The Fetal Basis of Adult Disease: Role of the Environment

It is recognized that between two-percent and five-percent of all live-born infants have a major developmental defect. Approximately 40-percent of these defects are thought to be due to the effect(s) of an adverse exposure of a genetically pre-disposed fetus to intra-uterine environmental factors. It is now clear that in many cases the fetus is more sensitive than the adult to the same environmental insults. Exposure to environmental agents during early development can result in death, structural malformation, and/or functional alteration of the embryo/fetus. These toxicant-induced pathogenic responses are most likely the result of altered gene expression associated with altered cell production and cell differentiation involved in the establishment of cell lineages leading to the structural and functional character of the tissues, organs, and systems that arise from these lineages.

The National Institute of Environmental Health Sciences (NIEHS) has a significant program that addresses the role of developmental exposures on structural malformations i.e., classical birth defects and on functional alterations whose effects are readily observable early in development. The purpose of this PA with a set aside of funds and a Special Emphasis Panel review by the NIH Center for Scientific Review is to stimulate research in an important and emerging area of developmental toxicology: the effects of *in utero* exposures that cause permanent functional changes that are not overtly, grossly teratogenic yet that result in increased susceptibility to disease/dysfunction later in the life span. This program announcement seeks to encourage the application of the new high-throughput functional-genomic, metabonomic, proteomic, and bioinformatic technologies to pursue an understanding of these latent effects of *in utero* environmental insult.

The underlying scientific hypothesis behind the fetal basis of adult diseases has been developed by epidemiology studies and emphasized by Dr. David Barker in the United Kingdom. Most of the supporting studies in this area have concentrated on grossly altered nutrition *in utero* and its striking influence on multiple aspects of adult health and disease risk. Dr. Barker has shown that during development fetuses respond to severe malnutrition by favoring the metabolic demands of the growing brain/CNS and heart at the expense of other tissues. The growing brain/CNS and heart tissue may not, however, escape entirely unscathed. The long-term consequences of this response are that the fetus is protected from death, is live-born, but is more prone to diseases later in life. In support of the Barker hypothesis, epidemiology studies have shown that markers of malnutrition, such as low birth weight, small for gestation age, frank intrauterine growth retardation (IUGR) or clinically abnormal thinness at birth strongly predicts the subsequent occurrence of hypertension, hyperlipidemia, insulin resistance, type 2 diabetes, ischemic heart disease, breast cancer or prostate cancer in adult life. Fetuses that are clinically malnourished during the first trimester of development are three times more likely to be obese as adults. In addition to malnutrition, environmental exposures present during *in utero* development can have profound influences on fetal growth. Evidence has been presented in human populations that gross, heavy exposure to PM10 air pollution containing carcinogenic PAHs can be correlated with increased IUGR with a peak impact in the earlier portion of the first trimester—a most vulnerable period of the cell lineage expansion, differentiation, and cell interactions events of organogenesis and first growth.

The concept of fetal programming of structural-functional formations during development has been proposed to explain these findings and the resultant research area is referred to as "Fetal Basis of Adult Disease" (FeBAD) research. "Programming" is the term used to describe lifelong changes in function that follow a particular event in an earlier period of the life span. While epidemiology studies have identified the phenomenon of metabolic programming, little is known about the mechanism(s) by which fetal insults lead to altered programming and to disease later in life. In addition, emphasis thus far has been on alterations in nutrition during development with virtually no focus on the role that exposures to environmental agents, such as air or water pollution, either alone or in combination with qualitative alterations in macro- or micro-nutrition (i.e. soy protein, phytoestrogens, isoflavones or other chemicals in herbal supplements or dietary sources), might have on this phenomenon.

There is, however, evidence that some environmental agents, especially those with endocrine agonist or antagonist activity, may alter developmental programming via alteration in gene expression or gene imprinting that do not result in malformations but in functional deficits that do not become apparent until later in life. In the reproductive tract, the classic example of this phenomenon in the environmental area is the diethylstilbestrol (DES) story. In humans, *in utero* exposure to DES leads to an increase in vaginal adenocarcinoma around the time of puberty. In mice, neonatal DES exposure leads to an increase in uterine adenocarcinoma in adulthood. While the direct connection has not been made between *in utero* programming changes due to DES and later life disease, it is known that DES (in the animal studies) results in altered gene expression in the uterus that is irreversible without any noticeable gross alterations in uterine morphology. Other examples in the reproductive area include developmental exposures of the monkey to androgens that lead to polycystic ovary syndrome-like effects in the adult, data (still considered controversial) showing that environmental estrogens, such as DES, methoxychlor and bisphenol A, cause alterations in gene expression in the rat prostate that are irreversible and are correlated with increased prostate cancer, and data showing a link between *in utero* exposure to dioxin and endometriosis later in life in primates and rodents.

Cardiopulmonary diseases in postnatal life have also been linked to prenatal exposure. The most well-known example is the association between low birth weight (which is associated with poor maternal nutrition and perhaps corticosteroid exposure) and cardiovascular disease (e.g., myocardial infarcts) and predictors of future cardiovascular disease, such as hypertension and atherosclerosis, and complex metabolic disease, such as diabetes. In addition, studies have shown that maternal smoking is associated with deficits in lung function and with asthma symptoms in the offspring. Data indicate that these associations are independent of smoking status after birth.

Some forms of neurodegenerative disease may have their origins in *in utero* exposures. For example, there is preliminary evidence that a bacterial stimulus (endotoxin) can produce cytokines that impair the development of the mesencephalic dopaminergic systems during pregnancy. This attenuation of the dopamine neurons during fetal development leaves the offspring with fewer dopaminergic neurons at birth and at possible increased risk for Parkinson's disease in later life. In a similar vein, there is preliminary evidence that exposure to environmental neurotoxins during dopaminergic development enhances the susceptibility to accelerated dopaminergic cell death during aging via the common molecular mechanism(s) of the alteration of stress-activated signal transduction pathways, expression of differentiation transcription factors, survival factors or phenotype marker proteins in the nigral dopaminergic neurons. Similarly there is evidence that *in utero* exposure to polycyclic biphenols (PCBs) leads to altered thyroid function and subsequent learning disabilities later in life. In all instances data are needed to show that the *in utero* exposures actually lead to an altered programming at the molecular level and that the disease/dysfunction is a direct result, albeit, temporally discordant in its onset and/or progression, of that altered programming.

Another promising area for investigation is how environmental prenatal exposures might alter immune system programming. The development of the immune system, including the development of the repertoire of reactive lymphocytes that will exist in postnatal life, begins prenatally. Alterations of the fetal immune environment might pre-program the highly sensitive fetal immune system for aberrant immune regulation, leading to a loss of tolerance to self-antigens and

resulting in an increased risk for autoimmune disease. These changes might manifest in adult life and perhaps only after a second exposure to related environmental chemicals. There is evidence, for instance, that mice exposed prenatally to estrogenic compounds appear to develop normal immune systems. However, when stimulated with certain environmental chemicals, they can show an increased susceptibility to autoimmune disease. Similarly, there is evidence in humans and experimental animals that prenatal exposure to immunosuppressive drugs can lead to immune alterations in the mature animals, including development of autoantibodies and a higher risk of autoimmune disease in susceptible animals.

Based on the epidemiology data that support the Barker Hypothesis and the preliminary data showing alterations in gene expression and imprinting due to *in utero* exposures to some environmental agents, we propose that exposure to certain environmental chemicals as well as altered nutrition, or in combination with altered nutrition, will in some situations, not lead to easily identifiable structural malformations, but instead to alterations in developmental programming expressed as a permanently altered gland, organ or system potential. These states of altered potential would be a result of changes in gene expression, due to altered imprinting, and the underlining methylation-related protein-DNA relationships associated with chromatin remodeling. These effects may occur in a time specific (i.e. vulnerable window) and tissue specific manner and such alterations may be irreversible. The end-result is an animal that is sensitized such that it will be more susceptible to diseases later in life. The environmental insult could act via a one hit or two/three hit scenario. That is, there could be an *in utero* exposure that would lead by itself to pathophysiology later in life or there could be *in utero* exposure combined with a neonatal exposure (same or different compound(s) or adult exposure that would trigger the pathophysiology. The pathophysiology or functional change that results from the exposures/insult could lead to: a) the occurrence of a disease that otherwise would not have happened, b) an increase in risk for a disease that would normally be of lower prevalence, or c) either an earlier onset of a disease that would normally have occurred or an exacerbation of the disease. Finally, the pathophysiology could have a variable latent period from onset in the neonatal period, to early childhood, to pubertal, to early adulthood to late adulthood depending on the toxicant, time of exposure and tissue/organ affected and potentially transgenerational effects.

Research Approaches Relevant to this PA: 1) To provide a sound mechanistic understanding of fetal programming of adult disease, studies supported by this initiative must involve whole animal developmental exposures during gestation. Applicants can propose studies using transgenics, model organisms, or rodent models. For the purpose of this initiative, human studies (clinical or epidemiology) are not responsive; 2) Applicants must study an environmental agent/chemical/stressor to which there is human exposure and the potential for *in utero* exposure. This includes any endocrine active chemical(s) or organic solvents, particulate matter (PMs), pesticides, nutritional supplements, phytochemicals or metals. Nutrition alone cannot be used as an *in utero* exposure but can be studied in conjunction with another exposure; 3) Applications must propose studies that focus on *in utero* exposures, but additional exposures at other time points (e.g., exposure beginning *in utero* and extending to postnatal period; exposure *in utero* followed by adult exposure) can be included; 4) This initiative requires the use of the new technologies of gene expression profiling, and where appropriate, the examination of epigenetics (methylation, imprinting and chromatin remodeling); 5) Applications must link *in utero* exposures to changes in gene expression that are tissue specific and irreversible. These changes in gene expression will then need to be measured in the adult and correlated with the diseases/dysfunction studied; 6) A specific adult onset disease or dysfunction must be the focus of the application with emphasis on the role of *in utero* exposure and changes in gene expression in the fetus to the adult onset or severity of the disease. Applications that are not focused on a specific adult disease or dysfunction are not responsive. For example, applications that focus on *in utero* exposures as triggers of diseases of childhood or puberty are not responsive to this specific P; 7) Applications must focus on one of the following four emphasis areas: the reproductive tract, the pulmonocardiovascular system, the brain/nervous system or the immune/autoimmune system. Diseases of tissues or organ systems other than the four described here are not responsive to this specific announcement. The diseases of special interest to NIEHS with respect to this initiative include reproductive/hormonal (fertility, endometriosis, fibroids, premature menopause, polycystic ovary syndrome, prostate/ovary/breast cancer) cardiopulmonary (heart disease, atherosclerosis, hypertension, chronic obstructive pulmonary disease, adult asthma) and brain/CNS (neurodegenerative diseases—Parkinson's, Alzheimer) and immune/autoimmune (altered immune responsiveness, systemic or tissue specific autoimmine diseases of adulthood). It may be possible to submit applications to this initiative with an emphasis on other diseases as long as they are related to one or more of the above noted four emphasis areas. It should be noted that these are all adult onset diseases; 8) Critical areas of expertise that are required of applicants include developmental biology/toxicology, disease pathophysiology and gene expression profiling, including data analysis and interpretation of global gene expression alterations; 9) The National Cancer Institute (NCI) is interested in funding research aimed at understanding the effects of biological, chemical, and radiologic exposures *in utero* that cause permanent functional changes that result in increased susceptibility to cancer in adult life. The specific etiologic agents of interest include microorganisms such as herpes virus, HHV8/KSHV, cytomegalo virus, papilloma virus, polyoma virus, and bacterial infection such as chlamydia, to name a few. Chemical agents that have been characterized as carcinogenic, or are suspected to be carcinogenic in humans including polycyclic aromatic hydrocarbons, nitrosamines, heterocyclic amines, aromatic amines, DES and estrogens, as well as metals and metalloids such as chromium, mercury and arsenic are of interest to NCI. Additionally, exposures to radiologic agents from all externally applied sources as well as exposure to internally deposited radionuclides that would result in *in utero* exposures are of interest in this context. Studies which link *in utero* exposures to such agents that result in permanent, alterations in gene expression in tissues of the reproductive system, the pulmono/cardiovascular system, the brain/nervous system, or the immune/autoimmune system leading to or resulting in cancer in those organ sites later in the adult life of the exposed fetus are sought by the NCI; 10) The NCI is also interested in funding research aimed at understanding the consequences of fetal exposure to toxicants, hormone agonists, or antagonists that alter the expression or function of the steroid nuclear receptor superfamily of genes (androgen, estrogen, progesterone, glucocorticoid, Vitamin D3, thyroxine) in normal or cancerous organs of the male and female reproductive tract and/or immune system. In addition, the consequences of environmental carcinogen or tobacco smoke exposure of mice during gestation that result in long lasting immune function deficiencies or inflammatory responses, in so far as they are related to the development of hematological malignancies, or cancers of the reproductive system, the brain/nervous system and/or the lung. Studies which link fetal exposure to toxicants or other substances affecting somatic stem cells that later populate organs such as the mammary gland, the prostate gland and the lung, and that have cancer as the endpoint, are sought by the NCI.

This PA will use the NIH exploratory/developmental (R21) award mechanism. As an applicant, you will be solely responsible for planning, directing and executing the proposed project. The R21 grant award mechanism supports innovative, high-risk/high-impact research requiring preliminary testing or development; exploration of the use of approaches and concepts new to a particular substantive area; and research and development of data upon which significant future research may be built. Applications will be considered high-impact if they demonstrate the potential for groundbreaking, precedent setting

significance, and high-risk because they either lack sufficient preliminary data to ensure their feasibility, or involve the use of a new model system or technique.

This PA uses just-in-time concepts. It also uses the modular budgeting format (see http://grants.nih.gov/grants/funding/modular/modular.htm). Specifically, if you are submitting an application with direct costs in each year of $250,000 or less, use the modular format. This program does not require cost sharing as defined in the current NIH Grants Policy Statement at http://grants.nih.gov/grants/policy/nihgps_2001/part_i_1.htm.

It is anticipated that approximately $2 million in FY 2004 and FY 2005 will be available to fund grants in response to this PA. An applicant for an R21 grant may request a project period of up to three years and a budget for total direct costs, including third party facilities and administrative costs, not to exceed $100,000 per year. Because the nature and scope of the proposed research will vary from application to application, it is anticipated that the size and duration of each award will also vary. Although the financial plans of the NIEHS provide support for this program, awards pursuant to this PA are contingent upon the availability of funds and the receipt of a sufficient number of meritorious applications.

Prospective applicants are asked to submit a letter of intent that includes the following information: 1) Descriptive title of the proposed research; 2) Name, address, and telephone number of the Principal Investigator; 3) Names of other key personnel; 4) Participating institutions; 5) Number and title of this PA.

Although a letter of intent is not required, is not binding, and does not enter into the review of a subsequent application, the information that it contains allows NIEHS staff to estimate the potential review workload and plan the review.

The letter of intent is to be sent by the date listed at the beginning of this document. It should be sent to:

Dr. Cindy Lawler, Scientific Program Administrator, Cellular, Organ and Systems Pathobiology Branch, Division of Extramural Research and Training, NIEHS, P.O. Box 12233 (EC-23), 111 T.W. Alexander Drive, Research Triangle Park, NC 27709, 919 316-4671, fax: 919-541-5064, e-mail: lawler@niehs.nih.gov.

Applications must be prepared using the PHS 398 research grant application instructions and forms (rev. 5/2001). The PHS 398 is available at http://grants.nih.gov/grants/funding/phs398/phs398.html in an interactive format. For further assistance contact GrantsInfo, 301-435-0714, e-mail: GrantsInfo@nih.gov. Applicants should note that R21 applications have a page limitation of 15 pages for the Research Plan.

Application Receipt Dates: Applications submitted in response to this PA will be accepted on August 12, 2003 and 2004.

Contact: Dr. Cindy Lawler, Scientific Program Administrator, Cellular, Organ and Systems Pathobiology Branch, Division of Extramural Research and Training, NIEHS, P.O. Box 12233 (EC-23), Research Triangle Park, NC 27709, USA, 919-316-4671, fax: 919-541-5064, e-mail: lawler@niehs.nih.gov; Jerry Heindel, Scientific Program Administrator, Cellular, Organ and Systems Pathobiology Branch, Division of Extramural Research and Training, NIEHS, P.O. Box 12233 (EC-23), Research Triangle Park, NC 27709, USA, 919-541-0781, fax: 919-541-5064, e-mail: heindelj@niehs.nih.gov; J. Patrick Mastin, Scientific Program AdministratorCellular, Organ and Systems Pathobiology BranchDivision of Extramural Research and Training, NIEHS, P.O. Box 12233 (EC-23), Research Triangle Park, NC 27709, USA, 919-541-3289, fax: 919-541-5064, e-mail: mastin@niehs.nih.gov; Annette Kirshner, Scientific Program Administrator, Cellular, Organ and Systems Pathobiology Branch Division of Extramural Research and Training, NIEHS, P.O. Box 12233 (EC-23), Research Triangle Park, NC 27709, USA, 919 541-0488, fax: 919-541-5064, e-mail: kirshner@niehs.nih.gov; Carol L. MacLeod, Cancer Biology Division, National Cancer Institute, 6130 Executive Boulevard, Rockville, MD 20892, USA, 301 435 1878, fax: 301 480 0864, e-mail: macleodc@mail.nih.gov; Mary Frances Picciano, Senior Nutrition Research Scientist, Office of Dietary Supplements, National Institutes of Health, 6100 Executive Boulevard, MSC 7517 Suite 3B01, Bethesda, MD 20892-7517 (Courier Service use Rockville, MD 20852), 301-435-3608, fax: 301-480-1845. Reference: PA No. PAR-03-121

Genetics, Behavior, and Aging

This program announcement (PA) solicits novel research integrating genetics, behavior and aging. Human and non-human studies are needed to advance our understanding of the genetic and environmental influences and processes affecting variability in behavior and its functional sequelae with age. This includes studies that help elucidate the relationships of levels and change in behavior to health, functional competence, and quality of life of older adults. This PA is framed around two broad categories of questions: (1) gene-to-behavior questions concerning the nature and role of genetic influences on behaviors at older ages, and how these genetic effects vary with age; and (2) questions about dynamic processes including gene-environment interactions, gene-environment covariation, age-related genetic effects, and how behaviors interact with and affect genetic expression. The behaviors that are eligible for study under this PA should be critical to quality of life among the aged, either as outcomes or as mediators of physical or cognitive health and function. Examples of relevant behavioral domains include, but are not limited to, social behaviors, resilience, vitality, adaptivity, personality, vulnerability to stress, health behaviors, social cognition, human and social capital accumulation, economic savings for retirement, risk-taking, happiness, coping, caregiving, cognitive abilities, cognitive flexibility, cognitive reserve, learning, and functional abilities. This PA is intended to stimulate methodologically rigorous research integrating genetics, other biological sciences, and the behavioral and social sciences. To be considered responsive to this announcement, interdisciplinary perspectives must be unambiguous, the relationship between the behaviors or social processes under study and healthy aging should be articulated, and the proposed study should be embedded within a well articulated set of questions or hypotheses generated from social science and behavioral research. This announcement updates and replaces a previous PA, Behavior Genetics in Adulthood and Old Age (PAS-98-076, issued May 21, 1998).

Behavior and age-related changes in behavioral processes are integral to how well we age. Many behavioral phenotypes, such as resilience, cognitive and functional abilities, social connectedness, happiness, longevity and loneliness are intrinsic to maintaining health and quality of life. Behavior also plays a critical mediating role (e.g. smoking, alcohol use, exercise, risk taking behaviors, adherence, social engagement) in health and disease. Understanding the causes of variation in behavioral development, plasticity, stability, adaptation and change with age is essential to maintaining and enhancing quality of life throughout old age.

Family and twin studies on aging have demonstrated the importance of genetic influences for variation in a large array of behavioral phenotypes related to personality, well-being, functional abilities, cognitive aging, longevity and health. More recent findings based on the longitudinal twin design indicate the importance of genetic influences on functional stability and the importance of environments for change. To move beyond these findings innovative studies are needed that investigate genetic effects within the context of the dynamic aging processes in which they are expressed. This will involve diverse approaches that: integrate molecular and quantitative methods, focus on behavioral systems for which known or candidate genes are identified, explore social processes that affect individual environments, include measures of biological intermediaries of the behaviors, and use non-linear analytic approaches to study genes, social factors and environments in developmentally dynamic ways.

The underlying conceptual model is multifactorial, highlighting the combined action of multiple genetic and environmental influences where phenotypic variation arises as a function

of genotypic and environmental differences between people within a particular population. Features of this model are an assumption that environmental influences, ranging from intracellular conditions to larger socio/cultural effects, and genetic influences operate through the same causal field of biological structures and processes. The intricacies of this causal field can lead to complex relationships between genetic factors, environmental influences, and phenotypic outcomes. These complexities include time-related changes in the relative influence of genetic and environmental factors, non-linear interactions among genes, interactions and correlations between genes and environments and environmentally induced gene expression.

The need to examine genetic and environmental influences and behaviors in the context of dynamism of interactive aging systems is increasingly apparent, and unprecedented opportunities to do so are now available. Dramatic advances have been made by molecular geneticists in the explication of hereditary phenomena, by quantitative geneticists in the assessment of aggregate effects of genes and environments, by behavioral and social scientists in identifying intermediary phenotypes (endophenotypes) and in defining and measuring complex behavioral domains, and by statisticians in the measurement of change. Progress in understanding gene-behavior relationships in aging will rely on integrating the theoretical models and methodologies of these research domains to provide powerful tools for combining reductionist approaches (that explore the nature of specific genetic and environmental influences) and integrationist approaches (that explore effects within the larger context of complex systems). Among many examples, improved strategies now exist to identify genes and map quantitative trait loci (QTL); to assess specific genetic and environmental sources of variation; to quantify these specific effects relative to background variation due to the aggregate influence of still-anonymous genes and environments; to conceptualize and examine non-linear and dynamic processes such as epistasis, gene-environment interaction, gene-environment correlation, and behaviorally or environmentally induced genetic expression; to investigate how social worlds and behavioral factors modulate gene expression; to characterize population differences according to sequence (SNP) and haplotype diversity; to detail the structure of behavioral domains; to measure phenotypic change; and to assess age-related changes in influence of both specific and aggregate genetic and environmental domains.

A wide range of designs is relevant to the objectives of this PA, including augmented family studies with combinations of twins, parents, siblings, children and adoptees; sibling studies using highly selected samples for phenotypic indices of similarity/dissimilarity; extended pedigrees; special populations (i.e. inbred groups, cultural and genetic isolates); sub-populations such as the oldest-old; studies that utilize the extensive genome databases and genetic analyses resources that are becoming available; and animal model studies using cross-fostering, selective breeding, inbred strains, recombinant inbred strains or specific genotypic manipulations (e.g. transgenic, knockouts, knock-ins). Research is also encouraged that builds upon ongoing studies of aging cohorts whereby supplemental data collection would allow new hypotheses to be addressed at the intersection of genetics, behavioral and social science and research.

Major methodological considerations should be well articulated, including the implications for aging of the behavioral phenotypes being studied, documentation of solid measurement characteristics, presentation of power analyses to reveal that sample sizes suffice for analyzing the genetic effects being studied, and clear descriptions of the analytical procedures to be employed must be provided. The research team should be multidisciplinary and, at a minimum, reflect expertise in genetics (molecular and/or quantitative), and the social/behavioral sciences.

Among the many research avenues pertinent to studying the behaviors of relevance to this PA are 1) Studies that explore the genetics of behavioral interventions and address how genetic differences moderate responses among the elderly to health promoting behaviors (e.g. exercise, social connectedness, cognitive training); 2) Studies to elucidate behavior-gene (i.e. individuate loci and QTLs) relationships for behaviors affecting quality of life with aging. Novel, hypothesis-driven research is needed to: a) investigate how genes or QTLs implicated in aging processes (e.g. oxidative stress) affect behavioral function and change, and b) explore age effects in the genes or QTLs implicated in behavioral functioning (e.g. DRD4 and novelty-seeking, APOE and cognitive function); 3) Behavioral genetic designs (using human or animal models) that combine quantitative and molecular techniques to resolve variance and covariance structures more finely than has previously been accomplished by quantifying the influences of specific genes, QTLs and specific measured environments; 4) Behavioral genetic studies (using human or animal models) that investigate genetic variation and QTLs affecting rates and shapes of change in behavioral functioning; 5) Studies of specific environmental influences in genetically informative research on aging. Despite the importance of dynamic processes involving gene-environment interaction and covariation, measured environments are rarely included in human studies. Research is needed that incorporates critical features of diverse environments (i.e. social, economic, physical and cultural environments) into behavioral genetic studies of aging for the purpose of analyzing gene-environment dynamics; 6) Studies (using human or animal models) to investigate gene by environment interaction. Examples include research exploring whether and how social environments or enriched experiences (quality of education, etc.) mediate progression to disease among those with genetic predispositions to disease; studies investigating the effects of socially or experientially enriched or restricted environments on genetic expression; research testing whether protective health effects conferred by education prevail in the presence of genetic risk, and genetic research that builds upon and bridges findings from established fields of environmental inquiry (e.g. health disparities) to explore gene by environment interactions; 7) Studies elucidating the genetic regulation of neural mechanisms, and their modulation by environmental circumstances, that impact upon cognitive function, cognitive reserve and flexibility, learning, and memory; 8) Studies to investigate gene by environmental covariation. Individuals shape and select their environments throughout development, and these processes are affected by many factors including age, and aging transitions such as retirement, chronic care giving, bereavement, isolation and functional loss. Research is needed that develops analytical models by which to study gene-environment covariation in the context of these age-related changes in abilities to define and select one's environment.

This PA will use the NIH R01 award mechanism. As an applicant, you will be solely responsible for planning, directing, and executing the proposed project.

This PA uses just-in-time concepts. It also uses the modular as well as the non-modular budgeting formats (see http://grants.nih.gov/grants/funding/modular/modular.htm). Specifically, if you are submitting an application with direct costs in each year of $250,000 or less, use the modular format. Otherwise follow the instructions for non-modular research grant applications.

The National Institute on Aging (NIA) intends to commit at least $2 million for an initial round of funding of applications of high scientific merit in FY 2004. An applicant may request a project period of up to 5 years. Because the nature and scope of the proposed research will vary from application to application, it is anticipated that the size and duration of each award will also vary. Although the financial plans of the NIA provides support for this program, awards pursuant to this PA are contingent upon the availability of funds and the receipt of a sufficient number of meritorious applications.

An overarching goal of this PA is to encourage innovative research integrating knowledge and methodologies from genetics, gerontology, and the behavioral and social sciences. Applications should articulate the interdisciplinary dimensions and components of the proposed research, and explain how the collective

expertise of the research team meets these interdisciplinary requirements with regards to the specific aims to be investigated.

The sharing of unique resources such as phenotypic data, DNA, and genome scans in a timely manner contributes greatly to progress in understanding the genetics of complex phenotypes. The NIH encourages data sharing (http://grants.nih.gov/grants/guide/notice-files/NOT-OD-03-032.html) and requires all applications from October 1, 2003 that request $500,000 or more in direct costs in any single year to address data sharing. For this PA, investigators are encouraged to submit a data-sharing plan regardless of the size of the requested budget. The NIH data sharing and implementation guidelines policies can be found at http://grants.nih.gov/grants/policy/data_sharing/data_sharing_guidance.htm. NIH staff will evaluate the adequacy of the proposed data sharing and access plan, and consider it in making award decisions. Staff will also consider waivers as appropriate to the conditions of data collection. Also, except under circumstances where the data are likely to be of unique value and importance to other investigators, staff will accept requested costs under $500,000 a year as sufficient reason for a waiver. The sharing plan approved by NIH staff, after negotiation with the applicant when necessary, will become part of the terms and conditions of the award. NIH staff will also evaluate compliance with the sharing plan and scientific progress in the non-competing continuation of the grant award application.

Applications must be prepared using the PHS 398 research grant application instructions and forms (rev. 5/2001). The PHS 398 is available at http://grants.nih.gov/grants/funding/phs398/phs398.html in an interactive format. For further assistance contact GrantsInfo, 301-435-0714, e-mail: GrantsInfo@nih.gov.

Applications submitted in response to this PA will be accepted at the standard application deadlines, which are available at http://grants.nih.gov/grants/dates.htm. Application deadlines are also indicated in the PHS 398 application kit. Submit a signed, typewritten original of the application, including the checklist, and five signed photocopies in one package to: Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 1040, MSC 7710, Bethesda, MD 20892-7710, USA, Bethesda, MD 20817 (for express/courier service)

Applications must be received by or mailed on or before the receipt dates described at http://grants.nih.gov/grants/funding/submissionschedule.htm. The CSR will not accept any application in response to this PA that is essentially the same as one currently pending initial review unless the applicant withdraws the pending application. The CSR will not accept any application that is essentially the same as one already reviewed. This does not preclude the submission of a substantial revision of an application already reviewed, but such application must include an Introduction addressing the previous critique.

Contact: Angie Chon-Lee, Behavioral and Social Research Program, NIA, Gateway Building, Room 533, Bethesda, MD 20892-9205, USA, 301-594 5943, fax: 301-402-0051, e-mail: Chon-LeA@nia.nih.gov; Marilyn M. Miller, Neuroscience and Neuropsychology of Aging Program, National Institute on Aging, Gateway Building, Suite 350, 7201 Wisconsin Avenue, Bethesda, MD 20892-9205, USA, 301-496-9350, fax: 301-496-1494, e-mail: Millerm@nia.nih.gov. Reference: PA No. PAS-03-128

Gene-Environment Interactions Influencing Alcohol-Related Phenotypes and Diseases

The National Institute on Alcohol Abuse and Alcoholism (NIAAA) is seeking research grant applications on the role of gene-environment interactions underlying susceptibility to alcohol-related phenotypes including alcohol dependence, relapse, withdrawal; alcohol-induced organ damage including neurodegeneration, cirrhosis and other liver diseases, pancreatitis, cardiomyopathy, immune disorders, cancers, and alcohol-induced birth defects. This solicitation specifically encourages multidisciplinary approaches to study how environmental conditions, such as chemical, infectious, physical, nutritional, and social behavioral factors, impact genetic predisposition to alcohol-related diseases. Identification and characterization of gene-environment interactions will offer better opportunities to effectively target prevention, intervention and treatment strategies. The National Institute of Environmental Health Sciences (NIEHS) is collaborating on this PA and is seeking research grant applications on interactions between alcohol effects and environmental agents.

Alcoholism and alcohol-related diseases are the result of complex interactions of multiple genetic and environmental factors. Several genetic factors have been associated with alcohol-related behaviors, alcohol-induced birth defects, and alcohol-induced organ damage; however, the role of environmental factors in modifying the risk of developing alcohol abuse, alcoholism, and/or organ damage remains undefined.

Genetic factors leading to differential risk for alcoholism were demonstrated using twin and family studies. In addition, functional polymorphisms of alcohol dehydrogenase (ADH2) and aldehyde dehydrogenase (ALDH2) genes have been shown to have a significant impact on alcohol metabolism in the liver, and thus, may contribute to vulnerability to alcohol abuse and dependence, alcohol-related liver diseases and cancers.

Genetic and environmental interactions modulate an individual's susceptibility to certain diseases/disorders. For example, male children who have a polymorphism in the monoamine oxidase A gene conferring low enzyme activity show non aggressive behavior when raised in a non-abusive environment. However, male children with the same polymorphism show aggressive and antisocial behavior when raised in an abusive environment. Males with normal enzyme activity do not become violent offenders when raised in the same abusive/maltreated environment. Similar results have been found in animals. For example, mice lacking a functional corticotroping-releasing hormone 1 receptor do not differ from wild-type mice in alcohol intake under stress-free conditions; however, after repeated stress, the knockout mice increase their alcohol consumption. Also, monkeys with a polymorphism in the regulatory region of the serotonin transporter gene show no differences from the wild-type monkeys when reared with their mothers. However, monkeys with the polymorphism that were nursery raised have attention and orientation deficits.

The dynamic multi-level interactions between genetic and environmental components are responsible for the heterogeneity and complexity of alcohol dependence phenotypes. Therefore, it is necessary to use multi-disciplinary approaches to decipher the underlying mechanisms for alcohol abuse, alcohol dependence and alcohol related disorders. Comprehensive designs and methodologies for both human and animal studies of gene-environment interactions are of crucial importance to identify alcohol-related genes and environmental factors, and their interrelationships. Human studies using informative populations such as twins, multi-generation families and migrants, as well as children at high risk or low risk to develop alcohol dependence can provide unique opportunities and advantages to study neurobiological and behavioral consequences of gene-environment interaction. In addition, animal models can be used to study gene-environment interactions requiring genetic and environmental manipulations that are impractical or ethically impossible in humans. Controlled genotypes can be devised in genetically modified or chemical-induced mutant animals. Therefore, animal models can also offer unique opportunities to explore the role of gene-environment interactions as the means of understanding the pathways to alcohol-induced diseases.

There are several approaches for analyzing the effects of environmental factors in experimental animals, including differential gene expression using cDNA microarrays, RAGE, and SAGE; as well as proteomic methods to determine changes in protein levels and protein modifications. The role of genes implicated in the response may be further studied by developing transgenic and gene-targeted animals, as well as,

by using other gene expression strategies including RNA interference (RNAi) and retroviral-mediated gene transfer techniques.

Epigenetic changes are known to be involved in the etiology of a large number of diseases such as schizophrenia, cancer, and alcohol dependence. Recent evidence shows that there is an association between the GABAA receptors and alcohol dependence that is modulated by genetic imprinting. There are numerous types of epigenetic modifications on both DNA and nucleosomes, including methylation and acetylation, which could affect the expression and regulation of alcohol-related genes. NIAAA seeks proposals that will examine environmental factors that alter the epigenetic status of genes that may affect gene expression leading to alcohol-induced diseases.

The purpose of this PA is to encourage multidisciplinary research that will investigate gene-environment interactions influencing susceptibility to alcohol abuse and dependence, alcohol-related behaviors, and alcohol-induced organ damage in both animals and humans. NIAAA seeks research projects that include, but are not limited to:

1) studies of changes in gene or protein expression by investigating animal models such as knock-out and other genetically modified animals under a variety of environmental conditions to identify candidate genes, or their corresponding proteins, that may be associated with susceptibility to alcohol abuse and dependence, relapse, withdrawal, alcohol-induced organ damage including neurodegeneration, cirrhosis and other liver diseases, pancreatitis, cardiomyopathy, immune disorders, cancers, and alcohol-induced birth defects; 2) studies to identify allelic variants (polymorphisms) and determine the functional relevance of an identified gene or protein for increasing or decreasing susceptibility to alcohol abuse and dependence, alcohol-related phenotypes, and other alcohol-induced diseases under different environmental conditions; 3) studies using genetic epidemiological, psychiatric and behavioral genetic and molecular genetic methods to determine the interaction of genetic, biological, and social factors in the development of risk to alcohol abuse and dependence; 4) studies to develop and/or utilize statistical modeling approaches to identify the contributions of genetic and environmental factors to the individual risk, their interrelationship and their developmental trajectories; 5) studies to determine the genetic and environmental risk and protective factors that influence individual drinking behaviors including children and adolescent underage drinking across populations, socio-cultural backgrounds, and environmental exposures; 6) studies to determine the environmental factors that alter the epigenetic status of genes, thus increasing vulnerability to alcohol abuse and dependence, alcohol-associated behaviors, and alcohol-induced organ damage;

7) studies to determine the functional relevance of candidate genomic markers associated with an increased susceptibility to alcohol abuse and dependence, alcohol-related phenotypes, and alcohol-associated medical conditions; 8) studies to identify potential links between alcohol exposure and expression of functional polymorphisms of neurotransmitters and their receptors under various environmental conditions such as stress and cross-fostering, to understand the development of excessive drinking behaviors; 9) studies to determine how genetic variations between individuals and among various populations impact on how environmental influences may differentially alter alcohol metabolism.

NIEHS seeks research grant applications in which the goals are to determine gene-environment effects on disease susceptibility in response to specific exposures or which involve Environmentally Responsive Genes, for example, as defined in the Environmental Genome Project (http://egp.gs.washington.edu/). NIEHS seeks applications that focus primarily on exposure to environmental agents and in which attention to alcohol abuse is secondary or minor.

This PA will use the NIH R01 and Exploratory/Developmental Research Grant (R21) award mechanisms. As an applicant, you will be solely responsible for planning, directing, and executing the proposed project. Applications using the R21 mechanism may request a project period of up to two years with a combined budget for direct costs of up $275,000 for the two year period. For example, the applicant may request $100,000 in the first year and $175,000 in the second year. The request should be tailored to the needs of the project. Normally, no more than $200,000 may be requested in any single year.

This PA uses just-in-time concepts. It also uses the modular as well as the non-modular budgeting formats (see http://grants.nih.gov/grants/funding/modular/modular.htm). Specifically, if you are submitting an application with direct costs in each year of $250,000 or less, use the modular format. Otherwise follow the instructions for non-modular research grant applications. This program does not require cost sharing as defined in the current NIH Grants Policy Statement at http://grants.nih.gov/grants/policy/nihgps_2001/part_i_1.htm. Exploratory/developmental grant support is for new projects only; competing continuation applications will not be accepted. Two revisions of a previously reviewed exploratory/developmental grant application may be submitted as defined in NIH Policy at http://grants.nih.gov/grants/policy/amendedapps.htm.

Applications must be prepared using the PHS 398 research grant application instructions and forms (rev. 5/2001). The PHS 398 is available at http://grants.nih.gov/grants/funding/phs398/phs398.html in an interactive format. For further assistance contact GrantsInfo, Telephone (301) 435-0714, Email: GrantsInfo@nih.gov.

Application Receipt Dates: Applications submitted in response to this PA will be accepted at the standard application deadlines, which are available at http://grants.nih.gov/grants/dates.htm. Application deadlines are also indicated in the PHS 398 application kit.

Application Processing: Applications must be mailed on or before the receipt dates described at http://grants.nih.gov/grants/funding/submissionschedule.htm. The CSR will not accept any application in response to this PA that is essentially the same as one currently pending initial review unless the applicant withdraws the pending application. The CSR will not accept any application that is essentially the same as one already reviewed. This does not preclude the submission of a substantial revision of an application already reviewed, but such application must include an Introduction addressing the previous critique.

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