of individuals. That is, an individual's function at age 70 years might be a poor predictor of that individual's function at age 80, 85, and 90 years.

Consistent with Finkel's presentation, studies by McGue and colleagues reveal only modest to moderate genetic influences, in all domains, late in life.[36] Instead, these influences are more important in one's initial level of function than in age- or time-associated changes, whereas environment appears to play a larger role in age-related change. Lifestyle factors have been measured in the Danish cohorts and found to be heritable, but it is not clear whether these factors directly affect outcome or are selection effects that could confound assessments of risky or protective exposures. For example, heavy drinking is detrimental to health, but moderate drinking has been associated with lower rates of cardiovascular disease, dementia, and cognitive decline. In a recent study by McGue and colleagues (submitted), moderate drinkers perform better on the MMSE and maintain that better performance over time, compared with individuals

[36] McGue M, Christensen K. Social activity and healthy aging: a study of aging Danish twins. *Twin Res Hum Genet*. 2007;10:255-265.

who do not drink. To control for potential confounding, McGue and colleagues focused on monozygotic twins who were discordant for drinking status. They found that twins who drink moderately perform better than their identical twins who do not drink at all, suggesting a causal effect of moderate drinking on outcomes. In another study, socioeconomic status (SES) effects on health disappear when data are controlled for monozygotic twin status.[37]

Studies have established that individuals who try alcohol before age 15 years are at high risk for becoming alcoholic.[38] Similar results were found with other behaviors such as smoking, problems with police, and sexual intercourse.[39] Thus, even if early alcohol use is eliminated, other problems might appear later on. McGue and Iacono propose that early alcohol use or another problem behavior is a marker of genetic risk and that expression of that marker starts a cascade of events that can exacerbate early genetic risks. Thus, a multivariate perspective on risk and protection might be critical.

Studies in a Northern Russian Correctional System

Elena L. Grigorenko, Ph.D., Yale University

With the great strides in technology development, genotyping is no longer a problem, but data processing is. As a result, investigators interested in the influence of genetics on behavior might know what they have, but they still do not know what is missing. Although large-scale GWAS are valuable, these studies require prohibitively large sample sizes to capture small effects. Thus, specialized, narrowly defined samples are also valuable. Moreover, many discussions at this workshop have focused on gene-environment interactions, but gene-gene or allele-allele interactions are also important. Determining which genes are expected to interact depends on how the interaction is defined. For example, in a single neurotransmitter system, different genes control the production, delivery, release, and reception of a neurotransmitter, but these gene activities constitute a pathway, not an interaction. Thus, it is also important to determine what agents in this or other pathway are agents for interaction. Focusing on a single pathway can be too narrow of an approach, as noted by Vaccarino's work on fibroblast growth factor genes, but the literature does not appear to offer much on how to distinguish interactions from sequential cascades of events.

Grigorenko and her colleagues work with the correctional system in a district comprising 200,000 individuals in northern Russia. They have focused on adolescent males, and they have demographic records, biospecimens, and longitudinal data on recidivism and reoffending. They also have collected some postmortem samples for expression studies. The population in this district is homogeneous, consisting of Northern Slavs, and it has been homogeneous since the sixteenth century. Grigorenko's group has looked for possible distinctions between violent and nonviolent offenders, focusing on four dopaminergic genes with suggested involvement in criminal behavior: *COMT, MAOA* and *monoamine oxidase B (MAOB*), and dopamine beta-

[37] Osler M, et al. Socioeconomic position and twins' health: a life-course analysis of 1266 pairs of middle-aged Danish twins. *Int J Epidemiol*. 2007;36:77-83. Epub 2007 Jan 24.

[38] Grant BF, Dawson DA.. Age at onset of alcohol use and its association with DSM-IV alcohol abuse and dependence: results from the national longitudinal alcohol epidemiologic survey. *J Substance Abuse*. 1997;9:103-110.

[39] McGue M, Iacono WG. The association of early adolescent problem behavior with adult psychopathology. *Am J Psychiatry*. 2005;162:1118-1124.

hydroxylase (*DβH*). They also have looked at maternal rejection, using a Swedish tool developed and validated based on Russian populations.[40]

The results of these studies have yielded no surprises in terms of distributions of allele frequencies. Although large tables of allele frequencies arose, there were no systematic differences that distinguished violent from nonviolent behavior or interacted with maternal rejection. Likewise, distributions of haplotype frequencies yielded interesting differences, but again, these differences were only sporadic. Regression analyses of gene-environment (maternal rejection) interactions, however, yielded consistent interactions across various polymorphisms in *MAOA* and *MAOB*. These genes are extremely close to each other physically and could form a single haplotype block.

To further explore these interactions, Grigorenko and colleagues employed the Multifactor Dimensionality Reduction (MDR),[41] which allows for the inclusion of several alleles in one analysis. This system reliably classifies marker combinations based on their importance. When MDR considered all alleles at once, it revealed important interactions among *COMT*, *MAOA*, and *DβH* alleles. When MDR was focused only on the one, two, or three best predictors, however, it consistently yielded four markers that distinguished violent from nonviolent criminals: two *COMT* alleles and two *DβH* alleles. Both the *COMT* and *DβH* genes showed substantial effects in regression analyses, in the absence of social factors such as maternal rejection. Moreover, in an analysis on a reduced dataset, maternal rejection still had some value, but *COMT* and *DβH* polymorphisms appeared to be better at sorting nonviolent criminals from violent ones.

These studies point to the importance of empiricism, of considering various allelic combinations and their interactions, and of representing a gene by more than a single polymorphism (whether functional or not).

Personality Differences in Animal Models II

Stephen Suomi, Ph.D., NICHD

As discussed earlier, young rhesus macaques raised by their peers and not by their mothers tend to be hyperattached when placed in larger social groups. This hyperattachment impedes normal development, as these monkeys explore their environments less and play less competently, and these deficits can be maintained in later years. The consequences of differential rearing appear to affect several biological and behavioral systems, particularly under challenging conditions. Peer-reared monkeys are more fearful in new or mildly challenging settings or in social isolation, and they exhibit higher HPA activity, as measured by cortisol, ACTH, and CSF 5-HIAA. These differences are maintained well into adolescence when these monkeys are in challenging environments. Moreover, they tend to become more impulsive and aggressive as they mature,

[40] Perris C, et al. Development of a new inventory assessing memories of parental rearing behaviour. *Acta Psychiatr Scand*. 1980;61:265-274.

[41] Hahn LW, et al. Multifactor dimensionality reduction software for detecting gene-gene and gene-environment interactions. *Bioinformatics*. 2003;19:376-382.

Moore JH. Computational analysis of gene-gene interactions using multifactor dimensionality reduction. *Expert Rev Mol Diagn*. 2004;4:795-803.

Ritchie, et al. Multifactor-dimensionality reduction reveals high-order interactions among estrogen-metabolism genes in sporadic breast cancer. *Am J Hum Genet*. 2001;69:138-147. Epub 2001 Jun 11.

and differences in 5-HT metabolism between mother-reared and peer-reared monkeys tend to increase as these monkeys get older. Differences have been observed in the levels of nerve growth factor and brain-derived neurotrophic factor, in serotonin-binding potential, and in cerebral blood flow in particular brain regions. Thus, early experiences in these monkeys have consequences not only at the levels of behavior and emotional regulation but also at the levels of hormonal output, neurotransmitter metabolism, and brain structure and function.

Suomi and his colleagues have also assessed these differences, using a battery of neonatal tests based on the Braselton Neonatal Assessment Scale. These tests are standardized to assess reflexes and early infant temperaments, and they translate well between humans and monkeys because of the nonverbal nature of what they measure and the age at which they measure them. On the basis of these tests, activity, state control, and measures of auditory and visual orientation conducted during the first month of life can predict which individuals will become aggressive and impulsive and exhibit low levels of CSF 5-HIAA. As is the case with other measures, peer-reared monkeys carrying the short 5-HTT allele show large deficits on these tests, whereas those carrying the long allele perform slightly better than normal, and these differences disappear among monkeys reared by good mothers. Studies of MAOA polymorphisms have shown similar effects, with monkeys carrying the less efficient form behaving similarly to monkeys with short 5-HTT alleles.

When peer-reared monkeys are maintained in stable social groups, they tend to do better; even peer-reared females carrying the short allele can become good mothers in these situations. Suomi and his colleagues also have shown that alternative nursery-rearing procedures, in which peer-reared monkeys are given attachment or mother figures and allowed to associate with peers for about 2 hours each day, produce better outcomes than continuous exposure to peers during early life. Thus, compensatory mechanisms are at work in this population. Studies of interactions among rearing, 5-HTT genotype, and dominance hierarchies in the troop are under way.

This work emphasizes the importance of early experiences; good parenting can play a large role in protecting individuals from polymorphisms that would otherwise place them at risk for later biological and behavioral problems. In addition, as shown by cross-fostering experiments, good parenting tends to be passed from one generation to the next in a nongenetic manner, which might explain why problematic polymorphisms or alleles can persist in the population with no ill effects. In a stable environment, a monkey carrying a short allele but raised by a good mother will go on to be a good mother to her own offspring, some of whom carry that short allele. However, if something happens to that environment that hinders the ability of a female to act as a good parent—for example, the death of an old matriarch, a food shortage, increased predation—the stress brings about deleterious effects that place offspring with problematic alleles at risk for subsequent pathology.

The Fragile Families Study

Sarah Meadows, Ph.D., Princeton University; Daniel Notterman, M.D. (by teleconference), Princeton University

The Fragile Families Study (FFS) is a collaborative project designed to determine the capabilities and circumstances of unwed parents; the nature of the relationship between unwed parents and their children; how parents' capabilities and relationships influence the health and development

of themselves and their children; and how interactions among environmental stressors, parents' capabilities, and genetic factors affect the health and development of the parents and their children. The FFS is a multistage, stratified probability sample of approximately 5,000 new births from 1998 through 2000 in 20 cities with populations of 200,000 or more. Interviews were conducted with both parents at birth, and followup interviews were conducted by phone when the children were 1, 3, and 5 years old. Child assessments of cognitive and socioemotional development were collected during in-home interviews when the children were 3 and 5 years old. Interviews are under way for the 9-year followup. The FFS data oversamples nonmarital births (3:1), producing a large sample of "high-risk" children. Response rates are high—about 88 percent among unmarried mothers at birth, 75 percent among unmarried fathers at birth, and 85 percent of unmarried mothers and 69 percent of unmarried fathers by year 5. Data also include interviews with child care providers and teachers at years 3, 5, and 9; qualitative interviews in four cities (birth to year 3); prebirth maternal medical records; and DNA collected from mothers and children at year 9. Core data are publicly available.

The FFS is ideal for studying gene-environment interactions. For example, the data have been used to assess the idea of "at risk." Research thus far shows that children born to unmarried parents are exposed to multiple environmental risks, including low parental education (35 percent with less than a high school diploma), high levels of paternal incarceration (about 50 percent), and high levels of violence in the home (about 13 percent). About 25 percent of the study population was near poor, and about 75 percent were poor at some point in time by the time the child was 5 years old.[42] Similar results were observed with material hardship—that is, insufficiency of food, housing, or medical care. Thirty-two (32) percent of the families moved at least once, and 16 percent moved three or more times by the time the child had turned 3.[43] Residential mobility usually represents a break from neighborhood ties and family and social resources. In addition, the average number of transitions in relationships doubled among mothers who were cohabitating when their children were born, and the number tripled among mothers who were visiting partners or in no relationship when their children were born.[44] All these factors have been associated with poor outcomes such as maternal mental health problems, maternal stress, poor parental cooperation, harsh parenting, and higher levels of aggression in children.

Yet certain genetic polymorphisms are also associated with behavioral outcomes, and without genetic information, environmental stressors are imprecise and effects most likely underestimated. Candidate genes were chosen for FFS based on three criteria: An obvious polymorphism, an established linkage between polymorphisms, and a straightforward analysis of genotype. Thus, the FFS focuses on 5-HTT, MAOA, COMT, and DRD2, and investigators hope to add more genes in the future. As stated by others at the workshop, 5-HTT has been associated with depression and anxiety; DRD2 has been associated with substance use and alcoholism; and MAOA and COMT have been associated with antisocial behavior, aggression, anxiety, substance

[42] Fragile Families Research Brief. "Mothers' and Children's Poverty and Material Hardship in the Years Following a Non-Marital Birth." January 2008: No.41. Available at: http://www.fragilefamilies.princeton.edu/briefs/ResearchBrief41.pdf.

[43] Fragile Families Research Brief. "Mothers' Residential Mobility Following the Birth of a Child." December 2007;No. 40. Available at: http://www.fragilefamilies.princeton.edu/briefs/ResearchBrief40.pdf.

[44] Beck A, Cooper C, McLanahan S, Brooks-Gunn J. Family Structure and Mothers' Parenting. Fragile Families Working Paper. 2008.

use, and alcoholism. The literature on possible relationships between 5-HTT and alcohol and substance abuse is somewhat inconsistent.

The FFS uses Oragene to collect DNA from study participants; developmentally delayed children use sponges. DNA samples are sent to Princeton University, where they are deidentified and coded. So far, the response rate for DNA collection is 92 percent in two cities. The yield and quality of DNA samples are high. Investigators hope to have 8,000 samples from a racially, ethnically, and socioeconomically diverse group of mothers and children by the year 2010.

Future plans for the FFS include assessments of gene-gene and gene-gene-environment interactions both within and between generations. Investigators also are considering ways to handle gene-environment interactions in the context of population-level studies and to address cumulative effects of exposure to environmental stressors in gene-environment studies.

Discussion

Workshop participants first discussed the FFS. One participant noted that about 60 percent of the FFS population is admixed and that the frequency of COMT genotype varies across racial and ethnic groups. For example, the COMT-Val allele is more common among Africans than Caucasians. Participants cautioned that the FFS and other population-based studies will need to develop strategies to address problems of population stratification for this and other candidate genes. Notterman acknowledged ascertainment bias as a broad problem in genetic studies and commented that FFS investigators hope to move quickly to an approach that encompasses more of the "gene neighborhood" and focuses less on single polymorphisms.

Participants also pointed out that one of the criteria for candidate gene selection—known relationship between the gene and behavior—might not work well in the long run. None of the genes examined in the Caspi and Moffitt studies had a direct relationship to behavior. Instead, the biology of these genes and how they work in the context of stressors must be understood. Moffitt suggested including a stressor that will help FFS investigators examine biological systems important to environmental outcomes rather than focusing on behavior alone. Physical child abuse, breastfeeding, and lead exposure are examples of environmental stressors that could be tied to biological systems. At present the FFS has maternal medical records and nursery records, but it does not have blood samples to examine some of these stressors. Although FFS also does not have formal data on how much time mothers spent with their children, interviews do include some questions about what mothers do with their children (for example, "How often do you read to your children?").

In considering ways to integrate genetics into existing lifespan studies, it is important to address how public access to these data will be maintained and whether genetic risks should be disclosed to study participants. The National Research Council will have a workshop in Fall 2008 to address issues of access. Hauser noted that WLS releases all possible data to the public and suppresses identifying information. He added that he is interested in having other researchers work with genetic data in the WLS. Other participants pointed out that investigators are now required to release their datasets publicly when they publish with many journals. In addition, the NIH requires that all GWAS it supports place their data in the database of Genotype and Phenotype (dbGaP), and policies and procedures are in place to determine what information

accompanies the data. Principal investigators from these studies also can decide where else to deposit their data.

With respect to disclosure, the WLS release form states that investigators will not disclose information about genetic risks because the study is not equipped to address the consequences of what is learned about an individual's genotype. Moffitt added that she and her colleagues convened focus groups of parents to assess their concerns regarding genetic testing. Most focus group participants expressed pragmatic concerns, which Moffitt and colleagues addressed with a brochure. For the most part, the parents were not concerned about confidentiality. Other participants noted that most of the information learned now does not appear to have clinical import at this time.

Discussion turned again to the advantages of candidate gene studies versus GWAS. Richard Suzman reiterated the importance of both and the need to have these studies interacting with each other. Once genetic loci have been identified and associated with behavioral outcomes, they should be studied further in large-scale replicative studies. Heckman pointed out that many early-childhood intervention studies exist but have not collected genetic data. Supplemental studies could be added to these, but the interventions, which might not have anything to do with genetics, could still modify genetics. Other participants agreed on the need to combine studies; for example, conducting large, longitudinal studies and putting those together with genome projects that assess SNPs across the entire genetic code.

Some participants suggested that a standardized array or chip be developed for candidate gene studies, which could help in the building of a cumulative record that could, in turn, be useful for large-scale studies. Other suggested arrays using the Illumina platform or allowing researchers to use their own arrays for specific studies. As costs decrease, it will be increasingly possible to employ genome-wide scans, and this is being planned for FFS. It is important that the platform used for these studies be sufficiently well textured to permit analysis of copy number variants and tagSNPs. With some platforms, the genetic power in most populations is nearly complete for alleles with a frequency greater than 5 percent. For these alleles, power calculations are close to those achievable by direct sequencing of the alleles. Furthermore, sequencing costs are decreasing rapidly as their speed increases, and within a few years it is likely that very dense sequence information will be available for samples such as those developed in FFS. While GWAS on sample sets of the size of FFS would be at the margins of usable statistical power, a dense genome-wide scan could be regarded as being a cost-effective way of providing a major resource (database) that would allow rapid exploration of emerging markers. This approach addresses the problem of failing to including important polymorphisms on limited or custom arrays. The importance of a cumulative record was emphasized, and a journal of negative results in gene-environment interactions over the lifespan was suggested.

Workshop participants also cautioned that the field often moves at such a rapid pace that developers cannot catch up. By the time a chip is developed, more is known about the candidate genes in question. This problem is addressed by using a platform that provides very dense genetic information (one such platform is the Affymetrix SNP Array 6.0; others are available or in development). Grigorenko noted that the problem is not so much the generation of biological data but the processing of it. Because most models are not flexible enough to address

complexities, investigators have a large amount of genetic data but are unsure of how to handle it.

Efforts to integrate genetics into lifespan studies also should consider which point in the lifespan represents the most fruitful place to merge these studies. Most epidemiological literature has focused either on early-life events or later life outcomes, but participants agreed that efforts should not focus only on one time point but on dynamic interactions over time. Lifespan studies offer the advantage of controlling for certain variables and examining changes from a particular point in time. Investigators can learn about underlying biological processes and the types of structures that become important later in life, and they can develop new models. Participants added that early-life studies continue to be essential in these efforts, that measurement should be emphasized and should account for heterogeneity, and that mortality data should be incorporated in models of change as individuals leaving a population are different from those still living.

Mendelian randomization experiments were suggested as one way to integrate genetics into lifespan studies. For example, interactions between childhood illness and SES differences could be teased out further by following individuals homo- and heterozygous for alleles associated with a predisposition to particular illnesses. About 98 percent of genetic influences are set *in utero,* and these types of experiments could evaluate the association of particular genes with the subsequent arrival of environmental factors. However, it should be noted that the effects of a particular polymorphism are not determined in isolation from other genes or biological processes, and such an experiment most likely would not consider the entire haplotype.

FOAs that promote interdisciplinary collaboration to study large datasets also were suggested as a way to integrate genetics into lifespan studies. “Cross-talking” FOAs, aimed toward geneticists, psychologists, demographers, economists, and others, were suggested.

Future studies of gene-environment interactions should distinguish between predictive and causative interactions. Human genome sequencing projects and HapMap were sold as ways to identify predictors of disease, but that has not happened. It is likely that all genome projects together will yield tiny bits of information about causal effects.

GENE EXPRESSION AND EPIGENETIC MECHANISMS

Epigenetics and the Long-Term Effects of Early Experience

Frances A. Champagne, Ph.D., Columbia University

Individual differences in stress response to early environmental experiences can predict lifetime risk for psychopathology, obesity, and cognitive decline. Human studies have shown that secure attachment in early life is protective, whereas disorder is associated with high risk for adult psychopathology. These studies also have shown that low parental care and high, overprotective parental control are associated with increased risk for depression, and abuse or neglect has been associated with cognitive impairments and with high risks for physical and psychiatric disorders. Yet although these studies show a strong correlation between early-life experiences and later outcomes, they do not show direct causality.

Maternal separation and complete maternal deprivation have been used extensively in experimental work both in primates and rodents. As discussed by others at the workshop, these manipulations lead to increased stress response, poor cognitive ability, impaired social ability, and hyperactivity. Yet these manipulations are invasive. Work in humans, primates, and rodents has taken a different approach, focusing on the natural variations in maternal care.[45] This work has demonstrated that during the first week of infants' lives, maternal licking and grooming (LG) of pups occurs about 10 percent of the time, with profound effects on development. The frequency of LG is a stable trait and follows a normal distribution between low LG mothers and high LG mothers. Low LG has been associated with many processes, including increased corticosterone response to stress, decreased expression of the glucocorticoid receptor (GR) in the hippocampus, decreased dopamine in the accumbens, decreased synaptophysin, and increased latencies to maze learning. In cross-fostering experiments, Champagne and colleagues have found that the quality of the foster environment, not the character of the biological mother, predicts outcomes. They also have found that the effects of LG behavior persist into early adulthood.

Epigenetic mechanisms might play a role in this phenomenon. Other speakers have discussed the genome and the importance of the DNA sequence, but the structure of the DNA, or the epigenome, is also an important source of information and serves as an on/off switch. In the cell nucleus, DNA is wrapped around histones, and it must be unwound from these proteins and become available to RNA polymerase and other transcription factors to be expressed. This unwrapping is facilitated by acetylated histone tails. DNA methylation modifies the structure. Methyl groups bind cytosine residues at certain points in the gene sequence, and in so doing they become physical barriers to transcription factors, decreasing transcription. Methyl groups also attract other proteins that can further silence DNA by altering the acetylated histone tails. DNA methylation is a stable modification of DNA and can be transmitted to daughter cells.

The offspring reared by high LG mothers are more efficient at shutting down the stress response once stress ends. The offspring reared by low LG mothers exhibit much more methylation within the promoter of the GR gene in the hippocampus, and this may explain the decreased hippocampal GR expression seen in the offspring reared by low LG mothers. In general, little methylation of the GR promoter is seen late in gestation, but complete methylation is seen at birth. Yet this methylation is sustained in the offspring reared by low LG mothers, contributing to a divergence in methylation between these offspring and the offspring of high LG mothers by postnatal day 6. The maintenance of high levels of methylation is sustained at weaning and in adulthood, 2 to 3 months later. This work demonstrates that experiences in early life can have long-term effects on gene expression, and these effects can be stably maintained into adulthood. Social experiences can alter the epigenome and thus regulate gene expression, and neural

[45] Liu, et al. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic pituitary-adrenal responses to stress. *Science*. 1997;277:1659-1662.

Champagne FA, et al. Variations in maternal care in the rat as a mediating influence for the effects of environment on development. *Physiol Behav*. 2003;79:359-371.

Pruessner, et al. Dopamine release in response to a psychological stress in humans and its relationship to early life maternal care: a positron emission tomography study using [11C]raclopride. *J Neurosci*. 2004;24:2825-2831.

Hane AA, Fox NA. Ordinary variations in maternal caregiving influence human infants' stress reactivity. *Psychol Sci*. 2006;17:550-556.

systems regulating stress response, and ultimately risk of psychopathology, can be regulated by these epigenetic mechanisms.

To determine whether these effects can be reversed, Champagne and colleagues conducted experiments with a histone deacetylase (HDAC), which silences gene expression by removing acetyl groups from the histone tail. When offspring reared by low LG mothers were treated with the HDAC inhibitor trichostatin A, they exhibited decreased stress response and other behaviors similar to that seen in the offspring reared by high LG mothers. In addition, increasing methyl donors in pups reared by high LG mothers leads to decreased GR expression and increased stress response. Social experiences later in life also can reverse the effects of maternal LG. When pups reared by low LG mothers are placed in enriched social environments, they behave like pups reared by high LG mothers. On the other hand, when pups reared by high LG are placed in impoverished or isolated environments, they behave like pups reared by low LG mothers.

These effects are passed on to the next generation. The effects of later social experiences have not been studied yet at the molecular level. Champagne and colleagues also investigated the effects of altering maternal care, using the paradigm of gestational stress. Under normal conditions, high LG mothers have offspring who exhibit high LG behavior. If these mothers are subjected to gestational stress, however, they become low LG mothers, and their offspring, in turn, become low LG mothers. Other stressors include variable foraging demand, social isolation, and food restriction. This work is consistent with other studies demonstrating the transmission of the effects of maternal care to future generations.[46]

Expression of oxytocin and the alpha isoform of the estrogen receptor has been implicated in the transmission of the effects of maternal care to future generations. High methylation and, thus, low expression of these genes have been observed in females reared by low LG mothers. These females go on to exhibit low LG behavior, and the cycle continues. Maternal care also can be influenced by communal nursing, experience, and juvenile social experience. Although Champagne and colleagues have not examined the epigenetics of these other influences, the factors do appear to affect oxytocin, which can be affected through multiple genes.

This work indicates that some effects associated with maternal care can be passed down in a way that looks genetic but could arise from epigenetic environmental variables. This concept has several implications. Epigenetic mechanisms can mediate the developmental effects of early experiences by regulating long-term suppression of genes involved in stress response. They also provide potential therapeutic targets in adult rats. In addition, these studies suggest that experiences beyond so-called “critical periods” can ameliorate the effects of early “adverse” experiences. Moreover, preconception, prenatal, and perinatal environments can alter epigenetic

[46] Miller L, Kramer R, Warner V, Wickramaratne M, Weissman M. Intergenerational transmission of parental bonding among women. *J Am Acad Child Adolesc Psychiatry*. 1997;36:1134-1139.

Benoit D, Parker KC. Stability and transmission of attachment across three generations. *Child Dev*. 1994;65:1444-1456.

Ney PG. Transgenerational child abuse. *Child Psychiatry Hum Dev*. 1988;18:151-168.

Francis, et al. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science*. 1999;298:1155-1158.

Champagne F, Meaney MJ. Like mother, like daughter: evidence for non-genomic transmission of parental behavior and stress responsivity. *Prog Brain Res*. 2001;133:287-302.

patterns, and these alterations can be transmitted to future generations; epigenetic alterations, for example, the transgenerational effects of early nutrition, have been implicated in longitudinal studies. Epigenetic patterns also can change over time. For example, chromosomal methylation patterns are similar in young twins but diverge as the twins get older.

Gene x Social Environment Interactions: Bioinformatic Strategies for Discovery

Steven W. Cole, Ph.D., University of California, Los Angeles School of Medicine

As discussed throughout the workshop, social environments exert their effects in part by changing the expression of genes. These effects are mediated by "social signal transduction pathways" in which social environments first influence brain activity, which in turn affects peripheral neuroendocrine function, which influences cellular signal transduction, transcription factors, and other factors that modulate transcription, and ultimately, the activity of the genome. Cole and colleagues have attempted to understand these pathways in viruses, which are simple genomic systems. For example, individuals displaying the shy, socially withdrawn phenotype tend to die from HIV infection 2 to 3 years before other infected patients do. High threat perception has been associated with more neural fibers in the lymph nodes and, in turn, the activation of beta-adrenergic signaling pathways in lymphocytes, which activates factors such as CREB/ATF and inhibits other transcription factors. The resulting changes in the expression of key human genes leads to changes in HIV gene expression, which alters the equilibrium between virus replication and the host's ability to inhibit it.

In looking at viral systems, Cole and colleagues have attempted to determine what design principle leads the human body to change gene expression in a way the virus can adapt to. They ask which genes in the human genome are sensitive to social processes and targeted by social pressures, which transcription control pathways are intermediate, and which genetic polymorphisms modulate these influences. Cole and colleagues have built computer models that track the flow of information from the social world to the genomic world. Their approach develops theories about regulatory relationships based on laboratory molecular biology, scans those theories across the entire human genome to yield *in silico* predictions, and then cross-validates those predictions using empirical genome-wide transcriptional data, or microarrays. This approach thus represents a middle ground between candidate gene studies and the exhaustive process of a hypothesis-free genome-wide scan of genes and SNPs.

One of their bioinformatic approaches reverses the normal flow of biological information to ascertain transcription control pathways that mediate environmental effects based on their downstream impact on gene expression profiles. Under normal conditions, something in the environment leads a molecule to bind to a receptor, which triggers transcription factors that bind DNA and turn on expression by recruiting the transcription machinery. Sequences in the gene promoter determine which transcription factors will bind. One can reverse this biological flow, but there are challenges. An expressed gene often has more than one putative promoter sequence; thus, investigators must look at several genes. Cole and colleagues' models look at multiple promoters, identify those that are activated in a social experiment, and determine differences among binding motifs. This allows them to home in from approximately 20,000 promoters to about 200 transcription factors with reasonable confidence, and from there they can predict which transcription factors modulate gene expression under certain conditions. The models can display promoter elements that are differentially activated within a set of differentially expressed

genes. This model can be used for just about any social exposure. An investigator can start with his or her topic and obtain gene expression data that can be used to make inferences about upstream transcriptional mediators, thus narrowing an investigator's search. It is not a perfect model—investigators must still try to obtain tissue samples to get that gene expression data—but it opens a pathway of possibility.

In one example of this approach, Cole's group analyzed gene expression in lymphocytes isolated from stressed individuals, finding an altered equilibrium of activity between the anti-inflammatory GR pathways and the proinflammatory nuclear factor kappa B (NF-κB) pathways. Inflammation thus proceeds without the normal checks and balances. In one study with John Cacioppo, this approach identified altered GR/NF-κB regulation of gene expression under conditions of social isolation.[47] Social isolation appears to induce glucocorticoid resistance; the body produces normal levels of cortisol, but the signal triggered by this cortisol does not get through to the genome. Similar shifts have been observed in the parents of children with cancer compared with matched controls.[48] Thus, differences in the gene expression profile mediated by transcription factors become apparent, even when differences in hormone levels outside the cell are not.

Other generalizations observed by Cole and colleagues in analyses of social stress include increased CREB/ATF signal transduction. These alterations have been observed in socially isolated individuals, parents of cancer patients, sleep-deprived individuals, and ovarian cancer patients with high levels of depression and low levels of social support. Cole and colleagues also have seen reductions in the activity of transcriptions factors, such as interferon response factors, that are key defenders against viral replication. They also have looked at the effects of genetic polymorphisms, for example, in the GATA binding site of the interleukin 6 promoter. A polymorphism upstream of the transcription start site replaces a guanine residue with a cytosine residue, effectively knocking out the GATA binding site. Activation of GATA-dependent gene expression is less effective in individuals carrying the cytosine polymorphism. This has been shown in cell culture, where activation in response to norepinephrine treatment occurs to a lesser degree in cells carrying the cytosine polymorphism.

Cole and colleagues have identified more than 1,200 polymorphisms predicted to change the access of the GR to genes, and similar numbers for other socially sensitive transcription factors. Next steps will involve empiric verification of gene-environment interactions in human clinical studies and in *in vitro* mechanism studies. The availability of a sequenced human genome, combined with information about transcription factor binding motifs and receptor-mediated signal transduction pathways provide new opportunities for making theory-driven predictions about the effects of socioenvironmental factors on gene expression, physiology, and disease pathogenesis.

[47] Cole SW, et al. Social regulation of gene expression in human leukocytes. *Genome Biol*. 2007;8:R189.
[48] Miller GE, Chen E, Sze J, et al. A genomic fingerprint of chronic stress in humans: Blunted glucocorticoid and increased NF-kappaB signaling. *Biological Psychiatry*. 2008 (in press).

Expression of Risk Genes in the Human Brain: Development, Maturation, and Schizophrenia

Joel E. Kleinman, M.D., Ph.D., National Institute of Mental Health (NIMH)

The work by Kleinman and colleagues aims to discover the cellular and molecular mechanisms that lead to schizophrenia, using postmortem brains. This work has several objectives, including the following:

- Comparing expression of susceptibility genes in schizophrenic brains with normal controls;
- Examining the effects of allelic variations of susceptibility genes on expression of these genes and related molecules; and
- Determining variation in expression patterns of susceptibility genes during normal human brain development.

Kleinman and colleagues have collected 973 brains since 1977. More recently they have received a number of specimens from the NICHD. They also have gathered clinical histories, medical records, and the results of toxicology and neuropathology screens. Toxicology has been done on brain and/or blood and, in some cases, they have performed segmental hair analysis. Some brains in this collection also have undergone molecular biology screening (pH, *in situ* histochemistry hybridization, ribosomal RNA ratios, and capillary electrophoretic analysis yields).

Allelic variation might be associated with brain- and primate-specific alternative transcripts of genes, many of which have not been identified previously. Colleagues of Kleinman have identified risk alleles or haplotypes associated with schizophrenia and/or cognition. Kleinman and colleagues have followed up this work by using postmortem human brains to see if risk alleles/haplotypes affect mRNA expression and/or protein levels. For example, they have found that COMT enzymatic activity in the prefrontal cortex is higher in individuals with the COMT-Val/Val genotype compared with the COMT-Val/Met or COMT-Met/Met genotypes.[49] Another SNP of interest in COMT is a synonymous coding SNP, in which a nucleotide has changed but the resulting amino acid is the same. Although the SNP does not change the amino acid sequence of the COMT protein, it does change the structure of the messenger RNA such that less protein is produced.[50] Still, other SNPs in COMT[51] may affect function in a number of other ways. Disease risk alleles for other relevant genes, such as occur in GRM3 and neuregulin-1, may involve a number of different mechanisms including alternative transcripts of those genes.

Development and maturation of the brain may also involve the expression of alternative transcripts. To pinpoint the stage at which risk alleles act, Kleinman and colleagues have undertaken an ambitious lifespan study. This study involves 215 normal human brains taken from individuals aged 14 weeks *in utero* to 78 years. The population represented by these brain samples is predominantly male, 62 percent African American, 34 percent Caucasian, and 4

[49]Chen J, et al. Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am J Human Genet*. 2004;75:807-821. Epub 2004 Sep 27.
[50]Nackley AG, et al. Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. *Science*. 2006;314:1930-1933.
[51]Bray NJ, et al. A haplotype implicated in schizophrenia susceptibility is associated with reduced COMT expression in human brain. *Am J Hum Genet*. 2003;73:152-161. Epub 2003 Jun 11.

percent Hispanic and Asian. Smokers comprise 28 percent of the population. DNA, RNA, and protein have been extracted from the dorsolateral prefrontal cortex, which has been associated with schizophrenia, and analyzed by microarray. This work has yielded a database that will allow investigators to examine the putative effects of 650,000 SNPs on the expression of approximately 23,000 genes across the lifespan. This database will hopefully be released to the public in the near future.

Data from the normal development series described by Kleinman can also be used to test a number of hypotheses including mechanisms that may underlie gene-environment interactions of cannabis and COMT discussed in Moffitt's presentation. High use of marijuana in early adolescence has been associated with increased risk for schizophrenia.[52] The risk for individuals with the COMT-Val/Val genotype increases tenfold if they use cannabis in early adolescence, whereas the risk for those with the COMT-Val/Met genotype is slightly modified and that for individuals with the COMT-Met/Met genotype is not affected. These differences disappear among individuals who use a large amount of cannabis at age 18 or older.[4] Data from the Kleinman developmental series can be used to see if COMT val/met genotype effects expression of predicted genes that may affect response to cannabis.

Discussion

Workshop participants discussed pragmatic issues in conducting research on gene-environment interactions across the life course. Some participants advocated moving ahead with what can be done as opposed to waiting for methods to be perfected. Accessible tissues can be used as proxies for inaccessible tissues. For example, lymphocytes can provide a readout for systemic biological processes. Cole recommended adding the whole blood collection to large-scale social surveys. The BSR is working on this. Kleinman shared that his group has lymphocyte cell lines for all patients studied, but he cautioned that there are some limitations because some epigenetic patterns are passed through generations in cell culture. Other participants suggested adding biopsy sample collection, if possible, and others noted that once study participants have agreed to donate their DNA, obtaining consent for blood collection is easier. Workshop participants also pointed out that once study participants became comfortable with a biomedical protocol, they usually prefer it to psychosocial assessments.

In response to questions about the logistics of assessing gene expression in lymphocytes, Cole recommended collaborations for this type of work. He suggested that investigators use reverse-transcription polymerase chain reaction if they have strong biological hypotheses. Otherwise, they should examine the lymphocytes and look for systemic problems. Cole cautioned, however, that lymphocytes form a heterogeneous population of cell types and that different responses will activate different cell types. Thus, it is important to monitor which cell types are involved in the response of interest.

Cole also noted that gene expression profiles in the lymphocyte have not yet been correlated with those in the brain. It is not clear what techniques are available to assess what happens in the brain, but the BSR should support laboratory models and small studies as it moves its portfolio

[52] Caspi A, et al. Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. *Biol Psychiatry*. 2005;57:1117-1127.

forward. Microarrays, gene expression data, and a comparison of expression profiles will be helpful in moving between animal and human studies. For traits such as delayed gratification studied by Shoda and colleagues, Cole suggested finding an animal model and identifying markers of the trait. In response to other questions about embedding the types of assays Cole described into longitudinal studies and the number of measurements needed to assess biological responses over time, Cole suggested that investigators do what they can, using crude approaches, choosing the right point for measurement, and standardizing confounders as much as possible to rule out contamination issues.

The models developed by Cole and colleagues have not been used to assess time courses. Cole acknowledged that they have looked at gene expression after 24 hours and found no differences from baseline. He noted complexities in terms of what happens at night; for example, differences in central nervous system activity that affect information storage and changes in neuroendocrine and immune cell function.

Participants also suggested partnerships with established centers that track people over time, which can facilitate tissue collection. For example, a partnership with NIA-funded AD centers might allow Kleinman to obtain postmortem brains from patients with AD. These centers collect not only tissue but also large amounts of associated data.

GENERAL DISCUSSION

This workshop was one of many that explored the idea that genetic variations can explain or predict phenotypic variation and, thus, differences in nature. Although many in the public fear attempts to use fixed genetic traits to describe aspects of behavior, presentations and discussions at this workshop and others indicate that genetic traits can be modulated by experiences and environments over time. The integration of genetics into life course studies, and the subsequent exploration of how genetic variation can explain in part the phenotypic variation observed in these studies, will require investigators to ask and choose the right scientific questions.

The NIA has a large portfolio of population studies, including longitudinal and intergenerational studies, that can benefit from the collection of genetic data. Many, such as the HRS and WLS, have begun to collect these data. However, the best material from which to extract DNA has not been standardized. The HRS began collecting DNA by mouthwash but then discovered that Oragene is a better method. Although study investigators will make sure to collect DNA by Oragene from all its participants, they do not have plans to collect whole blood or other tissue because of the degree of participant contact required. McGue's studies make use of mobile phlebotomy services to obtain blood and DNA, and all interviewing staff receive training in phlebotomy. However, these services cost about $75 per sample. Hauser discussed his experience developing an ambitious agenda for comparing WLS with a statewide survey that mimics the National Health and Nutrition Examination Survey. This agenda proposed to use mobile clinics and collect biomedical data, but leaders were told to scale back because of funding issues. It may be that collection of blood and tissue for genetic data is unfeasible for large, population-based studies.

Workshop participants agreed on the importance of storing samples for later DNA extraction and analysis. Suomi shared that in all his studies, the study team collects biological samples,

subdivides them, and saves them. Creating this kind of collection not only allows them to repeat assays but also to conduct freezer experiments. McGue suggested a contractual agreement in which investigators could send samples to a facility that could extract DNA from part of the sample and store the rest. For example, his laboratory sends samples to Rutgers University under a contract with the NIMH and the National Institute on Drug Abuse. Another workshop participant suggested repositories focused on specific scientific questions. One example is a repository devoted to genes involved in AD. This repository stores blood and brain samples, isolates DNA, and makes DNA and samples available to researchers along with phenotypic information associated with those samples.

Databases also were discussed. One participant noted that many ongoing large studies might not have begun with social changes or parameters in mind, but they are now collecting this type of information. One example is the Framingham Heart Study, which, with support from the NIA, collects information on cognition now that participants are getting older. Because the NIH has determined that GWAS are necessary, it now requires studies supported by NIH funds to deposit these types of results into dbGaP. Investigators interested in genetic influences over the life course could query these types of databases.

The evolutionary advantage of polymorphisms that influence behavior should be studied further. Many of the polymorphisms discussed at this workshop appear to have detrimental consequences on behavior, but under certain circumstances, they may be adaptive or beneficial. To provide an example, Suomi described Chinese rhesus macaques, which are descendants of monkeys that migrated over the Himalayas. These monkeys are extremely aggressive, impulsive, and disruptive compared with Indian rhesus. The gene frequency associated with low 5-HT metabolism is about 75 percent in Chinese monkeys versus 25 percent in Indian monkeys. Suomi added, however, that Chinese monkeys can live in extremely harsh environments where humans are unable to live, thus increasing their chances for survival, suggesting that the benefit of certain polymorphisms may depend on the environment of the organism.

Many studies of genetic influences on behavior have focused on candidate genes, but heterogeneity among individuals makes it difficult to determine how much variation these genes account for. In addition, as has been demonstrated so far, the effects of candidate genes appear small, and SNPs on existing panels might prove uninteresting in larger studies. Samples collected by large studies could prove valuable in helping investigators identify what candidate genes do in the general population. Workshop participants also emphasized that even though genome analyses using existing gene chips have yielded negative data so far, they could still prove to be valuable tools. Asking the right question is important to obtain useful data from limited gene chips. However, workshop participants also noted the need to move beyond candidate genes.

Workshop participants pointed out that many gene-environment interactions discussed are static. However, workshop presentations suggest that the influence of some of these interactions on behavior is continuous, whereas the influence of others is plastic. Thus, studies should focus not only on the influence of early-life events but also on events throughout the life course. Suzman discussed the billions of people who are already “out of the starting gate” in terms of aging and emphasized that a focus on late-life events can provide some insight. Other participants added that there is a need for more studies to determine how environmental changes or shocks “get

under the skin." Yet others indicated the importance of not only identifying resilient versus at-risk individuals but also enhancing and developing retrospective measurements of early-life factors to enhance examinations of later life.

Although a large amount of data has been generated on personality, the components of personality cannot be associated directly with genetic differences, and the heritability of personality traits has not been established rigorously. Causal pathways that give rise to individual phenotypic differences must be understood within context and with respect to health processes, and problems such as reverse causality should be addressed. For example, Heckman described work developing a common set of measurements to assess how personality traits predict economic outcomes. Even after data are adjusted for reverse causality, some predictive traits have been identified. Conscientiousness was cited as an example in discussions about the influence of genetics on behavior over the lifespan. Workshop participants suggested that the definition of personality constructs such as conscientiousness or time preferences be revisited. At present, these constructs usually describe a collection of behaviors and outcomes, but an understanding of the underlying processes or mechanisms is needed. This type of understanding can increase the likelihood of discovering interventions that transform context or inhibit negative responses. Nonlinear dynamics also should be explored.

The same behavioral phenotype should be measured across multiple studies, either in different ways or in the same way to accommodate cross-talk among studies. Workshop participants discussed ways to link smaller studies with large, nationally representative studies that have data on phenotypes of interest. Yet how to standardize phenotypes is not clear, and many large studies have changed since their initial design with respect to the types of data collected. Consideration should be given to the ongoing debate about what to measure and how to measure it, and more should be done to refine phenotypes. Workshop participants expressed concern that some items currently measured might not be important. Others discussed the failure of consensus meetings to promote standardization of measurements. Calibration models, or calibration of phenotypic domains represented by reasonable measures, were suggested. Some workshop participants cautioned against the "risk of tyranny" in deciding what phenotypes matter, especially when it is not clear what phenotypes are most important. Suzman pointed out that the NIA is considering ways to pool studies and data, with a focus on a limited number of phenotypes. He added, however, that this should not prevent investigators from focusing on other phenotypes. Other workshop participants added that substudies can be carved from large ones.

Twin and sibling studies remain valuable. Traditional twin studies have examined genetic and environmental covariance structures, some have measures on personality traits such as conscientiousness, and some offer longitudinal data. These studies are therefore useful for evaluating gene-environment interactions over time. For example, the Swedish Adoptions/Twin Study of Aging has a family environment component in which participants are asked about their perceptions of their family. This study also offers retrospective and biological measures. Other studies focus on twins who were reared together or apart. One workshop participant suggested a design in which the advantages of a cohort design—attributable risk and real effect sizes in the population—are combined with the study of dizygotic twins, which would allow the examination of genetic differences and their links to different phenotypic outcomes while controlling for the twins being born at the same time and reared in the same environment. Studies of monozygotic

twins, which at one time were not considered useful for molecular studies, are proving useful for exploring epigenetic differences.

Workshop participants agreed that investment should not focus on one study design alone. Rather, investment should be made across a broad variety of designs, including multigenerational designs. One participant suggested a study of adopted children whose earliest experiences occurred in impoverished countries and how their experiences have changed throughout life. In such a study, one could examine how biomarkers have changed. Scott Hofer discussed measurement burst designs, which involve widely spaced intensive measurements. For example, one study obtained data every 6 months for 4 years to redefine or perfect the notion of cognitive functioning. In this study, participants were allowed to practice until they reached their best performance, and best performance was tracked over time. Such a design provided information about learning effects as well as daily systemic variations. Microarray studies could be integrated with this type of study.

As noted by Jennifer Harris, science has moved to the point where collaborations are needed between investigators with different skill sets. Such collaborations facilitate creativity and help investigators move beyond questions they have the expertise to answer. Workshop participants suggested the following strategies to facilitate interactions between potential collaborators and thus promote integration of genetics into existing lifespan studies:

- **Workshops focused on outcomes or risk factors of interest to the BSR.** Participants agreed that recent workshops, including this one, were extremely valuable, but they often ask participants to make a sweep from substantive science to policy. The BSR could convene workshops bringing together investigators from different disciplines who use different approaches to study a particular outcome or risk factor, such as child malnutrition or lead exposure, for which a large amount of data has been collected. Whether these workshops should focus on outcomes or risk factors was a point of debate. Some participants noted that focusing on a risk factor, which could have more than one outcome, could provide an evidence-based foundation for biologically plausible hypotheses about pathways between genetics and behavior across the life course. Others worried that focusing on a risk factor rather than an outcome would ignore other intervening factors, and some considered some risk factors to be outcomes. They also pointed out that outcome guidance would ultimately help investigators determine which genetic modules to focus on. One participant provided an alternative view by describing a genetic analysis workshop that had taken place years before. At this workshop, investigators from different backgrounds received a common dataset and question and applied their individual approaches to them and then discussed at a later point which approaches were most useful. A similar approach could be employed to develop a biological model that would guide the inclusion of genetics in life course studies.
- **Other workshops.** The BSR will hold workshops on the interactions between interventions and genetics as well as a workshop at the Association for Psychological Science for psychologists entering aging research. Suggested workshop topics included statistical methodologies used by different disciplines and new strategies for data analysis. One participant also suggested a workshop focused on the investments that already have been made in genetics and behavior.

- **Encouraging efforts to learn languages used across disciplines.** Workshop participants agreed on the importance of understanding terms used by people from other disciplines and suggested several methods for promoting understanding. For example, social scientists could attend mini-courses modeled after the RAND Summer Institute or Mini-Medical School. Courses in Seattle, Birmingham, and London are also available, and investigators can identify yet others through online searches. Discipline-specific 10- to 20-page primers were also suggested. One participant also suggested the book, *Molecular Biology Made Simple and Fun*, by David P. Clark, with an update by Lonnie Russell.
- **New project support.** An RFA could encourage investigators to think about methods of collecting genomic data for behavioral studies.
- **Special journal issues.** Participants from this workshop and the fall 2007 workshop on Refining Economic Phenotypes for Genetic Analyses could submit papers for a special issue.

APPENDIX 1: WORKSHOP AGENDA

NATIONAL INSTITUTE ON AGING
BEHAVIORAL AND SOCIAL RESEARCH PROGRAM

Genetic Methods and Life Course Development

A workshop to integrate life course and genetics research

Marriott Bethesda, 5151 Pooks Hill Road, Chevy Chase/Rockville Suites, Bethesda, MD
February 11-12, 2008

AGENDA

Three promising streams of research suggest this workshop. First, there is a growing body of research showing that early life experiences may have effects enduring across the life span and influence the trajectories of social and emotional competence or decline in old age. Second, there are an increasing number of well-crafted, longitudinal studies covering broad spans of development whose methods, observations, analyses and findings are helping to delineate patterns of behavioral and social development in mid and late life. Third, findings from genetic studies, using quantitative and molecular genetic approaches, are suggesting mechanisms that may account for continuities and discontinuities in social and emotional competence across broad spans of development. This workshop will explore areas where the integration of lifespan development and genetics can clarify developmental mechanisms that promote selected domains of social and emotional competence in aging. Where possible, the focus of this workshop will be on resilience and enhancement factors, or factors that lead to improved health and aging outcomes, rather than risk factors.

To address these issues, we plan to bring together a small number of researchers with experience in life course developmental research with researchers using various genetic approaches in order to build upon and synthesize the three lines of research outlined above.

Goals:

This workshop is intended to be a beginning to an ongoing dialogue between researchers in lifespan development and genetics with the goal of integrating the two areas of research. At this initial exploratory workshop we plan to address the following:

1. Identify questions related to behavioral and social factors of aging that are best answered through the integration of genetic and lifespan data.

2. Discuss whether or not there are lifespan studies in existence that could be tapped into and added onto in order to address these questions.

3. Discuss strategies and opportunities for integrating relevant genetic methods with existing lifespan studies to address key research questions and move science forward.

Monday, February 11

8:30 am **Continental Breakfast**

9:00 am **Introductions**

General Introduction and Goals	Erica Spotts and Matthew McGue, co-chair
Comments	Richard Suzman
Background of BSR Workshops in Genetics	Jennifer Harris

9:30 am **Overview** Chair and Discussion Leader Matthew McGue

Discussion of the conceptual issues involved in the integration of genetics and lifespan research with a focus on the unique role that genetic methods can play in explaining the influence of early life experiences on aging processes.

9:30 – 9:45	Terrie Moffitt
9:45 – 10:00	Stephen Suomi
10:00 – 10:20	Discussion

10:20 am **Break**

10:30 am **Stability and Change** Chair and Discussion Leader Elena Grigorenko

Long-term longitudinal genetic studies can delineate the role of both genes and environment in accounting for stability and change. In this section several researchers will present key findings from lifespan studies and then describe where their research could be advanced with the addition of genetic methods. Then work will be presented showing how quantitative genetic methods can expand our understanding of stability and change in aging research.

Questions for Discussion:

- Where should research on stability and change head and what genetic methods might be most applicable to current questions?
- Are there specific substantive areas that would most benefit from this type of analysis?
- What genetic methods are necessary appropriate for addressing these questions?
- What specific problems are involved in modeling phenotypic changes over time and how can genetics be added to these models?

10:30 – 10:50	James Smith
10:50 – 11:10	Robert Hauser
11:10 – 11:30	James Heckman
11:30 – 11:50	James Vaupel
11:50 – 12:10	Yuichi Shoda
12:10 – 12:30	Deborah Finkel

12:30 – 1:00 Discussion

1:00 pm **Lunch**

2:15 pm **Gene-environment Interplay** Chair and Discussion Leader Jennifer Harris

Genetic methods permit a better understanding not only of genetic mechanisms, but environmental ones as well. This session will focus on the complex interplay among genes and environments.

Social science has long dealt with the issues of selection into various social environments versus social environments causing particular outcomes. Genetically informed designs have illuminated the role of genetic and environmental factors in evoking reactions from the social environment, in developing and sustaining supportive social relationships, and in leading to engagement or withdrawal from the social world.

Genetically informed designs have also helped to clarify both environmental circumstances across the life span that moderate heritable risk in social and behavioral domains, as well as genetic factors that moderate individual responses to environmental stimuli.

Questions for Discussion:

- What behavioral and social domains of relevance to aging could benefit from an understanding of gene-environment interplay?
- How can an examination of gene-environment interplay add to our understanding of robust findings linking early experiences with later aging outcomes?
- What genetic methods can be brought to bear on key areas of interest?
- Once genes are discovered in other gene-finding endeavors, how can well-characterized studies of behavioral and social science play a role in elucidating gene function?

2:15 – 2:35 Matthew McGue

2:35 – 2:55 Elena Grigorenko

2:55 – 3:15 Stephen Suomi

3:15 – 3:35 Sarah Meadows/Daniel Notterman

3:35 pm **Break**

3:45 – 5:00 Discussion

6:00 and 6:15 **Catch shuttle to dinner**

6:30 **Dinner at Jaleo**
7271 Woodmont Avenue, Bethesda, Maryland (301-913-0003)

Tuesday, February 12

8:30 am **Continental Breakfast**

9:00 am **Gene Expression and Epigenetic Mechanisms** Chair and Discussion Leader Steve Cole

Epigenetic mechanisms can be set in motion by maternal treatment of offspring and are reversible later in development. New techniques in studying the expression of genes allows more precise estimates of the influence of some genes on behavioral and social development and on factors that influence that expression. There is an increasing likelihood that in some instances differential patterns of gene expression rather than different genotypes lead to differences in phenotypes. In other cases, differential genotypes play a dominant role in health outcomes. Understanding influences on gene expression will be crucial for understanding behavioral and social developmental processes of aging, as will understanding when gene expression and differential genotypes are important.

Questions for Discussion:

- How can these methods be used for advancing aging research? What types of questions can be addressed?
- More pragmatically, what is necessary to incorporate these methods into existing behavioral and social studies?
- What samples should we be collecting now?
- What can be done with pre-existing samples?

9:00 – 9:20	Frances Champagne
9:20 – 9:40	Steve Cole
9:40 – 10:00	Joel Kleinman
10:00 – 10:30	Discussion

10:30 am **General Discussion** Chairs and Discussion Leaders David Weir and Steve Cole

This will be a discussion with represented lifespan and longitudinal studies on issues related to integrating genetics with lifespan and longitudinal work. The participants listed represent particular lifespan or longitudinal studies; all attendees are invited to take part in the discussion.

This is a point at which we can revisit critical issues raised during the workshop, as well as a prime opportunity for crosstalk among representatives of longitudinal studies and genetic methodology. Most importantly, this is an opportunity for participants to provide guidance to NIA staff on how to promote this work.

Questions for Discussion:

- What substantive areas should be addressed? Each longitudinal study that is represented might mention one or two possibilities.
- What genetic methods are necessary to address these domains?

- What can NIA do to promote integration of longitudinal studies and genetic methods (e.g., resources, infrastructure, workshops)?
- What suggestions are there for adding genetics to smaller, richly-characterized studies of behavioral and social science?

Robert Hauser

Matthew McGue

Scott Hofer

Yuichi Shoda

Terrie Moffitt

Deborah Finkel

James Smith

11:30 am **Light Lunch**

12:00 pm **Continue with Discussion**

- Future directions: measurement and data needs, research gaps and emerging opportunities in the study of economic phenotypes of relevance to aging.

1:00 pm **Revisit the Introduction, General Discussion, Future Discussions & Wrap Up**

2:30 pm **Adjourn**

APPENDIX 2: PARTICIPANT ROSTER

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