



**Office of Research on Women's Health (ORWH)
Research Summaries, FY 2010***

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ORWH RESEARCH SUMMARIES, FY 2010

ADOLESCENT HEALTH

5P01 HD031921-15

The National Longitudinal Study of Adolescent Health (Add Health)

Harris, Kathleen M

University of North Carolina, Chapel Hill

\$200,000

Add Health is currently funded for Wave IV data collection. At the time the project began in 1994-1995, investigators selected a nationally representative sample of adolescents in grades seven through 12. Participants have been followed through adolescence and the transition to adulthood with three in-home interviews. Wave IV will include additional information that encompasses social, behavioral, and biological data. In addition to data contributed in earlier surveys, these subjects, who are now between the ages of 23 and 31 years, will provide biological data to capture the prevailing health concerns as well as biological markers of future chronic health conditions.

1 R34 MH086922-01A2

An Integrative Intervention for Binge Eating Among Adolescent Girls

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Virginia Commonwealth University, Richmond, VA

\$200,000

Many adolescents struggle with overeating (binge-eating). Adolescents with these eating problems are more likely than their peers to be depressed, anxious, and feel badly about their appearance. African American girls are especially at-risk for these eating problems. Effective treatments are urgently needed. This study will develop and evaluate an innovative intervention (LIBER8-Linking Individuals Being Emotionally Real) for ethnically diverse adolescent girls. This intervention will focus on teaching girls skills that help them reduce their problematic eating behaviors and improve their overall well-being. Binge and loss of control (LOC) eating affect a significant number of adolescents of all ethnicities and are associated with numerous psychological problems, including depression, anxiety, low self-esteem, body dissatisfaction, and weight concerns. African American women appear to be particularly vulnerable to binge eating disorder, and LOC and binge eating are prevalent among African American girls. However, empirically validated culturally sensitive treatments for these disordered eating behaviors are not available. Thus, this R34 application aims to develop a manualized and culturally sensitive intervention (LIBER8-Linking Individuals Being Emotionally Real) for African American and White adolescent girls targeting binge and LOC eating. They will target the intervention to adolescents with either or both behaviors. This intervention will integrate components of dialectical behavior therapy tailored to adolescents who engage in binge and LOC eating, such as mindfulness and distress tolerance skills training, with a core of cognitive-behavioral therapy (CBT). They will seamlessly integrate a key adolescent communication strategy, namely text-messaging, into therapeutic self-monitoring. They will evaluate the feasibility and acceptability of the intervention in a controlled pilot trial. This study is designed explicitly to gather

preliminary data to inform a subsequent larger randomized controlled trial. They hypothesize that this intervention will serve to reduce binge and LOC eating, as well as improve psychosocial functioning as evidenced by decreased depression, anxiety, eating disorder cognitions, and impulsivity, and improved quality of life.

NHANES Project For Adolescents And Girls

Troiano, Rick

NCI, Bethesda, MD

\$300,000

NHANES data from 2003-2006 show a dramatic decline in physical activity, especially for girls, as children move into adolescence. Poor sleep and loss of muscle strength are an issues for women as they age. The NHANES will measure physical activity, sleep and muscle strength for women and men across the life span from a nationally representative sample.

AGING

5 R37 AG030481-03

National Social Life, Health, and Aging Project

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\$200,000

They propose to collect a second wave of the National Social Life, Health and Aging Project (NSHAP) to obtain data on social networks and social support, marital and cohabitational relationships, attitudes, self-reported health and behavior, and cutting-edge biomeasures of physical function and health. The crucial contribution of Wave II will be in enabling analyses of trajectories; the availability to the community of scholars of such a broad-based, longitudinal data set will permit an examination of the health trajectories of older adults and inform new approaches to reducing morbidity and preventing disability and dysfunction as individuals age. The primary objective of the National Social Life, Health and Aging Project (NSHAP) is to establish an innovative, high-quality dataset for use by researchers studying the relationships between social processes and health among older adults. Wave I obtained questionnaire and biomeasure data on a nationally-representative sample of 3,005 community-dwelling adults ages 57-85 in 2005/6. They propose to collect a second wave in NSHAP to obtain data on social networks and social support, marital and cohabitational relationships, attitudes, self-reported health and behavior, and cutting-edge biomeasures of physical function and health. The crucial contribution of Wave II will be in enabling analyses of trajectories; the availability to the community of scholars of such a broad-based, longitudinal data set will permit an examination of the health trajectories of older adults and inform new approaches to reducing morbidity and preventing disability and dysfunction as individuals age. They propose to revisit respondents four years after their initial interview. Using these data, they can describe and model the distribution of changes in health, well-being, social networks, social participation and social context. In each case, they shall examine the distributions both for the entire sample and within subgroups defined by key sociodemographic characteristics such as gender, race/ethnicity, and socioeconomic status. They also propose to augment the sample by interviewing the spouse/cohabitating romantic partner. These data will allow us to characterize the impact of

marital and romantic relationships on health by examining the effects of one person's characteristics and behaviors on the health of the other. They will also analyze the partnerships themselves, and assess the relationship between characteristics of the partnership, such as support, closeness and mistreatment, and the health of each of the partners. In sum, they will explore their overarching hypothesis that older adults with strong functioning intimate relationships will show more positive (or less negative) health trajectories than those who have weaker relationships or lack such relationships altogether.

ALCOHOL AND OTHER SUBSTANCE ABUSE

5 R21 DA027145-02

An Ethnographic and Economic Investigation of the Fresh Start Program (Detroit)

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\$154,500

Treatment professionals and substance abuse researchers agree that both successful drug abuse recovery and exiting street prostitution require changes in social networks and accompanying economic independence. These changes can be both quantitatively and qualitatively described by studying street prostitutes who are engaged in the treatment and recovery process through the application of ethnographic and economic instruments and an accompanying mapping of changing social networks. The proposed work has implications for women's health and welfare and the prevention and treatment of sexually transmitted disease. Aside from journalistic accounts detailing the pitfalls of drug treatment, little research has been done on the recovery process as it actually proceeds within particular programs. Research is not always well incorporated into treatment settings, which may resist innovations due to internal organizational factors. Conducting research in criminal justice settings, including drug courts or programs administered through drug courts, is also problematic. Nonetheless, more such research is needed as treatment and recovery services become central features of the national response to substance abuse, especially in an era of prison downsizing. This will also require research that actively engages with communities and institutions. The proposed research will work collaboratively with multiple agencies to investigate the process of treatment and recovery as it occurs in women who participate in Fresh Start. Fresh Start is a substance abuse intervention program for female street sex workers who have come into repeated contact with law enforcement. Fresh Start is a coercive recovery-based program that serves as a direct contrast to voluntary, traditional, treatment-based programs. The program serves as an alternative to jail time for these women, who are arrested in periodic sweeps of neighborhoods where street prostitution is common. They predict that, prior to entering residential treatment through Fresh Start, women will have daily routines dominated by "street" networks that are relatively constricted and immersed in drug use and drug-using contexts. For those that enter residential treatment and stay in the program for a year, they predict that networks will remain relatively constricted, but will instead be largely immersed in treatment-dominated contexts. In the recovery stage, women will be engaging with the larger community, that is, interacting with more people who are not in treatment or recovery. In this stage, they predict that networks may grow more extensive and variable, as women begin to explore new avenues of social interaction and opportunity. Using interdisciplinary methods, they

will seek evidence of desired change in social networks, sociospatial contexts, and economic behaviors, resources and outcomes.

5 R21 AA018398-02

Interactive Effects Of Ethanol And Estrogen On Brain Vasopressin During Puberty

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\$186,875

Women who abuse alcohol are twice as likely to develop anxiety disorders compared with men, a phenomenon in which the underlying biological mechanisms are unknown. This proposal is focused on a specific candidate gene (AVP) and its downstream signaling pathways that are developmentally shaped during puberty. They expect these studies to show that AVP is permanently altered, either as a direct target for alcohol or indirectly through steroid hormone receptor signaling pathways. Thus, the value of this research lies in the potential for therapeutic approaches that would target specific genes and perhaps reverse brain damage caused by alcohol consumption during pubertal development as well as strengthen reasons for abstaining from alcohol during that time. Women who abuse alcohol are twice as likely to develop anxiety disorders compared with men, a phenomenon in which the underlying biological mechanisms are unknown. Their overall objective is to identify the interactive effects of alcohol and estrogen on arginine vasopressin (AVP), a well-established key molecular mediator of anxiety, in order to elucidate the molecular mechanisms predisposing women to increased risk of anxiety disorders. Adolescent binge drinking is a potential risk factor for the development of adult anxiety disorders due to the heightened stress reactivity that occurs as a direct result of increased circulating estrogens during pubertal development. Little is known about the long-term neurobiological consequences of alcohol consumption during puberty, which is a dynamic and important period of brain development that involves changes in cortical gray matter, synaptic connectivity, and increased neurogenesis. Exposure of alcohol during this critical period of extensive brain remodeling may result in permanent neuronal damage or disruptions in the formation of new neuronal connections, which might manifest as adult behavioral psychoses, including anxiety disorder. Their preliminary data show that 1) alcohol exposure during puberty increased AVP gene expression in specific regions of the brain. Therefore, the experiments proposed in Specific Aim 1 will directly test the hypotheses that there is a critical window of time during pubertal development when the AVP system is most vulnerable to the effects of alcohol and (2) that estrogen exacerbates the effects of alcohol on AVP gene expression. Also, their preliminary data demonstrate that alcohol treatment and estrogen receptor ligands increased AVP gene expression in neuronal cells derived from the hypothalamus, and gene expression is closely correlated with the activity of the gene promoter. Alcohol also activates estrogen-signaling pathways in the brain, which suggests that the underlying mechanisms for alcohol-induced changes in AVP may be mediated by estrogen signaling pathways. Therefore, the experiments proposed in Specific Aim 2 will directly test the hypotheses that (1) acute alcohol exposure increases AVP promoter activity in neuronal cells, (2) that there are specific regulatory regions of the AVP promoter that interact with alcohol, and (3) that estrogen and alcohol interact synergistically to increase AVP promoter activity. To date, specific molecular and neuroendocrine markers that are activated by alcohol during puberty have not been identified. This proposal is focused on a specific candidate gene (AVP) and its downstream signaling pathways that are developmentally shaped during puberty. They expect these studies to show that

AVP is permanently altered, either as a direct target for alcohol or indirectly through steroid hormone receptor signaling pathways. Thus, the value of this research lies in the potential for therapeutic approaches that would target specific genes and perhaps reverse brain damage caused by alcohol consumption during pubertal development as well as strengthen reasons for abstaining from alcohol during that time.

CANCER

1 R21 CA149803-01A1

A Decision Making Framework for Contralateral Prophylactic Mastectomy

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\$191,327

The rising incidence of contralateral prophylactic mastectomy (CPM) among patients with sporadic breast cancer for whom there is no established psychosocial or clinical benefit or cost data is a critical area of public health concern. The proposed study will evaluate the clinical benefits, risks and cost of CPM and prospectively evaluate the decision making process leading to CPM among women with sporadic breast cancer. The results of the study will be used to develop a clinical educational instrument that will enable patients with sporadic breast cancer and their providers to make more informed decisions regarding CPM which will serve to improve the quality of life of breast cancer survivors. The two specific aims are: 1a) Assess the association between CPM and disease-free survival (defined as contralateral breast cancer, distant recurrence and breast cancer mortality), 1b) Assess the health care resource utilization associated with CPM, which will include the surgical procedure, management of surgical complications and subsequent breast reconstruction and 2) Prospectively examine the psychosocial characteristics and decision-making process of women considering CPM. The hypothesis is that CPM will marginally improve disease-free survival, add substantial costs to the health care system and that patients with more cancer-related distress will be more likely to consider CPM. The findings from this study will form the basis for future research (e.g., intervention and/or observational studies) in this area that may be expanded to include multiple institutions. The proposed study is innovative because there are no studies to date that have prospectively evaluated the psychosocial factors that contribute to the decision-making process leading to CPM among patients with sporadic breast cancer. This research is important because of the rising incidence of CPM and the exposure of an increasing number of women to aggressive surgical management without established psychosocial or clinical benefit. The long-term impact of the proposed research is significant because it will lead to the development of an evidence-based clinical educational intervention that will enable patients with sporadic breast cancer and their providers to make more informed decisions regarding CPM and improve the quality of life of breast cancer survivors.

1 R03 CA141572-01A1

Can Lifestyle Modify Fatigue in Breast Cancer Survivors?

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\$89,000

With over 2 million breast cancer survivors in the United States, the investigators propose to evaluate the determinants of fatigue with a specific focus on new-onset and persistent fatigue among breast cancer survivors and an innovative exploration of dietary modifiers of fatigue. Fatigue is the most common and distressing symptom among breast cancer survivors and can persist for years after treatment, even in those clinically disease-free. Most studies among cancer survivors have evaluated the prevalence of fatigue using a cross-sectional design with limited longitudinal follow-up and none of the published studies to date have data on subjects before diagnosis. They propose to utilize the prospective Nurses' Health Study (NHS) cohort, which includes data on subjects before and after cancer diagnosis as well as a cancer-free comparison group, to evaluate the prevalence and trajectory of fatigue among breast cancer survivors not currently on treatment and the influence of lifestyle factors affecting the inflammatory pathway, a possible causative mechanism. They will examine both persistent (present before and after diagnosis) and new-onset fatigue (lack of fatigue before diagnosis and development of fatigue after diagnosis). Strengths of the NHS dataset include data collected prospectively before and after diagnosis, availability of a comparable control group, and extensive information on dietary and lifestyle factors before and after diagnosis with an excellent follow-up rate. Although the cause of fatigue among cancer survivors is most likely multi-factorial, increasing evidence suggests that inflammation is an important mediator. Breast cancer survivors with fatigue have higher levels of pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-1), and circulating T lymphocytes compared with survivors without fatigue. In addition, dietary intake can modify inflammatory biomarkers relating to cardiovascular disease and the metabolic syndrome. Therefore, if inflammation is a mediator of fatigue, lifestyle factors that modify inflammatory pathways may also influence fatigue among survivors. Using data both before and after cancer diagnosis for survivors and cancer-free comparisons, they propose analyses to characterize risk factors for persistent and new-onset fatigue among breast cancer survivors and lifestyle factors that may modify the development and persistence of fatigue. Their findings could lead to new methods of coping with a debilitating symptom and identifying women who might best respond to interventions to decrease fatigue. Their application is novel in examining dietary modifiers of fatigue. Specifically, they hypothesize that among breast cancer survivors that the prevalence of persistent fatigue is similar to that in matched controls without cancer, but that new-onset fatigue is more common among breast cancer survivors. They hope to identify predictors of new-onset, but not persistent, fatigue, such as age at diagnosis, employment status, and lack of physical activity and overweight at diagnosis. Finally, they will evaluate dietary modifiers of fatigue including a higher intake of cereal fiber, whole grains, and n-3 fatty acids and lower intake of saturated and trans-fatty acids.

1 R21 CA152839-01

Microbial Exposures across the Lifespan and Cancer Risk in Women

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\$220,500

Exposure in early life to harmless microbes, as occurs through contact with children, pets, and farming environments, could prevent certain cancers in later life through a calibrating effect on the immune system. This study, based in a large cohort of California women, will examine whether age-specific markers of microbial exposures are associated with the risk of developing

malignancies that together account for a large proportion of the cancer burden in US women, including cancers of the breast, endometrium, colon, thyroid, and melanocyte. If this study finds that these microbial exposures do reduce cancer risk, their results could justify the investigation of new cancer prevention strategies using harmless surrogates of important microbial exposures. Early-life exposure to harmless microbes, as occurs through exposure to children, pets, and farm animals, is increasingly understood to affect childhood health, and mounting evidence suggests that these exposures also influence health in adulthood. The likely pathway for this influence involves a calibrating effect of early-life microbial exposures on the immune response so as to reduce the likelihood of chronic inflammation in later life. As chronic inflammation is known to interact with hormone levels and is suspected in the cause of several cancers in women, including breast, endometrial, and colon cancers, microbial exposures represent important targets of cancer prevention studies. They have preliminary evidence that early-life exposure to farming and preschool or kindergarten protects against breast cancer risk later in life, justifying more detailed study of these exposures for breast and other cancers, including how the risk associations might vary with age-specific exposures across the lifespan. They have a unique opportunity to examine relationships between microbial exposures and cancer risk in over 60,000 women participating in the California Teachers Study (CTS), a cohort of female California teachers and administrators followed prospectively for cancer incidence since 1995. Their study aims to measure associations between selected markers of microbial burden at a range of ages and risk of several cancer outcomes, including in situ breast cancer, estrogen receptor (ER)-positive and ER-negative invasive breast cancer, type 1 endometrial cancer, cutaneous melanoma, colon cancer, and, to the extent possible, papillary thyroid cancer. To accomplish these aims, they will conduct a secondary data analysis in the CTS, using a nested case-control design, using exposure data collected from 60,878 cohort members who provided information about five markers for microbial exposure, including characteristics of their home environment at ages 6 months, 3 years, 5 years, 12 years, and 30 years. They will use unconditional logistic regression to measure associations adjusted for confounders such as age and socioeconomic status. This unified approach will enable us to compare and contrast in a single cohort the associations of early- and later- life markers of microbial exposures with risk of hormone-dependent cancers (e.g., ER-positive breast, endometrial, and papillary thyroid cancers), hormone-independent cancers (e.g., melanoma and ER-negative breast cancer), and cancers linked strongly to chronic inflammation (e.g., breast and colon cancers). If they find that these understudied environmental exposures do reduce risk of specific cancers or groups of cancers, their results could justify the investigation of new cancer prevention strategies using harmless surrogates of important microbial exposures.

N01 CP11005

Costa Rica HPV-16/18 Vaccine Trial (CVT)

Hildesheim, Allan

NCI, Bethesda, MD

\$550,000

The ORWH has supported infrastructure costs associated with the Costa Rica HPV Vaccine Trial (CVT) since its inception. In FY09, support in the amount of \$400,000 was provided. These funds were utilized to support continued follow-up and clinical management of the 7,466 women enrolled in this community-based, randomized, phase III clinical trial and for the enrollment of participants into the extended follow-up phase of the trial (planned for an additional 6 years

beyond the initial, 4-year blinded phase). More specifically, funds provided by ORWH in FY09 supported the following activities: 1) Continued blinded follow-up screening of trial participants, 2) Referral of participants with evidence of high-grade disease to colposcopy and treatment, 3) Initiation of 4-year study visits (final visit under the blinded design - Approximately 2,000 such visits of expected total of 7,000 were performed in FY09), 4) Consenting of women into their Long-Term Follow-up Study (Approximately 2,000 women of expected total of 7,000) were consented in FY09, 5) Initiation of recruitment of new control group for the Long-Term Follow-up- Study (Approximately 700 women of expected total of 3,000) were recruited in FY09, and 6) Additional collection of specimens from the vulva, anus, and oral cavity to allow for the evaluation of vaccine efficacy at sites other than the cervix. The activities funded by ORWH in FY09 and preceding years have resulted in several important publications in the peer-reviewed literature.

1 R03 CA141318-01A1

Nuclear Pore Complex Architecture and Drug Resistance in Ovarian Carcinomas

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\$84,750

The proposed studies are concerned with a novel mechanism by which ovarian carcinoma cells acquire resistance to cisplatin. While ovarian tumors initially respond well to cisplatin and carboplatin, 70 to 80% of advanced stage ovarian cancers will develop resistance to the drug. The proposed studies will investigate the role of changes in nuclear pore architecture and patterning that may contribute to the acquisition of drug resistance by ovarian cancer cells. While ovarian carcinomas initially respond well to treatment with platinum drugs, the majority relapse and acquire resistance. In ovarian carcinomas, they have observed reductions of NUP62 in resected tumor tissue from ovarian carcinomas, and redistribution of NUP62 among subnuclear fields of nuclear pore complexes (NPCs). Further, enrichment of NUP62-depleted NPCs renders ovarian carcinoma cells resistant to cisplatin in culture. The studies suggest the hypothesis that survival advantages conferred by the enrichment of NUP62- and/or NUP214+ NPCs may be exploited by tumor cells. To advance this hypothesis, they propose: 1) To investigate alterations in the accumulation and distribution of NUP62 and NUP214 in ovarian carcinomas, and to decipher how these factors correspond to tumor parameters; and, to investigate how changes in expression or accumulation of either NUP62 or NUP214 influences distribution of the other nucleoporin among NPCs. 2) To investigate how knockdown of NUP62 confers resistance to cisplatin; specifically, to decipher how altering the distribution and prevalence of NUP62+/NUP214- and NUP62-/NUP214+ NPC populations influence survival signaling through NF- κ B signaling pathways. The proposed studies impact the basic biology of epigenetic regulation and may also illuminate a new approach to improving the prognosis of ovarian carcinomas treated with platinum drugs. As the patterning and architecture of NPC populations influences the sensitivity of ovarian carcinoma cells to cisplatin, small molecules may be developed that modify NPC architecture to enhance its therapeutic effectiveness. These agents may be employed to reduce the number of cells that survive and/or become latent in response to therapy, and also to chemosensitize relapsed tumors that have acquired platinum resistance.

1 R21 CA135570-01A2

Estrogen and Skin Cancer

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\$228,750

There is an increase societal pressure in the US to remain young looking. Several studies carried out in post-menopausal women demonstrate the effectiveness of topical estrogen in reversing the signs of aging including thinning, dryness and wrinkling. As a result younger pre- and peri-menopausal women are turning to topical creams containing estrogen as anti-aging lotions. Their preliminary studies using female Skh-1 hairless mice found a significant increase in the number of tumors in mice treated topically with estrogen immediately following UVB exposure compared to mice treated with vehicle control. These data indicate that increased levels of estrogen in the skin combined with UV exposure may act to enhance initiation and promotion of UV- induced skin cancers. These findings also suggest that the use of lotions and creams containing estrogenic compounds on sun exposed sites by younger women may be contributing to the increase in the number of skin tumors being diagnosed in women under the age of 40. The current studies are designed to determine the effect of topical estrogen treatment of previously UVB exposed skin on tumor development and progression from benign lesions to frank malignant squamous cell carcinomas. Americans live in a culture that glorifies youth. According to market researcher FIND/SVP, the anti- aging products market is expected to hit \$56 billion by 2007. Studies in post-menopausal women have found that hormone replacement therapy is effective at reversing the dryness and wrinkling that affects aging skin. Based on these studies, there is increasing interest in the use of topical creams containing hormones such as estrogen to prevent or reverse some of the normal cutaneous aging processes in younger pre-menopausal women. While exposure to these creams may be beneficial cosmetically, the effect of applying estrogen to sun exposed sites for prolonged periods of time, on skin cancer development is not known. Their preliminary studies using female Skh-1 hairless mice found a significant increase in the number of tumors in mice treated topically with estrogen immediately following UVB exposure compared to mice treated with vehicle control. These data indicate that increased levels of estrogen in the skin combined with UV exposure may act to enhance initiation and promotion of UV-induced skin cancers. These findings also suggest that the use of lotions and creams containing estrogenic compounds on sun exposed sites by younger women may be contributing to the increase in the number of skin tumors being diagnosed in women under the age of 40. Most studies have examined the effects of topical or systemic estrogen on the skin in post-menopausal women, however the reality is that younger pre-menopausal women are applying topical estrogen containing creams on their faces previously exposed to UV light to prevent/reverse the signs of aging. Two specific aims are proposed to test the hypothesis that topical estrogen application to previously UVB exposed skin accelerates skin carcinogenesis. Studies in specific aim 1 will use the Skh-1 hairless mouse murine model of UVB induced skin carcinogenesis to determine the effects of clinically used topically applied estrogen (Estrogel(R)) on UVB induced skin tumor development in previously UVB exposed female skin of intact (pre-menopausal) and ovariectomized (post-menopausal) mice. Studies in specific aim 2 will determine the effects of topically applied estrogen (Estrogel(R)) on UVB induced skin tumor progression in female Skh-1 skin of intact and ovariectomized mice. The studies carried out in these aims will determine whether topical estrogen increases the number of UVB induced skin

tumors that develop and also whether it differentially enhances the progression of benign UVB-induced tumors to malignant squamous cell carcinomas in intact (pre-menopausal) and ovariectomized (post-menopausal) mice.

1R21 CA152433-01

Family Cancer Literacy to Promote Mammography Screening among Navajo Women

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\$132,301

Among American Indian and Alaska Native (AI/AN) women, breast cancer is more likely to be diagnosed at an advanced stage and the 5 year breast cancer survival rates are lower than any other ethnic group. Among Navajo women scheduled for a mammography screening appointment, the no show rate is markedly high (80%). This study proposes to develop a family-based cancer literacy intervention that includes culturally and linguistically appropriate education about breast cancer to promote mammography screening among Navajo women. This proposal describes a community-based participatory research study to develop and pilot test a new behavioral intervention to promote mammography screening among Navajo women. From a public health perspective, the intervention has the potential to reach many Navajo women, as 80% of women scheduled for mammography appointments do not follow through. These women (over 1,500 each year) are referred to the Nation Breast and Cervical Cancer Prevention Program (NNBCCPP). A key barrier toward implementing cancer prevention and control efforts in the Navajo community is a lack of cancer literacy or cultural and conceptual knowledge regarding cancer. Other barriers to screening are fear of cancer, stigma of cancer (even talking about cancer) often experienced by the patient, family and community, and lack of knowledge about the etiology of cancer and importance of early detection. Therefore, communication about cancer is impeded within Navajo families and the community. This proposal builds on their successful partnership and collaboration with Dini College (the Navajo tribal college). The proposed study is designed to assess the feasibility and potential efficacy of a cancer-literacy focused, family-based intervention on completion of mammography screening for Navajo women. The intervention will include culturally and linguistically appropriate educational materials about cancer (e.g., the Navajo Cancer Glossary). The project will be implemented in two phases. During Phase 1, they will develop the family cancer literacy intervention with feedback from their community advisory committee. In addition, the Cancer Literacy Measure will be adapted for Navajo women through focus groups and individual interviews. Phase 2 will consist of a formative evaluation of the intervention. The NNBCCPP patient and a female family member will be randomly assigned in pairs to the control condition (existing NNBCCPP health education services, N=40 pairs) or to receive these health education services plus the family cancer literacy intervention (N=40 pairs). They will assess the intervention's feasibility and acceptability as indicated by the recruitment and retention rates and qualitative ratings of treatment acceptability. In addition, they will examine the effect of the intervention compared with the control group on the proportion of women who complete mammography screening at 3-month follow-up documented by NNBCCPP records. They will also examine changes in Cancer Literacy Measure scores from baseline to 3-month follow-up among both patients and family members. They expect that as a result of this project, they will have developed a replicable, feasible, and acceptable intervention, the efficacy of which can be tested in future large-scale randomized clinical trials. In addition, the adapted Cancer Literacy Measure could be used in future cancer

prevention and control projects within the Navajo Nation. The overall objective is to reduce breast cancer morbidity and mortality among Navajo women.

2 U19 GM061388-11

Pharmacogenetics of Phase II Drug Metabolizing Enzymes

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\$250,000

Breast cancer is the most frequent cancer of women and depression is the most common major psychiatric illness. Drugs are available to treat both of these serious illnesses, but many patients fail to respond and some suffer serious adverse drug reactions. The Mayo Clinic Pharmacogenomics Research Network (PGRN) will apply modern pharmacogenomic techniques to help make it possible to "individualize" the drug therapy of breast cancer and depression. The Mayo PGRN is an integrated, multidisciplinary, pharmacogenomic research effort based on a decades-long focus at Mayo on the pharmacogenetics of phase II (conjugating) drug metabolizing enzymes. The Mayo PGRN began by applying a "genotype-to-phenotype" research strategy that included, sequentially, gene resequencing, functional genomic, mechanistic and translational studies. During the present funding cycle, the Mayo PGRN has also incorporated the use of genome-wide techniques and pharmacogenomic model systems, with a special emphasis on functional mechanisms responsible for genetic effects on drug response. They have used that approach to study the pharmacogenomics of the endocrine therapy of breast cancer and selective serotonin reuptake inhibitor (SSRI) therapy of depression - research that grew out of the contribution of phase II enzymes to the biotransformation of the estrogens that play such an important role in breast cancer and biotransformation of the neurotransmitters that are central to the pathophysiology and treatment of depression. Recently, they have performed pharmacogenomic genome-wide association (GWA) studies of breast cancer, and they will soon perform similar studies of the SSRI therapy of depression. They propose to continue this genome-wide focus during the next funding cycle, with both clinical and model system GWA studies of the drug therapy of breast cancer and depression, always including replication as well as functional and mechanistic studies. They also propose two "Network Resources", one designed to provide access to "Next Generation" DNA sequencing for all PGRN Centers and the other focused on pharmacogenomic ontology. In summary, the studies in this application build on Mayo PGRN strengths in DNA sequencing and functional genomics - while incorporating genome-wide techniques - to provide insight into the role of inheritance in variation in the efficacy and side effects of drugs used to treat breast cancer and depression.

1 R21 CA139201-01A2

Mitochondria: A Novel Genetic Modifier for Breast Cancer Risk

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\$180,248

Given the fact that mitochondrial produces energy and generate reactive oxygen species (ROS), inherited variations in mtDNA might represent a newly described mechanism of cancer predisposition. Inherited variations in mtDNA may be extremely relevant for breast cancer, as oxidative stress has consistently been regarded as a risk factor for breast cancer. The study will further their understanding of the genetic events leading to the development of breast cancer;

explore the genetic basis linking mitochondrial, ROS and breast cancer; find the clues for breast cancer racial disparity, and eventually provide a means of identifying a subgroup that are most likely to develop breast cancer. From a clinical perspective, the long-term application of this information to risk assessment and thus to the prevention and early detection of breast cancer in families as well as population will be significant. Somatic mutations in mitochondrial DNA (mtDNA) have been regarded as a hallmark of cancer. However, the role of germline variations (polymorphisms) in mtDNA in cancer development is largely unknown. The mitochondrial genome is highly polymorphic among individuals and exhibits significant geographic and racial difference. It has been suggested that some mtDNA variants could have adverse effect by increase the generation of ROS. The accumulation of those adverse effects over time may increase individual's cancer risk. Besides the sequence variations in mtDNA, the copy number of mitochondria might also affect cancer risk by disturbing crosstalk between mitochondrial and nucleus and consequently altering nuclear DNA stability. It has been proposed that the copy number of mitochondria per cell reflects the gene-environmental interactions between unknown hereditary factors and levels of oxidative stress. However, whether the copy number of mitochondria could be a predictor of human cancer development remains to be determined. Variability in MtDNA sequence and copy number of mitochondria might be extremely relevant to breast cancer because oxidative stress has been suggested to play a significant role in breast cancer etiology. Considerable efforts have been made to discover breast cancer susceptibility genes. However, few have been identified to date. The dilemma might be due to the fact that some of the susceptibility alleles might not reside in nuclear DNA, but in mtDNA. More intriguingly, the geographic and racial difference of mtDNA polymorphisms might have implications in breast cancer racial disparity because African American women are at disproportionately high risk for many oxidative stress-related medical conditions, including breast cancer. Therefore, the investigation of the role of mitochondrial as a predisposition factor of breast cancer could have significant impact. In current proposal, they plan to utilize the valuable biospecimens and data collected through an ongoing case-control study (7R01CA100598) to comprehensively investigate the associations between mtDNA polymorphisms/haplogroups and breast cancer risk in both Caucasian American (CA) and African American (AA) women. They will also examine the associations between the copy number of mitochondria and breast cancer risk. In further analysis, they will study whether mtDNA polymorphisms/haplogroups and copy number of mitochondria are associated with aggressive clinical characteristics of breast cancer. Because the proposed research is nested within an ongoing study, the objectives can be addressed in a timely and cost effective manner. The study will further their understanding of the genetic events leading to the development of breast cancer; explore the genetic basis linking mitochondrial, ROS and breast cancer; find the clues for breast cancer racial disparity, and eventually provide a means of identifying a subgroup that are most likely to develop breast cancer. Such individuals may then be targeted for specific intervention programs such as chemoprevention and dietary modification.

5R21CA135226-02

Brcal, Sporadic Breast Cancer And Aging Women

Avraham, Hava Karsenty

Beth Israel Deaconess Medical Center

\$149,600

By defining the targets that are altered in mutated BRCA1-linked breast and ovarian cancers and providing insights into the BRCA1 pathways, this study may lead to potential new therapeutic strategies for the prevention, early diagnosis and treatment of familial breast and ovarian cancers. In addition, results from this work will enhance their understanding of the molecular events that drive breast and ovarian cancers in aging women, and may link BRCA1 and beta-catenin to oxidative stress and breast oncogenesis. The risk of developing breast cancer increases as women get older. The maintenance of DNA represents a fundamental and continuous challenge to every cell in the body. Genomic instability is a hallmark of most cancers as well as a hallmark in aging. Recent evidence strengthened the link between the maintenance of genome integrity, cancer susceptibility and aging. These conditions can be caused by germline mutations in BRCA1, which is an essential caretaker protein in the surveillance of DNA damage. Impaired oxidative stress response plays an important role in breast oncogenesis. Beta-catenin was shown to be a co-factor for the FOXO family, which promotes survival by inducing cell cycle arrest and quiescence in response to oxidative stress. They observed that wild-type (WT) BRCA1, but not mutated BRCA1, interacts with beta-catenin and increases beta-catenin protein expression by promoting lysine-6-linked ubiquitination. Oxidative stress reagent H₂O₂ increased colocalization and the interaction of BRCA1 with beta-catenin in the nucleus. WT-BRCA1, but not mutated BRCA1, protected the nuclear active form of beta-catenin during oxidative stress responses. The expression of this form of beta-catenin was lower or absent in most of BRCA1 familial breast cancer tissues. Therefore, they hypothesize that: 1) BRCA1 acts as a sensor in regulating beta-catenin mediated oxidative stress and FOXO function; and 2) low expression of WT-BRCA1 or mutations in BRCA1 leads to impaired response to oxidative stress and causes genomic instability, resulting in increased risk of breast cancer in women. Therefore, they aim to examine the effects of BRCA1 on beta-catenin protein expression and stability and to analyze the role of BRCA1 in beta-catenin mediated oxidative stress response. Thus, they specifically propose the following aims: Aim 1: To investigate the role of Brca1 in the expression and distribution of beta-catenin and its targets (cyclin D1 and c-Myc) during mammary gland development in Brca1 mutant mice, in which Brca1 exon 11 is specifically deleted from the mammary glands by using the Cre-loxP system. Aim 2: To characterize the role of BRCA1 as a sensor in regulating the beta-catenin and FOXO interaction during oxidative stress signaling. Results from this work will enhance their knowledge of the molecular events that drive sporadic breast and ovarian cancer development and progression in aging women.

5R21CA140936-02

Improving Flexible Sigmoidoscopy In Women By Optical Analysis Of Microvasculature
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Northshore Univ Health system Res Inst
\$167,750

Flexible sigmoidoscopy as a colorectal cancer screening test is widely available but inaccurate at detecting premalignant polyps in women, largely because women's polyps tend to be located out of reach of the flexible sigmoidoscope. They believe that assessing the superficial blood supply in the visually normal rectum may be uniquely able to sense these lesions further up in the colon of women. If successful, this relatively inexpensive, easy-to-use test may be an adjunct to flexible sigmoidoscopy thereby allowing accurate and cost-effective colorectal cancer screening for women. Despite a myriad of screening tests available, colorectal cancer (CRC) remains the second leading cause of cancer deaths among Americans. Approximately half the population

does not undergo any CRC screening because of cost, access and concerns about discomfort with both the procedure and colonic purge. Flexible sigmoidoscopy (endoscopic evaluation of the distal colon) is performed in the community and has many advantages over other recommended tests (e.g. colonoscopy, CT colography) such as being relatively inexpensive, more widely available (performed by primary care physicians) and proven efficacy at decreasing both CRC mortality and incidence. However, flexible sigmoidoscopy is insensitive in women given their predilection for proximal neoplasia. Indeed, while flex sig identifies two-thirds of advanced adenomas in men, it only detects one-third in women highlighting the need for adjunctive approaches. Their multi-disciplinary CRC prevention group has focused on bridging novel optical technologies to clinical practice. Using 4-dimensional elastic light scattering fingerprinting (4D-ELF), they published that in CRC models, the peri-cryptal capillary blood content was increased prior to any histological abnormalities (a phenomena they termed EIBS (early increase in blood supply). They developed an endoscopically- compatible fiber-optic probe and demonstrated that EIBS was detectable at a distance from neoplastic lesions. In the rectum, EIBS was detectable in patients harboring advanced neoplasia elsewhere in their colon. Importantly, rectal EIBS was more robust in women (~60% increase versus neoplasia-free controls) than men (~25%) for proximal advanced neoplasia (that was not visualizable by flexible sigmoidoscope). They, therefore, hypothesize that rectal EIBS measurement will detect advanced proximal neoplasia in women. They will obtain rectal EIBS analysis on women undergoing colonoscopy. They will identify diagnostic EIBS parameters and determine the impact of demographic factors (e.g. age, race, smoking, medication use) on these markers. This data will be used to formulate a prediction rule for advanced proximal adenomas. They will then prospectively validate this prediction rule on a separate cohort of women simulating real world flexible sigmoidoscopy screening conditions prior to full colonoscopy. This will provide the rationale to performing future multi-center trials of rectal EIBS as an adjunct to flex sig in women. If successful, this practical and relatively inexpensive approach may be pivotal for the resurgence of flexible sigmoidoscopy as an accurate, cost-effective and patient-friendly CRC screening option in women.

5R21CA135532-02

Regulation Of Breast Cancer Progression By Fak Expression In Tumor Macrophages

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\$164,700

By focusing on the role of FAK in both macrophages and tumor cells, this work will uncover novel features of macrophage - tumor cell synergy that contribute to breast tumor behaviors. In addition to providing critical information about how FAK inhibitors should be used to treat breast cancer patients, this work will potentially identify new strategies for targeting distinct cellular compartments within the tumor that can be exploited therapeutically to control tumor growth and progression. It is anticipated that, through the knowledge gained from these studies, there will be a significant reduction in mortality from breast cancer. The growth and metastatic spread of solid tumors is controlled by signals emanating from tumor cells as well as by immune cells and fibroblasts in the surrounding stroma, components of the extracellular matrix, and soluble growth factors and cytokines. While this complexity creates challenges for therapeutic intervention, it also provides unique opportunities by making available a number of distinct cellular and molecular targets that can be exploited to control tumor growth and progression. The

focus of this proposal is on Focal Adhesion Kinase (FAK), a protein tyrosine kinase whose expression is significantly increased in many late-stage cancers, including breast cancer. They hypothesize that FAK expression in two components of the tumor microenvironment, the tumor cells and tumor-associated macrophages (TAMs), plays a critical role in promoting breast tumor progression and metastasis. They will use mouse models of breast cancer to gain an understanding of how FAK expression in breast carcinoma cells and/or the ancillary tumor-associated macrophages controls primary breast tumor growth and metastatic spread. By combining genetic manipulation of these mice with FAK inhibitors currently in Phase I clinical trials, they propose to 1) determine how the loss of FAK expression in macrophages alters or ablates macrophage functions that drive breast tumor growth/progression and metastasis (Aim 1); 2) assess how the dual modulation of FAK expression in breast tumor cells and in tumor-associated macrophages alters breast tumor growth and metastasis (Aim 2A); and 3) assess how systemic inhibition of FAK expression alters breast tumor growth and metastasis (Aim 2B). Successful completion of this study will provide new insights into features of the tumor that can predict a clinical response to the FAK-targeted drugs currently in clinical trials and the optimal timing for these treatments. More globally, they will learn about mechanisms through which tumor cells and other cells within the tumor microenvironment communicate to promote breast tumor growth and metastasis. They anticipate that this work will help to move the paradigm for breast cancer treatment away from the tumor cells per se and toward the full complement of factors that contribute to tumor growth and metastasis.

5R21CA135237-02

Chemoprevention Of Tamoxifen-Induced Endometrial Cancer By Black Cohosh And Red C
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University Of Illinois At Chicago

\$172,698

The selective estrogen receptor modulator, tamoxifen, is very effective in treatment and prevention of breast cancer; however, it causes menopausal symptoms and has carcinogenic effects on the endometrium. They hypothesize that red clover and black cohosh, both frequently used for the alleviation of menopausal symptoms, will reduce tamoxifen-induced endometrial cancer due to their cancer chemopreventive properties. Chemoprevention of Tamoxifen-induced Endometrial cancer by black cohosh and red clover Breast cancer is the most common cancer in women. The selective estrogen receptor modulator tamoxifen, which antagonizes estrogen in breast tissue, is efficacious in the treatment and prevention of breast cancer. In tamoxifen treated patients, botanical dietary supplements such as red clover and black cohosh extracts are frequently used for the alleviation of tamoxifen related menopausal symptoms. Very few studies about the modifying effects of these botanicals on tamoxifen's safety and efficacy have been reported. Tamoxifen's major side effect is an enhanced endometrial cancer risk. Tamoxifen's ER1 mediated uterotrophic activity and its reactive metabolites are believed to be responsible for this effect. Black cohosh and red clover contain anti-oxidative, anti-proliferative, anti-inflammatory, and detoxification enzyme inducing compounds, which could inhibit the initiation or retard the promotion and progression of cancerous cells. The central hypothesis of this project is that black cohosh and red clover reduce the carcinogenic effects of tamoxifen on the endometrium by inhibition of cell proliferation (Aim 1) and through enhancing detoxification pathways (Aim 2). To support this hypothesis they propose the following specific aims: 1. What is the effect of red clover or black cohosh on tamoxifen-stimulated endometrial cancer? Recent

data suggest that black cohosh and red clover can attenuate tamoxifen-stimulated endometrial cancer growth by inhibiting cell proliferation. They will measure the influence of these botanicals on tamoxifen stimulated endometrial tumor growth in ovariectomized athymic nude mice, an established endometrial cancer model for studying estrogenic influences. The mechanism of interaction will be examined by analyzing the expression of pro-proliferative genes and proteins important for tamoxifen mediated tumor promotion in vivo and in vitro. To further identify active compounds, they will examine the anti-proliferative effect of isolated compounds in endometrial cancer cells and in an immature rat model. 2. What is the effect of black cohosh or red clover on detoxification pathways of reactive tamoxifen metabolites? Their data indicate that both botanical upregulate the cellular antioxidative response machinery, thus reducing the carcinogenic effect of tamoxifen's reactive metabolites. They will study the ability of these botanicals to induce the detoxification enzymes, quinone reductase and glutathione-S-transferase, in the uterus and liver of adult rats. They will also analyze whether black cohosh and red clover prevent tamoxifen induced oxidative stress in these animals. Additionally, they will examine the effect of the botanicals on tamoxifen's metabolism to active or reactive metabolites in the blood. To elucidate the compounds responsible for the various effects, isolated constituents will be assayed in vitro. The completion of these specific aims will provide an overall picture of the effect of these botanicals and purified compounds on the efficacy of tamoxifen and on tamoxifen induced endometrial cancer, which is of importance considering the increasing number of breast cancer survivors and women at high risk undergoing tamoxifen treatment.

5R21CA135303-02

Nir Hypoxia Imaging Of Breast Tumor Response To Neoadjuvant Chemotherapy In Vivo

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Dartmouth College

\$173,800

This project will develop and evaluate dynamic Near-Infrared (NIR) tomographic oximetry for characterizing the response of locally advanced breast cancers to neoadjuvant chemotherapy by assessing the temporal variation in tumor oxygenation during hyperoxic gas inhalation. NIR oximetry acquired longitudinally during the course of therapy will be correlated with pathological endpoints in order to determine whether early prognostic biomarkers of treatment response can be identified in the dynamic NIR oxygenation signatures that could be used to customize breast cancer treatment decisions to individual patients in the future. Near-infrared (NIR) multi-spectral imaging is a unique tool for characterizing tissue composition in the female breast. The major advantage of this modality is its ability to provide images of tissue oxygen saturation (StO₂) as well as total hemoglobin concentration (HbT), water fraction (H₂O%) and elastic scattering parameters. Because microcirculation and oxygenation play such major roles in tumor progression and regression, assessing their variation in response to neoadjuvant chemotherapy may reveal early prognostic biomarkers of treatment response that could be used to alter and/or optimize the course of treatment on a more individualized patient basis. Assessing dynamic contrast enhancement in tumor oxygenation after hyperoxic gas inhalation with NIR spectral tomography appears to be feasible and may provide easily-acquired, low cost image signatures for predicting therapeutic response to chemotherapy in the breast. The overall goal of this proposal is to develop and evaluate dynamic NIR tomographic oximetry for characterizing the response of locally advanced breast cancers to neoadjuvant chemotherapy by assessing the

temporal variation in tumor oxygenation during hyperoxic gas inhalation. They hypothesize that tumors with initially larger and faster changes before and after breathing 100% oxygen will have better clinical responses to neoadjuvant chemotherapy. This hypothesis will be quantitatively assessed by 1) advancing the current NIR multi-spectral tomography system to image dynamic oxygenation changes within the tumor, induced by breathing 100% oxygen, with a 0.1 Hz image frame rate, 2) quantifying the tumor oxygenation response with respect to hyperoxic inhalation at different times during the course of therapy, and 3) quantifying the pathological and clinical outcomes of response in order to test for correlation with oximetry changes recorded early in the treatment course. Dartmouth College, through the Norris Cotton Cancer Center at the Dartmouth-Hitchcock Medical Center, has significant resources to leverage in order to conduct the proposed study. A group of investigators which includes clinical specialists in diagnostic radiology, surgical oncology, medical oncology, surgical pathology and medical engineering has been configured to develop and evaluate technology for breast imaging for cancer detection, diagnosis and therapy monitoring since 1999. The proposed project is an important component of the research of this group. In addition to the principal investigators, Professor Shudong Jiang and Dr. Peter A. Kaufman, MD, (Medical Oncology), Professors Keith D. Paulsen, Brian W. Pogue and Dr. Wendy A. Wells, MD, (Department of Pathology) will be significant collaborators engaged to accomplish the proposed specific aims, as an adjunct to currently funded grants involving breast imaging research.

5R03CA139545-02

Targeting The Phosphoinositide Kinase Chain To Prevent Breast Cancer Metastasis

Kunz, Jeannette

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\$76,750

This research project employs cancer cell lines and mouse models of cancer metastasis to uncover the signaling mechanisms that control a cell's ability to move. The migration of cells is important for proper tissue formation, immune function and wound repair, but when aberrantly regulated can also form the basis of devastating human diseases including cancer, atherosclerosis and allergies. Their long-term goal is to better understand the signaling mechanisms that control cell migration and to use this information to develop new therapeutic approaches for the prevention of human disease. Breast cancer is the most commonly diagnosed form of cancer in women 40-55 years of age and it is the second major cause of cancer deaths behind lung cancer for all women. Metastatic breast cancer, where cancer cells spread by motile mechanisms and establish tumors at distant vital sites, is much harder to eradicate and is the primary cause of patient death from breast cancer. Understanding the molecular principles that determine the efficiency of tumor metastasis is therefore critical to the prevention and treatment of breast tumors. Traditional cancer therapeutics are aimed at preventing tumorigenesis of normal breast tissue and inhibiting growth of established cancers. However, few therapeutic strategies target cell migration and invasion, although the pathological deregulation of these processes is a major cause of morbidity associated with the disease. Cell migration and invasion are coordinately regulated by the small GTPase Rac1 and the localized production of the lipid phosphatidylinositol-4,5-bisphosphate (PI4,5P2). The hyperactivation of Rac1 signaling has been observed in many cancers, particularly in cancers of the breast, and this is directly linked to increased metastatic potential and poor patient survival. A role for PI4,5P2 signaling in cancer progression has so far not been reported. However, recent evidence described in the preliminary

studies section of this proposal has established that PIPK1a, a member of the Type I phosphatidylinositol-4-phosphate kinase family, which generates PI4,5P2, is a critical regulator of cell migration and cell-matrix adhesion. They have defined a biochemical pathway in which PIPK1a mediates Rac1 activation in response to integrin and growth factor signals. Rac1, in turn, controls signaling to downstream effectors, including a second member of the PIPKI family, PIPK1b, to promote the assembly of F-actin and of focal adhesion sites necessary for migration and invasion. These results therefore establish a pathway in which PIPK1a is the pinnacle of a signaling cascade that links transmembrane receptors to the regulation of actin and focal adhesion assembly during cell motility. Because cell migration and adhesion are critical for cancer metastasis, PIPK1a may be a target for the prevention of cancer progression. The long-term goal of these studies is to validate PIPK1a as a target for therapeutic intervention in metastatic disease using tissue culture cell models and the athymic nude mouse model of breast cancer. The proposed research also involves pilot studies designed to assess the efficacy of a newly identified natural small-molecule inhibitor of PIPK1a in the control of breast cancer progression. They will use a combination of basic research, chemical genetic and in vivo approaches to systematically address the role of the PIPK1a pathway in cell migration and invasion in a 3-dimensional matrix, in anchorage-independent growth, and in cancer progression in vivo using the athymic nude mouse. The proposed research not only has the potential to impact therapeutic design to prevent breast cancer metastasis, but will also advance their understanding of signaling mechanisms that may be critical for breast cancer metastasis.

5R21CA140916-02

Mitochondrial Catalase As A Treatment For Metastatic Breast Cancer

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University Of Washington

\$205,823

The project is designed to determine the ability of mitochondrially targeted catalase to suppress metastatic breast cancer in mice. The chance of developing invasive breast cancer during a woman's lifetime is approximately 1 in 8 and more than 40,000 women die of metastatic disease each year. Inherent or acquired tumor drug resistance and dose-limiting toxicity limit many agents used in the treatment of invasive breast cancer. Therefore, an important goal is the development of novel non-toxic therapeutic agents that are active against this deadly disease. They have preliminary data showing that mitochondrial catalase (mCAT) reduces metastatic progression of primary breast cancer in mice, suggesting that targeting mitochondria with catalase could be a potential strategy to treat or prevent metastatic breast cancer in women. The aims of this proposal are 1) to further characterize the ability of mCAT to suppress breast cancer metastasis in mice; and 2) develop an inducible system in mice for controlling the expression of mCAT in a time and cell dependent manner. The data generated in this proposal would confirm their preliminary observations and provide the rationale for developing and/or testing clinically relevant mitochondrial-specific drug delivery systems for treating metastatic breast cancer.

5R21CA141112-02

Gender Selectivity To Colon Cancer Chemoprevention By Nsaids

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\$167,750

Colorectal cancer is one of the major public health issues in US with life time risk of being diagnosed with this cancer is about 6%. This cancer usually develops slowly (10- 15 years) through multiple genetic and phenotypic transitions from normal colonic mucosa to adenoma and then to carcinoma. This protracted progression provides ample time for interventions such as endoscopic screening and removing adenomatous polyps. This has been promising but only about half of the at-risk population (age >50) receive any sort of effective screening. This underscores the need for developing alternate cancer prevention strategies such as chemoprevention. Number of studies shows that nonsteroidal anti-inflammatory drugs (NSAIDS) exert chemopreventive benefits against CRC. However, the overall efficacy is relatively modest (30-50% risk reduction) and requires more than a decade to show significant benefits. In addition, the use of NSAIDS has been shown to be linked to severe side-effects including ulcers, GI bleeding, hemorrhagic strokes etc, thereby causing some uncertainty in its use for preventing CRC for average risk use. To improve the risk-benefit analysis, it is therefore critical to selectively target subjects that can efficiently respond to chemopreventive efficacy of nonsteroidal anti-inflammatory drugs (NSAIDS). It has recently been shown that women with CRC may respond to dietary nutrients or pharmacological agents differently than men as they may have differing pathologies, risk factors and hormone status. The epidemiological studies suggest an improved chemopreventive response in women to NSAIDS although there are discordant reports in the literature. Thus, the issue of whether women are more sensitive to NSAID chemoprevention is unresolved with possibility that NSAIDS type, dose etc may play a role. The proposed studies will address the role of estrogen in gender selective chemopreventive efficacy of NSAIDS. These findings will have an important bearing on the healthcare recommendations for colon cancer chemoprevention which have to be cognizant of this gender selective efficacy for maximum cost-benefit potential of NSAIDS. Colorectal cancer (CRC) is the second leading cause of cancer deaths among Americans. With proper screening and removal of adenomatous polyps, CRC risk reduction has been very promising. However, only ~50% of the at-risk population (age >50) receives any sort of screening and many undergo tests with suboptimal sensitivity. This underscores the need for developing alternate cancer prevention strategies such as chemoprevention. Of the myriad of purported agents, nonsteroidal anti-inflammatory drugs (NSAIDS) have reliably shown a positive outcome. Indeed, epidemiological, experimental and clinical trials unequivocally point to the CRC preventive benefits of NSAIDS. However, the efficacy is relatively modest (30-50% risk reduction) and requires more than a decade to show significant benefits. In addition, the use of NSAIDS has been shown linked to severe side-effects including ulcers, GI bleeding, hemorrhagic strokes etc, thereby cautioning that the risks may outweigh the benefits of aspirin and NSAIDS in preventing CRC for average risk use. To improve the risk-benefit analysis, it is therefore critical to selectively target subjects that can efficiently respond to chemopreventive efficacy of NSAIDS and at the same time leave out the population least likely to benefit. It is conceivable that responsive patients could be targeted with lower efficacious doses to avoid associated toxicity. Gender is an important risk factor for CRC with women frequently having biological differences (higher prevalence of proximal lesions, DNA mismatch repair deficient tumors etc). Estrogen is a well-accepted chemopreventive agent against CRC. Moreover, their group has reported that women have altered susceptibility to both genetic and environmental CRC risk factors. The epidemiological data has some studies suggesting an improved chemopreventive response to NSAIDS although there are discordant reports in the literature. Thus, the issue of whether women are more sensitive to NSAID chemoprevention is unresolved with possibility that NSAID type, dose etc

may play a role. They recently conducted a chemoprevention trial using the NSAID celecoxib in a well-validated model of intestinal tumorigenesis, the MIN mouse. They noted that in this model, females were more responsive to the chemopreventive effects of celecoxib. The chemopreventive response was found to have regional propensity with stronger efficacy in the proximal intestine. Furthermore, celecoxib treated female mice had higher levels of mucosal estrogen receptor-2 (ER2) levels. They hypothesize that in colorectal cancer, NSAIDs present an increased chemopreventive efficacy in females which may be secondary to modulation of estrogen receptor ER2 expression.

5R21CA134882-02

Antagonism Of The Ah Receptor In Controlling Breast Cancer Growth And Invasion

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\$178,823

They hypothesize that the hyper-expression of a protein, called the aryl hydrocarbon receptor, and its binding to DNA contributes to the growth and progression of breast tumors. Here they propose that chemicals that impede the function of this receptor (i.e. antagonists) will be effective at downregulating this protein's activity and therefore will suppress breast tumor growth and metastasis. Screening of plant and marine natural product libraries will provide a source of novel antagonists that can be tested for their interaction with this receptor and their mechanism of interference with tumor growth, ultimately resulting in the development of therapeutic agents for the treatment of breast cancer. Historically, the aryl hydrocarbon receptor (AhR) has been studied for its transcriptional regulation of genes encoding cytochrome P450 enzymes, which metabolize environmental and endogenous substrates into toxic and mutagenic intermediates. Accumulating studies support the hypothesis that the AhR also plays an important role in malignant epithelial cell growth and invasion apart from its role in formation of mutagens and in the absence of environmental chemicals. This new paradigm is based on several key observations: 1) AhR expression is increased dramatically in carcinogen-induced rat and mouse mammary tumors and in 'spontaneous' human mammary tumor lines. 2) Constitutive AhR activation is indicated by nuclear AhR localization in rat, mouse, and human mammary tumors and by AhR binding to gene promoters in the absence of environmental chemicals. 3) Constitutively active AhR regulates the expression of multiple genes, including CYP1B1, CK21, and Slug, a master regulator of tumor invasion. 4) Recent studies suggest that increased AhR activity in mammary tumors also contributes to cell migration and invasiveness. 5) Molecular downregulation of the AhR suppresses breast cancer cell proliferation and reverts cells to a non-aggressive phenotype. Molecular and biologic strategies have provided significant evidence that the AhR participates, beyond mutagenesis, in multiple mechanisms that contribute to tumor formation, growth and invasion. Therefore, they can exploit their ability to examine effects of constitutively active AhR to determine how chemical antagonism of the AhR may translate into breast cancer prevention or a therapeutic approach to suppress tumor progression. Thus, they propose a new hypothesis: Targeting the constitutively active AhR with naturally occurring, non-toxic antagonists represents a feasible therapeutic approach to inhibit breast tumor growth and invasion. Three specific aims are proposed: 1. Investigate strategies to maximize antagonism of the AhR by examining the potential for synergistic interaction in mixtures of antagonists, performing a high-throughput screen for novel, potent antagonists from natural product extract libraries (NCI Natural Products Repository) and examining the 'chemical knockout' approach for

improving AhR inactivation. 2. Define the molecular mechanisms of chemical antagonism of the constitutively active AhR in a breast cancer model by establishing antagonist effects on AhR transactivation of endogenous gene expression and examining antagonist-mediated changes in AhR-DNA interactions. 3. Establish the functional consequences of chemically antagonizing the constitutively active AhR using optimal AhR antagonists. The translational impact of these studies lies in the ability of known and newly identified antagonists to suppress tumor growth and invasion. Here, potentially therapeutic AhR antagonists will be evaluated for their ability to block the biological outcomes of constitutive AhR activity in human mammary tumor cell lines. Collectively, these studies will provide the foundation for preclinical studies on the potential for potent AhR antagonists to prevent and/or treat breast cancer in vivo.

5R03CA141564-02

Role Of Micrnas In Initiation And Progression Of Breast Cancer

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Dartmouth College

\$79,000

MicroRNAs are a recently-discovered class of short non-coding RNA genes, which act as post-transcriptional negative regulators of gene expression. microRNA-mediated regulation of tumorigenesis is emerging as a new paradigm in the field of cancer biology. Their implemented in situ hybridization technology offers spatial resolution of miRNA expression unsurpassed by other techniques, which could be readily adapted to routine clinical practice to benefit patients and assist physicians in making crucial decisions. Breast carcinoma (BrCa), which is the second most prevalent cancer in women, is a complex, inadequately understood, and often fatal disease when not detected at early stages. A more detailed understanding of the molecular mechanisms and regulatory pathways at work will enormously assist in improving the design and target selection of therapeutic strategies. MicroRNAs (miRNAs) are evolutionary conserved, short non-coding regulatory RNAs that post-transcriptionally modulate gene expression by binding to their cognate target mRNAs via pervasive and versatile mechanisms. Altered expression of specific subsets of miRNAs has been linked to different types of hematologic and solid tumors. Independent studies using BrCa clinical specimens have identified a small subset of miRNAs, which are differentially detected between normal and tumor tissue specimens. Thus, the clinical value of these miRNAs as novel biomarkers for different aspect of BrCa management is being actively investigated. Importantly, functional analyses in cell line systems and xenograft transplantation in mouse models have revealed tumor suppressive and oncogenic functions of some of these miRNAs. This proposal focuses on miRNAs as potential tumor suppressive mechanisms to prevent breast carcinogenesis. They will utilize a genetic approach in mouse models of BrCa to test the hypothesis that global impairment of miRNA functions enhances tumor growth and aggressiveness. Of note, their experimental strategy will be similar to the one successfully used by Tyler Jacks and colleagues to uncover tumor suppressive roles of miRNAs in a K-Ras-driven mouse model of lung cancer. They will target chromosomal deletion of miRNA-processing enzyme Dicer in mammary gland epithelia using the Cre/LoxP system. The effects of global loss of miRNA functions will be studied in well-established mouse models of BrCa. Mammary gland restricted expression of Polyoma virus middle T antigen (PyMT), Neu/HER-2 or Wnt-1 causes BrCa with different latencies and histological features reminiscent of specific human BrCa subtypes. They expect that results of this proposal will uncover an etiological contribution of miRNAs and validate the use of these mouse models for future studies

concentrating on the role of individual miRNA in BrCa and development of miRNA-based therapeutic strategies.

5R21CA142537-02

Reactivation Of Breast Cancer Micrometastases By Senescent Bone Marrow Stroma

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\$171,600

The proposed study will investigate the induction of senescence in mouse bone marrow stroma by estrogen deprivation in vitro and in vivo as manifested by the secretion of inflammatory cytokines and loss of the capacity to support dormancy of breast cancer cells in an in vitro model and the loss of the capacity to support the dormancy of xenografted human breast cancer cells in the bone marrow microenvironment. Experiments will determine if treatment with estrogen or anti-inflammatory agents can restore the capacity of senescent stroma to support dormancy. More than a third of stage I-III breast cancer patients have bone marrow micrometastases at the time of diagnosis providing a source of recurrence. Most recurrences occur in post-menopausal women. Mechanisms of dormancy and recurrence are not well understood, but data suggest a dependence on a close association with bone marrow stroma. They hypothesize that stromal cells undergo senescence due to aging and/or post-menopausal estrogen deprivation and begin to secrete inflammatory cytokines that can stimulate dormant cancer cells to re-awaken. The broad, long-term goals of their investigations are to define mechanisms that govern the establishment of the dormant state in breast cancer cells in the bone marrow and to determine factors and mechanisms responsible for their re-awakening and recurrence of disease. They propose to determine if bone marrow stroma can undergo senescence when deprived of estrogen or treated with cytotoxins in vitro and in vivo in a murine model. Their specific aims are: 1. to determine if in vitro estrogen deprivation can induce a senescent phenotype in bone marrow stromal cultures incapable of supporting breast cancer dormancy in an in vitro model and 2. to determine if in vivo estrogen deprivation induces a senescent phenotype in bone marrow stroma rendering it incapable of supporting breast cancer dormancy in vitro and in vivo. They will establish and characterize the phenotype of secretory senescence by subjecting stromal monolayers to oxidative and hypoxic stress and estrogen deprivation and measure the expression and activation of TGF β , Cox-2, IL-6, IL-8 and SA- β Gal, known markers associated with senescence. They will determine if estrogen deprivation in vitro and in vivo and cytotoxicity in vitro can induce senescence measured by these molecular markers and by the loss of support of breast cancer dormancy in an in vitro clonogenic co-culture model and in a left ventricle injection bone marrow metastasis model. Experiments will also determine whether estrogen-deprivation renders stroma more susceptible to chemical injury and whether administration of Cox-2 inhibitors or estrogen can reverse these effects. These studies will establish a way of thinking about dormancy as a function of the senescent microenvironment and seek to reverse estrogen-deprivation-induced inflammation to maintain it.

5R21AI079439-02

Hpv Epidemiology And Response To Screening (Hearts)

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University Of California San Francisco

\$156,969

To the best of their knowledge, this is the first study regarding HPV and HPV disease among homeless and marginally housed women. Ascertaining prevalence and risk factors specific to this population will facilitate a better understanding of HPV among indigent US women, which could have implications for improvement in health care delivery, particularly regarding HPV vaccine uptake and effectiveness. Given that poor and marginally housed women use health services infrequently, the potential benefits of a prophylactic vaccine are of the utmost importance. HPV vaccine development and clinical research have focused on women from the general population and little is known about HPV among indigent women, many of whom experience repeated risk for sexually transmitted infections that continues through the span of their lives. The impact of repeated exposure to HPV, as well as the impact of co-infections like HIV, HCV, gonorrhea, and Chlamydia, on the natural history of HPV infection and HPV-associated disease, is unclear in this population. Moreover, the prevalence of HPV subtypes in this population is unknown, which precludes estimates of potential vaccine effectiveness. A better understanding of HPV among indigent US women could have implications for improvement in health care delivery, particularly regarding HPV vaccine uptake and effectiveness. They propose an exploratory study to assess the prevalence and variability of cervical HPV and cervical HPV disease (cervical intraepithelial neoplasia); associations with co-infections (i.e., HIV, HCV, gonorrhea and Chlamydia) and drug use (e.g., tobacco and crack cocaine); and the feasibility of a larger randomized study among homeless and marginally housed women. Individuals will be recruited from homeless shelters, free food programs and low-income single room occupancy hotels. In this way, study participants will not be limited to individuals who visit specific institutions, thus facilitating reliable estimates from a community-based sample.

CHRONIC FATIGUE SYNDROME

5 R01 AR053821-04

HERV-K18 as a Risk Factor for CFIDS

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Boston, MA

\$164,058

The etiology of Chronic Fatigue Syndrome (CFS) is far from understood and is likely due to multiple genetic components. Infection with EBV and treatment with IFN- α have been implicated in the pathogenesis. Their laboratory has shown that EBV-infection, and exogenous IFN- α , activate transcription of the env gene of a Human Endogenous Retrovirus, HERV-K18. This provirus is normally silent, but when induced it encodes a superantigen (S_{Ag}), which is a class of proteins that is capable of deregulating the immune system. Three alleles of HERV-K18 env have been documented, K18.1, K18.2, K18.3, whose gene products have S_{Ag} activity, but are predicted to differ biochemically and functionally. Their working hypothesis is that HERV-K18 is a risk factor for CFS. In a pilot study, the allele and genotype distributions of the HERV-K18 env gene were compared between various groups of CFS patients and healthy controls. Although only a limited number of samples were available in the various cohorts, the odds ratios that were obtained were statistically significant. The most intriguing interpretation of these data are that they provide genetic evidence for the unique etiology of at least one group of CFS patients. Thus, it may be possible to delineate different subtypes of CFS, depending on the

clinical history of the patients. It is now proposed to substantiate these pilot results, using a much larger cohort of 400 CFS patients associated with EBV that has been assembled by the co-investigator, Dr. Renee Taylor. Dr. Ben Katz, board certified in both Pediatrics and Pediatric Infectious Diseases, will clinically evaluate the patient cohort, and Dr. Inga Peter, a genetic epidemiologist and biostatistician, will oversee the statistical analyses. In addition, the expression pattern of the HERV-K18 SAg during active disease versus intermission will be measured. Furthermore, T cell stimulatory activity of this SAg, expressed on peripheral blood lymphocytes of patients during the course of the disease, will be tested *ex vivo*, using a T cell hybridoma reporter assay that has been developed in their lab. Since SAg-activated T cells produce massive quantities of chemokines, lymphokines and neurokinins, the expression of the HERV-K18 SAg could influence not only the immune system, but other organs as well. A positive association between CFS and either HERV-K18 alleles or expression patterns would open new avenues for the development of clinical treatments of this chronic disease. CFS is a disease that affects a significant number of people worldwide, yet the underlying mechanism(s) of pathogenesis remains unclear. The herpesvirus EBV and IFN- α have been suggested to be associated with CFS, although these concepts are far from accepted. They propose a novel genetic aspect in the EBV/ CFS association, namely the presence of certain HERV-K18 alleles that differ in their superantigen activity.

CARDIOVASCULAR DISEASE

5R21HL093450-02

Compromised Microcirculation In Women With Polycystic Ovary Syndrome

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John B. Pierce Laboratory, Inc.

\$223,143

Women with Polycystic Ovary Syndrome (PCOS) have greater risk for cardiovascular disease, in particular dysfunction of the peripheral circulation that can lead to hypertension and comprised glucose disposal. This research will determine the role of hyperandrogenism in microvascular responsiveness in women with PCOS, and the mechanisms by which testosterone may impact endothelial function. These studies also have broad public health implications because their findings on the effect of hyperandrogenism on endothelial function may provide insights that can improve cardiovascular health of all obese women and men. Polycystic ovary syndrome (PCOS) is the most common reproductive endocrinopathy in young women, affecting 6-10 % of women of reproductive age. Obesity, insulin resistance, hyperandrogenism and hyperestrogenism are core functional disorders of PCOS and place women at increased risk for microvascular dysfunction. Women with PCOS have greater circulating concentrations of endothelin-1 (ET-1), a potent vasoconstrictor in the microcirculation (including that of the skin), which can increase blood pressure and lead to endothelial damage. The central hypothesis of this proposal is that testosterone effects on ET-1 mediate the peripheral microvascular dysfunction associated with PCOS. This hypothesis will be tested using a prolonged skin heating model to study peripheral microvascular responsiveness. Local skin heating has been used extensively to study mechanisms controlling peripheral microcirculation under a number of physiological conditions, including obesity, insulin resistance and hypertension. The impact of testosterone or ET-1 on microvascular responsiveness to local heating has not been studied in women with or without

PCOS. This proposal seeks to provide this missing information via pursuit of two Specific Aims. Specific Aim 1 will apply dose-response curves to examine the mechanism by which ET-1 influences peripheral vasodilation. Specific Aim 2 will determine the mechanism by which testosterone affects peripheral microcirculatory responsiveness in women with and without PCOS. These studies will have broad public health implications because their findings on the effect of hyperandrogenism on endothelial function may provide insights applicable to cardiovascular health in women and men.

5R21HL093665-02

Sex Differences In Myocardial Ischemia Triggered By Emotional Factors After Mi

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\$193,750

Coronary heart disease is the major cause of death in American women and yet, much remains to be learned about the unique characteristics of this disease in women. Young and middle-aged women have higher mortality and complication rates after an acute myocardial infarction (MI) and higher burden of psychosocial risk factors. This study will evaluate whether emotionally triggered ischemia is more common in women than in men after MI, whether it is correlated with psychosocial risk factors that are common in women, such as depression and history of trauma, and whether it is associated with biological changes that may have prognostic significance, such as hemodynamic, neurobiological and inflammatory responses to stress. Coronary heart disease (CHD) is the major cause of death in American women, and every year a similar number of women and men die due to CHD. Growing evidence supports important differences in the pathophysiology, clinical presentation and prognosis of CHD between women and men; yet much remains to be learned about the unique characteristics of CHD in women. Young and middle-aged women have higher mortality and complication rates after an acute myocardial infarction (MI) compared with men of similar age. Reasons for these differences are unknown; they are not explained by traditional CHD risk factors, other comorbidity or treatments, and occur despite the fact that women have less coronary atherosclerosis and more preserved ventricular function than men. One third to two thirds of patients with CHD have myocardial ischemia that is induced by psychological stressors. Such ischemia is often painless and unrelated to severity of coronary artery disease; nonetheless it is associated with adverse outcomes. Emotional factors such as depression and psychological trauma are more common in women with CHD than in men and may predispose women to stress-induced ischemia. Depression, for example, is present in up to 40% of women with MI younger than 60 years. However, emotionally-triggered ischemia has hardly been studied in women before. The overall objective of this proposal is to evaluate differences in stress-induced ischemia between 50 women and 50 men younger than 60 years who were hospitalized for acute MI in the previous 6 months in Emory- affiliated hospitals. They hypothesize that myocardial ischemia due to emotional factors is more common in women than in men, while exercise-induced ischemia is as common, or even less common, in women. The aims of this study are: (1) Using single photon emission tomography (SPECT) [Tc-99m] sestamibi myocardial perfusion imaging, to compare myocardial perfusion during rest, during exercise, and during an emotionally stressful challenge in women and men. (2) To investigate biological mechanisms for the sex differences in ischemia induced by emotional stress, including differences in hemodynamic (blood pressure, heart rate), neurobiological (cortisol and autonomic nervous system) and inflammatory responses to the

stressful challenge. (3) To investigate behavioral/psychosocial explanatory factors for the sex differences in ischemia induced by emotional stress (depression, history of trauma, and socio-economic environment). Younger women with MI represent an understudied patient group despite their higher rate of adverse events compared with men. Investigation of this group will provide critical information for the prevention of CHD in women. Their study may uncover a unique pathway which may explain sex differences in the outcome of MI.

5 R01 TW008288-02

Weight, Diet, Genes and CVD Risk Factors (Hypertension and Diabetes)

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\$50,000

This study will examine the independent and combined effects of genetic predisposition and modifiable factors such as weight and dietary patterns on the risks of having hypertension and diabetes, two major cardiovascular disease (CVD) risk factors. The demographic and health trends in the Philippines exemplify those of other developing Asian countries where CVD-related morbidities and deaths are prevalent and increasing. Thus, studying the mechanisms that can lead to the development of hypertension and diabetes among Filipinos can provide critical information that may guide more tailored prevention efforts for these populations, potentially narrowing global health disparities. Cardiovascular diseases (CVD) are the leading causes of morbidity and mortality in the world (1- 3). Hypertension and diabetes, two of the major CVD risk factors, are complex diseases caused by the combined actions of genetic and environmental factors (4-8). Few studies have examined the interaction of these factors and fewer, if any, have looked at their effects in populations of developing Asian countries that are plagued with increasing levels of obesity and rapidly changing food environments (9, 10). The information gap may be due to the lack of population-based studies with adequate depth and detail. There is a paucity of information on dietary and adiposity trends derived from longitudinal studies and there are inadequate genetic data, especially among Asians who tend to develop CVD risk factors at lower body mass index thresholds (11, 12). Aims and Methods: The proposed study aims to understand how weight history, dietary patterns, and genetic variants independently and jointly affect blood pressure and fasting glucose among adult Filipino women (ages 38 to 71 yr in 2007) using the Cebu Longitudinal Health and Nutrition Survey (CLHNS), an ongoing community-based study of over 2000 women (and their infants) which began in 1983. This is a unique dataset that contains not only rich genetic information on these women but also dietary and anthropometric measurements obtained since baseline, recent blood pressure (1998-2007) and fasting glucose (2005) measurements, and other individual-, household-, and community-level data collected over a span of 24 years of rapid country-wide socio-economic changes. Specifically, using multivariate regression methods they will determine the: (a) effect of weight history (i.e. duration of overweight) on the risk of having hypertension and/or diabetes; (b) association between dietary patterns (identified through cluster analysis) and hypertension and/or diabetes; (c) independence and co-occurrence of hypertension and diabetes and how these relate to weight and dietary patterns; and (d) effects of genetic variants on hypertension and diabetes, focusing on gene variants that have been associated with hypertension or diabetes by previous association studies. Further, the study will explore significant interaction of effects among genetic variants, overweight history and dietary patterns in affecting hypertension or diabetes.

5 R21 HL093626-02

Mechanisms Underlie Inverse Gender Discrepancy in Ischemic Protection

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\$185,625

The proposed research will employ KATP channel mutant mice that are defective in the sulfonylurea receptor 2 (SUR2) to evaluate gender difference in ischemic protection, regulation of estrogen in sarcolemmal and mitochondrial SUR2 forms and obtain new insights in ion channel regulation in cardiovascular diseases. Myocardial infarction (MI) is a major health problem worldwide due to its acute nature and lack of effective prevention schemes. Gender difference in ischemic protection exists, with relatively lower MI incidences in pre-menopausal females than age-matched males. Emerging evidence indicates that the female-specific advantage in ischemic protection is mediated by estrogen. In the ischemic protection network, KATP channels (KATP) are postulated to play protective roles, but their relative importance remains to be controversial. Composed by a Kir6.2 pore and an SUR2 regulatory subunit, KATP activity is recorded in cardiac sarcolemmal or mitochondrial inner membrane. Their recent data show that disrupting the SUR2 gene at an earlier exon 3 causes an early lethality and the mutants only lived 8 days. However, disrupting SUR2 at middle exons 12-16 interrupts the SUR2 long forms, but the novel SUR2 short forms remain expressed. They have identified 2 splice variants that are generated by a rare intra-exonic splicing (IES) event in SUR2 mRNA to produce transcripts encoding the 55-kDa SUR2 short forms in heart mitochondria. Characterization of SUR2 KO has revealed an inverse pattern of gender difference in cardioprotection. Completed tests in KO males show that they are constitutively protected, with reduced infarcts after ischemia, while KO females have larger infarcts and cannot be preconditioned. mRNA levels of both IES variants markedly increase in the preconditioned KO males but they reduce dramatically in the preconditioned KO females. This interesting discrepancy offers a new platform of using SUR2 mutant mice to investigate gender difference in ischemic protection. The proposed research intends to explore the molecular mechanisms underlying gender difference in cardioprotection in relation to KATP channels, especially mitochondrial KATP. They hypothesized that estrogen modulates expression of sarcolemmal and mitochondrial SUR2 forms in mice. They further hypothesized that levels of the IES variants encoding the mitochondrial SUR2 short forms are critical to protection. In Aim 1, they will characterize ischemic protection in both genders of WT and KO mice, and study whether estrogen modulates expression of the SUR2 forms. In Aim 2, estrogen regulation in mitochondrial SUR2 will be investigated, and a 55A "rescued" female mouse model will be tested whether they can improve protection. Interactions of estrogen receptor 2 and the IES variants will be explored. Results from this research not only provide new insights in gender-specific response to cardioprotection but also identify new drug targets for future clinical treatments against MI.

CRANIOFACIAL

3-U01-DE-017018-06S2

Risk Factors for Onset and Persistence of TMD

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\$150,000

Risk factors for onset and persistence of TMD: Myogenous temporomandibular disorder (TMD), with or without arthralgia, ranks second only to headache as the clinical condition most likely to cause craniofacial pain and dysfunction in the U.S. population. During the last decade, a small number of epidemiological studies have attempted to quantify the incidence of TMD in populations of European heritage; however, no investigative team to date has undertaken a large-scale, hypothesis-driven, prospective study designed to identify biopsychosocial and genetic risk factors for the onset and persistence of this vexing pain disorder. They propose to conduct a comprehensive, prospective cohort study of the incidence of TMD in collaboration with an internationally recognized group of epidemiologists, pain researchers, and geneticists. Participants will be enrolled and followed prospectively at four research institutions and by their Data Coordinating Center (Battelle Memorial Institute). their three goals are to: a) undertake a five-year, prospective cohort study of 3200 initially TMD-free individuals recruited from major ethnic and racial strata at four study sites, quantifying incidence rates of first-onset-TMD; b) undertake a case-control study by recruiting 200 people with chronically symptomatic TMD identified during cohort recruitment whose history of TMD precludes them from the prospective study; c) to identify in both groups the individual and joint effects of predictors of TMD risk using a conceptual, causal model for TMD that they have developed based their own studies and other published research. Their preliminary epidemiological findings have led to the central hypothesis that pain amplification and psychological factors, both of which are influenced by genetic variants, represent causal risk factors that influence TMD onset and persistence. The outcomes of their proposed study will identify the primary socio-demographic, clinical, biological, psychological, and genetic risk factors for TMD onset and persistence. In so doing, they will obtain important and novel information regarding the etiopathogenesis of TMD, which will assist with the development of evidenced based pharmacological and behavioral interventions for TMD.

DIABETES

5R21HL093699-02

Gender Specific Complications Of Diabetic Autonomic Neuropathy: A New Mouse Model

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\$198,750

Diabetic Autonomic Neuropathy (DAN) is characterized by impairment of autonomic responsiveness of the heart and an increased incidence of arrhythmia and sudden death. Data suggest the hypothesis that menopausal women might be more likely to develop DAN and that hormone replacement therapy might protect the heart from development of DAN and decrease the incidence of arrhythmia and sudden death. Here they propose to test the hypothesis that the female Akita mouse might serve as an animal model for the study of Gender Specific Complications of DAN and the protective effects of estrogens against the development of diabetes and its secondary complications in the heart. Diabetic Autonomic Neuropathy (DAN) is characterized by impairment of autonomic responsiveness of the heart. DAN has been associated with an increased incidence of arrhythmia and sudden death in diabetics. Although the overall incidence of sudden death is lower in women than in men, the risk of sudden death

associated with diabetes in women is greater than in men. Studies in postmenopausal women demonstrated that combined estrogen/progestin therapy reduced the incidence of diabetes. Comparison of heart rate variability showed that the parasympathetic response of the heart was increased in young women compared with men; this difference was attenuated after menopause, but maintained in women on hormone replacement therapy (HRT). These data suggested the hypothesis that menopausal women might be more likely to develop DAN and that HRT might protect the heart from development of DAN and decrease the incidence of arrhythmia and sudden death. The Akita mouse manifests a gender difference in the development of diabetes: males develop severe hyperglycemia and secondary effects of diabetes, while females exhibit only a mild hyperglycemia. Using male Akita mice, they have previously developed an animal model for DAN that is characterized by the appearance of spontaneous ventricular arrhythmias following myocardial infarction (MI). Here they propose to test the hypothesis that the female Akita mouse might serve as an animal model for the study of Gender Specific Complications of DAN. Specifically, they will test the hypotheses 1) that ovariectomy of female Akita mice results in the development of the diabetic phenotype and secondary effects of diabetes as demonstrated by the development of hyperglycemia, proteinuria and a decreased parasympathetic inhibition of Isoproterenol-stimulated L-type Ca²⁺ currents, and that estrogen reverses this effect 2) that estrogen replacement protects ovariectomized female Akita mice against the development of spontaneous ventricular arrhythmias following MI and 3) that gene array studies will establish a subset of genes that are differentially expressed in ovariectomized mice who develop arrhythmias following MI, which might serve as candidate genes for the treatment and prevention of this effect of diabetes in women. Studies in this application propose to establish a unique animal model, which might offer a new gender specific therapeutic approach to diabetes and the complications of DAN.

5-U01-DK-057136-12

Look AHEAD: Action for Health in Diabetes

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\$100,000

Look AHEAD is randomized clinical trial examining the long-term health effects of an intensive weight loss intervention in approximately 5,145 overweight volunteers with type 2 diabetes. Participants are randomized to an intensive lifestyle intervention designed to achieve and maintain weight loss by decreased caloric intake and increased physical activity, or to a control program of diabetes support and education. The primary outcome of Look AHEAD is the aggregate occurrence of severe cardiovascular events (fatal and non-fatal MI and stroke and cardiovascular deaths) over a planned follow-up of 11.5 years. The original grant application provided funding for the first 7 years of the study (1 year for study design and 6 for execution of the trial). The present grant application is for an additional 7 years of funding to complete the Look AHEAD trial. All aspects of the study have proceeded extremely well - the sample of 5,145 was recruited on time; retention has been excellent and the intervention has been effective in producing initial weight loss and maintaining it over time. All 16 clinical sites have been successful in recruitment, retention, and delivery of the intervention and the DSMB has been very positive about the execution of the trial. The present application reviews the overall design of Look AHEAD, progress to date, and plans for the future. Specific Aims are to retain the cohort over time, continue to complete annual in-person visits and semi-annual telephone

interviews for outcome assessments and continue to administer the lifestyle intervention. These procedures will enable us to analyze the effects of the intervention on serious cardiovascular-related factors and complications, and cost-effectiveness of the intervention.

5U01 DK048489-17

Post DPP Follow-Up Study

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\$650,000

The Diabetes Prevention Program is a multicenter controlled clinical trial examining the efficacy of an intensive life-style intervention or metformin to prevent or delay the development of diabetes in a population selected to be at high risk due to the presence of impaired glucose tolerance (IGT). Development of diabetes, defined by 1997 ADA criteria, is the primary outcome while cardiovascular disease and its risk factors are important secondary outcomes. The DPP began recruitment in mid-1996. At the time of this application, total study exposure is a mean of approximately 3 years (range 2 to 5) with a total of approximately 10,000 patient years in the 3,234 volunteers in the 3-arm study. On the basis of a statistically significant and clinically compelling decrease in the development of diabetes in the life-style intervention and metformin-treated groups (58% and 31% reductions, respectively) compared with the placebo treated group, the DPP Data Monitoring Board and NIDDK ended the masked treatment phase of the study in May, 2001, one year earlier than originally planned. This application is designed to take further advantage of the scientifically and clinically valuable cohort of DPP volunteers and the large volume of data collected during the study. The highly compliant DPP cohort, including 45% minorities, is the largest IGT population ever studied. Moreover, the sub-cohort that has developed diabetes (n approximately 700) has been followed from near the exact time of diabetes onset. Clinically important research questions remain in the wake of the DPP. The carefully collected, centrally measured and graded data in this cohort should help to answer, definitively, a number of important questions regarding the clinical course of IGT and early onset type 2 diabetes. Specific aims include: 1. Examine the long-term effects and durability of prior DPP intervention on the major DPP outcomes including diabetes, clinical cardiovascular disease, atherosclerosis, CVD risk factors, quality of life and cost-benefit; 2. Determine the clinical course of new onset type 2 diabetes and IGT, in particular regarding micro-vascular and neurologic complications; 3. Determine the incidence of cardiovascular disease (CVD), CVD risk factors and atherosclerosis in new onset type 2 diabetes and IGT; and 4. Examine topics 1-3 in minority populations, men vs. women, and in older subjects in the DPP.

5U01-DK048489-17

Sex hormones & sex hormone binding globulin effects on diabetes risk in women in DPP

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\$350,000

To date neither phenotypic factors nor genetic factors that may contribute specifically to diabetes risk among women have been explored in the Diabetes Prevention Program (DPP). Associations of low estrogen status with increased diabetes risk, and associations of low concentrations of sex hormone binding globulin (SHBG) with increased diabetes risk, have been observed separately

in other studies. However, little published data connects these factors with formally ascertained conversions to diabetes mellitus, and prior studies have not specifically identified women at such high a priori risk as were selected for the DPP. Sex hormone binding globulin is a protein produced by the liver that binds to hormones with a steroid nucleus, with greatest affinity for sex steroids. This protein carries the majority of the circulating sex steroid mass in both men and women. The circulating concentration of SHBG is determined by a number of physiologic factors, in particular by the concentrations of sex hormones. In these relationships estrogen and testosterone act in opposite directions on SHBG concentration, with higher levels of estrogen resulting in reduced SHBG and higher levels of testosterone resulting in increased SHBG. Genetic effects on SHBG concentration are also recognized, and single nucleotide polymorphisms (SNPs) in the SHBG gene have recently been identified that produce changes in SHBG concentrations or function. The inter-relationships of menopausal status and/or sex hormone concentrations with diabetes risk among women at high baseline risk for diabetes are not well understood. Furthermore, the contributions of genetic effects on SHBG and its relationship with diabetes risk have not been well explored. Here they propose to evaluate associations of sex hormones and SHBG, plus genetic variants that affect SHBG, on diabetes risk in women in the DPP. They will evaluate 4 main hypotheses: (1) The relationship between baseline SHBG concentrations and risk of progression to prospectively ascertained diabetes differs between premenopausal and postmenopausal women. (2) Sex hormone levels will influence diabetes risk through effects on SHBG concentrations, and explain in part the effects of SHBG on diabetes risk in premenopausal and postmenopausal women. (3) Race/ethnicity or direct genetic variation in the SHBG gene will alter diabetes risk through effects on SHBG concentrations, independent of effects of sex hormones on SHBG. (4) The beneficial effects of treatment interventions in DPP are proportional to baseline SHBG and sex hormone concentrations, without differences in this effect across treatment groups.

DIETARY SUPPLEMENTS/CAM

1 R21 AT005377-01A1

Identification of novel phytoprogestins from hops and red clover

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\$235,500

Women are already taking phytoestrogens in botanical extracts for menopausal symptoms, and the incorporation of progestins may prevent hyperplasia and cancer of the uterus. As women search for more potent alternative estrogens to satisfy the need for menopausal symptom alleviation, the chance for hyperplasia in the uterus increases and makes the characterization of novel phytoprogestins crucial. Hormone replacement therapy (HRT) is the most commonly prescribed medication for the alleviation of menopausal symptoms. Unopposed estrogen replacement therapy increases the risk of developing endometrial cancer by 120% for every 5 years of use. To eliminate this risk in women with a uterus, the addition of progesterone to HRT in the form of combined estrogen/progesterone replacement has been implemented. Considerable evidence now indicates that the addition of synthetic progestins to HRT increases the risk of breast cancer as well as many other deleterious side effects. In response to the problems associated with HRT, millions of women are exploring the use of botanicals and dietary

supplements for the alleviation of climacteric symptoms. However, the use of botanicals with only plant-derived estrogens in the absence of progestins might increase the risk for developing endometrial cancer similar to estrogen alone. Two common supplements, hops and red clover, contain phytoestrogens that bind and activate estrogen receptors. Interestingly, when hops and red clover are given orally to ovariectomized rats, uterine weights are not significantly increased in animals treated with a crude extract but are significantly increased in animals given an equivalent dose of the pure phytoestrogen. The hypothesis of this grant proposal is that selective natural progesterone compounds can be identified from botanical extracts to generate a combined phytoestrogen-phytoprogestin alternative to traditional hormone replacement therapy. The presence of both estrogen and progesterone receptor agonists in one botanical extract may provide both the benefits of estrogens for alleviation of menopausal symptoms and the progesterone necessary to combat formation of uterine cancers. Selective and safer progestins might also be identified from botanical sources improving the overall behavior of the progestin used in HRT. In order to provide support for this hypothesis the following specific aims are proposed: Aim 1. Do botanical extracts contain phytoprogestins and what are the pure compounds responsible for the progesterone-like activity? Aim 2. Are phytoprogestins specific and selective for uterine progesterone receptors? Aim 3. Are phytoprogestins protective against uterine hyperplasia in an ovariectomized rat model? These studies will provide a clear justification for the use of botanicals that have the possibility of providing both estrogen and progestin-like activity but with more selective and safer profiles for the treatment of menopausal symptoms. Women are already taking phytoestrogens for menopausal symptoms, and incorporation of progestins may prevent hyperplasia and cancer of the uterus.

GENITOURINARY

1 R01 NR012011-01

Translating Unique Learning for Incontinence Prevention: The TULIP Project

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\$300,000

More than one in three US women suffer from the distressing, embarrassing, and often unreported problem of urinary incontinence (UI). A key committee of the 2008 International Consultation on Incontinence concluded that pelvic floor muscle training (PFMT) should be offered as first line therapy to all women with stress, urge, or mixed UI and that bladder training (BT) may be preferred to drug therapy. Conservative strategies are low risk and do not prejudice future treatments. They reasoned that such self-management practices should also prevent UI and conducted a RCT to test a prevention behavioral program. A group session presented an array of conservative self-management practices- PFMT, BT and the Knack Maneuver, which is a preemptive contraction to decrease stress UI and/or suppress urge UI. At 12-months post-intervention they found a two-fold UI prevention effect. Moreover, they found high and sustained adherence: 82% at 3 months post intervention and 68% at 12 months. At four years follow-up, sustained adherence of 70% was predicted by early self-efficacy. This intervention is novel because it enables women to adopt and sustain efficacious bladder health practices for incontinence prevention, whereas to date conservative management approaches have focused on treatment. Based on what they now know, these practices should be part of standard well woman

care, but it is not realistic to expect busy clinicians to provide this information within the confines of a brief encounter. They have developed a 15-minute DVD that is a condensed version of the prevention behavioral session; it is culturally sensitive and has yielded comparable levels of knowledge and self-efficacy. Using two sites (Michigan and Pennsylvania), they aim to compare the outcomes of the group behavioral program to the DVD version by randomizing 600 women aged 55 years and older to two arms of a comparative effectiveness trial. Follow-up will be at 3-months, 12-months, and 24-months post-intervention. (Aim 1). Controlling for age and BMI, they will test the hypotheses: HO1: There will be no difference in UI incidence demonstrated between groups (PRIMARY HO) HO2: There will be no difference in post-intervention self-management adherence between groups HO3: There will be no difference in post-intervention self-efficacy to adopt strategies between groups They will conduct an economic analysis comparing the two-hour session with the DVD version (Aim 2). Describing the costs and analyzing the willingness to pay and employment data will be the primary focus of this study in order to create the foundation for a future cost-effectiveness analysis, should trial hypotheses be confirmed. At 36-months post-intervention, they will conduct interviews to learn which intervention elements contributed to sustainability of adherence (Aim 3). Their long-range objective is to provide a UI prevention intervention suitable for wide-spread translation at the point of well woman care (annual visit).

5 U01 DK058229-10

Urinary Incontinence Treatment Network: DCC

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New England Research Institutes, Inc., Watertown, MA

\$100,000

This proposal is submitted in response to RFA-DK-06-501 for continuation of the Urinary Incontinence Treatment Network (UITN) Data Coordinating Center (DCC) at New England Research Institutes, Inc. The DCC is responsible for the scientific management of the studies, including directing, training, and monitoring the performance of Clinical Centers in enrollment, data collection, and data management as well as for all data analysis, and reports to the DSMB. In Phase I and continuing to Phase II, NERI has provided several unique and innovative tools and capabilities, including a proprietary Web-based data management system, an automated patient randomization system, and an electronic repository for UDS tracings. The DCC is also responsible for network communications and meeting support and provides a secure study website and a public website. DCC scientists play a leadership role in all network activities, including protocol development, standing committees and work groups, manuscript development and presentations. Phase II will focus on conduct of the TOMUS trial as well as continuation of the observational follow-up studies for the SISTER and BE-DRI studies (i.e., E-SISTER and E-BE-DRI) of Phase I. Primary Aims of TOMUS are to compare objective and subjective cure rates for stress incontinence at 12 and 24 months between the retropubic and transobturator midurethral sling procedures. Performance of these procedures is increasing rapidly with limited data available on safety and efficacy. Therefore, this study will compare the efficacy and safety of the retropubic and transobturator (inside-out and outside-in) procedures in a 2-arm RCT; 588 women with stress UI will be enrolled. Primary Aim of E-SISTER is to compare long-term (60 mos.) effectiveness and durability of the Burch colposuspension and autologous fascial sling for treatment of stress UI in a randomized cohort of 655 women. Primary Aim of E-BE-DRI is to examine long-term (26 mos.) durability of the addition of behavioral treatment to drug therapy

for treatment of urge UI in a randomized cohort of 307 women. The UITN is a multi-disciplinary, multi-center group of Investigators dedicated to high impact clinical research regarding the prevention, evaluation and management of UI to improve the quality of life for adults. The UITN is conducting 3 studies of treatments for both stress and urge urinary incontinence.

HIV

5-D43-TW-001039-12

AIDS International Training and Research Program

Adimora, Adaora A.

University of North Carolina Chapel Hill

Chapel Hill, NC

\$20,000

Fogarty trainees are serving in key leadership positions and are in the center of exciting and critical research activities. Working with their collaborating institutions they have assessed the priority health needs of their partner countries and propose a research training program that addresses the countries' research needs as well as the developmental plans of their collaborating institutions. This is the second competitive renewal application for the UNC AIDS International Training and Research Program. They propose to continue to provide training in three countries: The Peoples Republic of China, Malawi and Cameroon. Investigators at UNC have worked in China since 1979, Malawi since 1989, and Cameroon since 1998. The UNC AITRP has embraced several guiding principles. First, they use training to build strong ties to key in-country organizations. Trainees with guaranteed "return jobs" in these organizations are preferentially selected. Second, their training opportunities build on funded research projects and bridge many of the strengths of UNC. Wherever possible they combine basic, clinical and epidemiological training and research in order to build critical mass. Third, they have used the Fogarty training to promote international research, working with many collaborators and funding agencies. Fourth, they have developed south-to-south and international collaborations to facilitate training and ongoing research opportunities. For example, University of the Witwatersrand is a training site for Malawi personnel, and they have developed a strong collaboration with the London School of Hygiene and Tropical Medicine for training of physicians from Malawi (a former British protectorate). Fifth, they have looked for opportunities for evolution and innovation. Such efforts have been particularly important in the development of a new Department of Public Health at the Malawi College of Medicine (which has received dedicated Fogarty support), extensive research ethics and IRB training in China, and rapid technology transfer in all three UNC AITRP countries. Sixth, they are committed to in- country leadership and ongoing mentorship after the trainee has completed their program.

5U01 AI035004-17

Women's Interagency HIV Study (WIHS)

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\$220,869

Early in WIHS, the lower limit of detection for HIV-1 quantification was 4000 copies/ml. Some of these early timepoints have been requantified with LLD of <80 cps/ml, specifically women who initiated HAART during visits 1-7. However, the high LLD of 4000 cps/ml (and those few with LLD of 400 cps/ml) for many samples continues to be problematic as WIHS has initiated investigations into new areas. This has been particularly true for studies of elite suppressors and long-term non-progressors (LTNP) and for cardiovascular studies in which quantitative total exposure to virus cannot be calculated. Further, it has been difficult to find controls for the elite suppressors and LTNPs as their true viral load is not known, nor is the viral load of the potential controls. Therefore, they propose here to measure quantitative HIV-1 RNA for all women-visits at which the viral load is undetectable at 4000 or 400 copies/ml. They propose to do this with Taqman, the current platform for viral load measurements in WIHS. This will allow more precise definition of many categories of participants, necessary in several WIHS investigations, now and in the future.

1 R21 AI082689-01A1

Development of Antimicrobial Peptides as Topical Microbicides

Buckheit, Robert Walter

ImQuest BioSciences, Frederick, MD

\$21,428

They hypothesize that novel anti-HIV and anti-STI topical microbicides based on natural antimicrobial peptides collected in the Antimicrobial Peptide Database developed by the co-PI's laboratory (<http://aps.unmc.edu/AP/main.html>) can be discovered and improved through peptide engineering technology. During the R21 phase, they will methodically screen peptides from the database and define specific inhibitors of HIV and HSV-2 as well as broad based inhibitory peptides. These active agents will be further developed in order to understand their range and mechanism of anti-HIV action. Superior peptides identified in SA1 will be characterized in SA2 to provide a rationale for continued development in SA3 using various molecular strategies which will result in the improvement of the therapeutic index of the peptide agents, with and without other small molecule microbicides, in order to begin development of an effective microbicide product. This product will be formulated and evaluated in animal models and safety assessment studies in the R33 portion of the project. Their goal is to produce a female controlled preventative agent which can be utilized to prevent the sexual transmission of viral, bacterial and fungal organisms with a focus on inhibiting the transmission of HIV. The research data will be entered into the existing antimicrobial peptide database to facilitate the use by funding agencies, other researchers, students and the public. Project Narrative: Over 25 million people have died since the first case of AIDS was identified in 1981, and the number of people living with HIV worldwide continues to expand - from 35 million in 2001 to an estimated 40 million in 2007. Almost 5 million people worldwide became newly infected with HIV and an estimated 3.8 million human deaths were attributed to AIDS in 2007. They propose to identify HIV-1 and HSV-2 inhibitory antimicrobial peptides which are naturally produced by mammals through evaluation of peptides which are identified in the Antimicrobial Peptide Database developed at The University of Nebraska Medical Center. They intend to discover and develop inhibitors of HIV and other sexually transmitted infectious organisms for use as an effective topical microbicide product.

5 R21 AI079772-02

Development of a Novel Semen-Activated Prodrug as an Anti-HIV Microbicide

Buckheit, Robert Walter

ImQuest BioSciences, Frederick, MD

\$37,500

The S-acyl-2-mercaptobenzamide thioester (SAMT) inhibitors are low molecular weight compounds which target multiple steps in the HIV replication pathway, but primarily function to specifically inactivate cell-free HIV immediately upon exposure to the reactive compounds and to suppress the production of infectious HIV from virus-infected cells. These NCp7-targeted, virus inactivating compounds act by stripping coordinated zinc ions from the nucleocapsid (NC) protein in the infectious virion or maturing virus particle. In the process, the compounds irreversibly cross-link the nucleocapsid proteins rendering the virion noninfectious and defective. Thus, the NCp7 inhibitors interfere with two potential virus transmission mechanisms required for the infection of target cells in the vaginal environment. In the R21 phase of this proposal they propose to develop new microbicides composed of polymeric prodrugs for delivery of the SAMTs. This delivery mechanism limits the tissue absorption of the SAMT until it comes in contact with the viral inoculum in semen by attaching it to a high molecular weight biocompatible polymer. They will conjugate the SAMT inhibitors to the polymer carrier through enzyme-cleavable linkages that will release the active drug product in the presence of specific enzymes in semen. This delivery approach offers several advantages in the context of microbicide action since (1) the NCp7 inhibitors can inactivate cell-free and cell-associated virus in semen, they will target the virus before it can diffuse in an infectious form to or into tissue, (2) they will add moieties to the polymer backbone that will increase the stability of the SAMT inhibitors by decreasing the pH local to the conjugated drug by the Donnan effect, and (3) since microbicides will be used by women repeatedly over many years, a polymeric prodrug approach will allow precise control over the tissue concentrations and exposure to anti-HIV compounds, limiting the chance to develop viral resistance and limiting toxicity. Critical to the development of this prodrug approach, biological evaluations will be performed to confirm the efficacy of the SAMTs in the presence of seminal plasma and vaginal fluids. Additionally, the enzymatic activation of the compound from its prodrug form will be evaluated in specially designed in vitro assays to mimic the events which must occur in the vagina and to quantify the kinetics of drug activation and virus inactivation in the presence of semen and other appropriate biological matrices. Finally, the biological properties of both semen and vaginal fluids on the efficiency of transmission of HIV to target cells will be evaluated to define the potential synergies between the antiviral activity of constituents of semen and the biological activity of the thioester inhibitors.

5-D43-TW-001042-12

Emory AIDS International Training and Research Program

Del Rio, Carlos

Emory University, Atlanta, GA

\$20,000

Located in Atlanta, the Emory AIDS International Training and Research Program (AITRP) has established itself as an interdisciplinary training environment, that is producing highly qualified HIV/AIDS researchers. The collaborating countries of the Emory AITRP proposed for this application are Mexico, Georgia, Vietnam, Rwanda and Zambia. The specific aims of the research training program include: 1. To build academic capacity in partner countries through the

support of in-country education and training. 2. To build HIV/AIDS research human resource capacity through the support of degree-seeking, long-term training. 3. To fill identified gaps in partner country research training capacity through the provision of specialized medium and short-term training. 4. To build in-country capacity to conduct implementation science research that will allow their trainees to become involved in the evaluation of the impact of a variety of interventions that are currently occurring in their collaborating countries such as PEPFAR.

1-R21-AI-088601-01

Targeted siRNA Delivery as An Anti-HIV Microbicide

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\$21,428

Human immunodeficiency virus (HIV) is a highly lethal lentivirus which over a protracted course destroys the host's adaptive immune system leaving them vulnerable to numerous opportunistic infections. Unlike most viruses whose genome replicates independently of the host cell's genome, the HIV-1 genome integrates into and is replicated with the host genetic material. Therefore, even if therapeutic approaches can inhibit new virus production, the viral genome remains intact and competent. Therefore, strategies that can prevent the uptake and integration of the virus would be of tremendous clinical value. The vast majority of HIV infections occur as a consequence of viral transmission through mucosal surfaces, such as the vaginal mucosa. The delivery of siRNAs that specifically silence host factors required for early events in the HIV life cycle to lymphocytes in the vaginal mucosa could prove to be an effective means of protecting individuals from HIV infection and serve as a potential microbicide. One of the main challenges facing the clinical application of siRNAs as a genetic therapy is the ability to deliver siRNAs to the cytoplasm of the appropriate target cell types. They have recently developed a novel lipid nanoparticle that is coated with an antibody recognizing the integrin molecule LFA-1 which is broadly expressed on lymphocytes. These immuno-nanoparticles will be used to deliver siRNAs to lymphocytes present in the vaginal mucosa of humanized mouse models of HIV. Given the high level of sequence heterogeneity, the propensity of HIV-1 to mutate and the inability of anti-HIV siRNAs to target the incoming viral RNA genome and prevent integration, alternative therapeutic targets are required to prevent the transmission of HIV. Host factors that are necessary for early events in the HIV lifecycle but are dispensable for cellular functioning could prove to be an effective therapeutic alternative. Using a high-throughput RNA interference-based screening platform, they have identified a large number of potential therapeutic targets that could serve to inhibit HIV integration when silenced. However, these factors require extensive analysis and characterization to ensure their safety and efficacy. They will be combining the LFA-1-mediated cell-type specific vehicle to introduce siRNAs targeting therapeutically relevant host factors as a potential means to inhibit viral infection in humanized mouse models of HIV. These experiments will provide the preclinical groundwork necessary for the development of an effective RNAi-based anti-HIV microbicide. Heterosexual transmission is the leading cause of new HIV infections in the world. A microbicide providing true intracellular immunity would make a significant contribution to controlling the spread of this deadly virus.

1 R21 AI088586-01

Development of a novel nanoparticle pyrimidinedione vaginal polymeric film as an

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\$21,428

Pyrimidinediones (PYD) are highly potent small molecule inhibitors that have a dual anti-HIV mechanism of action: viral entry inhibition and non-nucleoside reverse transcriptase inhibition (NNRTI). The PYD compounds have shown in vitro subnanomolar levels of activity as an NNRTI and nanomolar levels of activity as inhibitors of entry occurring prior to chemokine receptor binding and fusion. However, as microbicides compounds are being developed, delivery issues that are part of the formulation of the compound have lagged behind causing a critical delay in product development. Due to low solubility and poor penetration through the mucosa to the target site of action, Pyrimidinediones face significant obstacles as microbicides. Strategic drug delivery design is essential for Pyrimidinediones to advance as viable microbicide products. They propose a combination of innovative drug delivery strategies to enhance PYD anti-HIV efficacy through polymer biochemistry formulations. Specifically, nanoparticle encapsulation has been used to overcome many of the challenges presented when using hydrophobic drug molecules; however, its use as a vaginal drug delivery system has not been investigated. In the R21 phase of this project, they propose to develop nanoparticle encapsulation of PYD as a novel drug delivery method to improve the potency of HIV inhibition activity by increasing long term drug release, protecting against enzymatic degradation, enhancing submucosal tissue penetration and cell localization. Additionally, they propose to further formulate the nanoparticle PYD formulation into a vaginally delivery polymer film dosage form. Such "quick dissolving" solid dosage forms have recently been proposed as an innovative alternative to address several acceptability and compliance issues observed in more traditional vaginal delivery systems (gels, creams, intra-vaginal rings). Their nanoparticle PYD film delivery approach offers several innovative advantages in microbicide development by suggesting enhanced apparent activity without active pharmaceutical ingredient (API) reformulation, conferring HIV protection over long periods of time through controlled drug release, making such a microbicide coitally-independent, and introducing a novel drug delivery method through vaginal films that addresses many of the acceptability issues with gels and other semi-solid dosage forms. Biological characterization and evaluation will be performed to confirm the efficacy of PYD nanoparticles in biologically relevant conditions. The encapsulation of PYD into biodegradable nanoparticles will be characterized and evaluated in specifically designed in vitro assays to determine drug targeting and release. Additionally, the anti-HIV efficacy of the nanoparticle PYD will be compared to unformulated PYD in biologically relevant in vitro assays to determine the optimal formulation. Finally, the formulation will be introduced into a solid vaginal film dosage form to evaluate its biological properties in HIV prevention.

5-D43-TW001038-12

AIDS international Training and Research Program

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\$20,000

The proposed Pitt AITRP training will substantially enhance the ability of Brazil, Mozambique, and India to conduct crucial HIV prevention research. They propose to continue the AIDS

International Training and Research Program (AITRP) at the University of Pittsburgh (Pitt). Their mission is to provide Brazilian, Indian, and Mozambican health professionals with multidisciplinary tools needed to conduct cutting-edge HIV prevention research in their countries. The Director and Co-Director are, respectively, Dr. Lee Harrison, Professor of Epidemiology and Medicine, and Dr. Phalguni Gupta, Professor of Infectious Diseases and Microbiology. An exciting change in their program is the addition of a site in Beira, Mozambique, which has striking training needs and where Pitt has established close collaborations with the Universidade Catolica de Mozambique. The addition of Mozambique and the training of a large cadre of well-trained Brazilian investigators over the past ten years allow us to dramatically reduce their training efforts in Brazil and shift resources to Mozambique. As a component of their training program, they will leverage the extensive training already provided to Brazil by conducting south-to-south training between these two Portuguese-speaking countries. Ongoing research in Brazil includes HIV vaccine trials, studies of effectiveness of antiretroviral therapy in public clinics, and changes in causes of death among HIV-infected patients. In India, ongoing projects include studies of genetic heterogeneity of Indian HIV strains, CDS suppression of HIV, HIV incidence studies to identify high-risk populations, and development of a novel *Clostridium perfringens*-based oral HIV vaccine. Research at their new site in Mozambique is currently limited and they will use the training provided by the Pitt AITRP to jump start a much-needed research agenda there. Trainees from all three countries will have access to the substantial HIV research activities at Pitt, including research in epidemiology, behavioral sciences, and laboratory sciences. During the next five years, they propose to establish an extensive training program in Mozambique; provide limited, selected training for Brazil; and provide laboratory and behavioral sciences training for India. Their successful track record during the first 10 years, the excellent training opportunities they propose, and collaboration with key institutions in their three countries assure that their program will continue to be highly productive.

1-R21-AI-082680-01A1

Phosphorothioate Oligonucleotides as Microbicides against HIV Transmission

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\$21,428

Developing interventions that inhibit the transmission of HIV infection are critical for halting the HIV epidemic. Topical prevention strategies usually termed microbicides have been proposed as one strategy to halt or slow down the HIV epidemic. They have identified novel lead microbicides that potently inhibit HIV and SIV infection/replication *in vitro*. During their previous submission they reported an oligonucleotide with a phosphorothioate backbone (OPB) that could inhibit HIVBaL or SIVmac251 infection and/or replication in human or simian PBMC, respectively. OPB also inhibited infection/replication in cell-free infections of P4-R5 MAGI cells by HIVBaL and HIVIIIIB. OPB exhibited no toxicity against PBMC or P4-R5 MAGI cells after 24h continuous exposure. Preliminary data suggested that OPB may also inhibit other viruses as it was also effective against influenza type A virus. Thus, their first generation OPB may be a potent microbicide against HIV that prevents infection at mucosal sites when topically applied. Their preliminary studies were carried out with a 13mer Poly T or Poly A oligonucleotide of OPB and this suggested that the effect was sequence independent and may even be mediated by the phosphorothioate deoxyribose sugar backbone. Indeed in their current

re-submission they present data on their next generation compound, a baseless phosphorothioate 2' deoxyribose backbone (PDB) that has more potent HIV inhibitory activity than OPB. A 14mer PDB they show here has no toxicity, is a potent inhibitor of HIV and has the advantage of being a TLR7/9 antagonist that inhibits HIV-induced IFN γ production. This later property is important as the establishment of HIV infection may depend on HIV-induced mucosal inflammation triggered by TLR. Importantly, they show that PDB is active when formulated in hydroxyethylcellulose (HEC) gel at pH 4.4, survives pH transition to a neutral pH, and in retains its activity in HEC for long periods. They hypothesize that PDB binds enveloped viruses and inhibits their infectivity by acting as a "chemical lectin". They further hypothesize that PDB can act as a microbicide against HIV and can prevent SIV vaginal infection of rhesus macaques. The studies planned in the R21 phase will further optimize and characterize the safety and effectiveness of PDB in vitro and its safety in the Swiss Webster mouse vaginal/cervical model of irritation. They will determine the optimal size and composition that remains effective against HIV and exhibits no toxicity. Finally, the mechanism of action of PDB will be investigated, the effect of inclusion into hydroxyethylcellulose gel will be tested and PDB's effect on the growth of commensal lactobacilli will be determined. Five specific milestones have been set for the progression from the R21 Phase to the R33 Phase. The R33 phase will test the effectiveness of PDB in preventing vaginal SIV infection, investigate the effect of seminal plasma and pH transition on the efficacy of OPB, determine its safety with human genital epithelial tissue, and investigate its effectiveness against HSV-2. The current application will allow for an extensive evaluation of PDB as possible novel microbicide candidates. The studies proposed here address the important public health problem of developing treatments that inhibit the transmission of HIV infection. The current application investigates a novel chemical that may be used to inhibit infection with HIV.

5 R21 AI082738-02

Microbicide delivery system to target lymphoid organs

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\$37,500

Sexual transmission of HIV-1 involves complex processes involving exposure of the female genital tract to virus or infected cells and their transport to other sites, including local lymph nodes, where the virus replicates and establishes infection. It has been shown that Langerhans cells (LC) and dendritic cells (DC) capture the virus either from the vaginal surface or from top epithelium layers and transport it to draining and local lymph nodes, where it infects CD4+ T-cells. Intense development of topical microbicides is underway with the ultimate goal of decreasing the sexual transmission of HIV-1. Current efforts have been directed to inactivating the virus either at the surface of the vagina before entry, or in the squamous or stroma layers of the vaginal epithelium. Their physical transport modeling predicts that molecular drugs delivered as topical gels cannot reach draining or local lymph nodes. One possible way to deliver drugs to lymphoid sites surrounding the vagina is to use drug-loaded nanoparticles. Their preliminary results provide evidence indicating that nanoparticles can be delivered to local lymph nodes via vaginal application in a mouse model. To further develop this platform for use as a microbicide or prophylactic strategy, they propose the following plans for the R21/R33 application. In the R21 phase, they will study the delivery of quantum dots having different surface chemistry, including conjugation with targeting molecules, to determine the mode of their transport to

different lymphoid sites. In the R33 phase, they will use drug-loaded nanoparticles and verify the applicability of this platform to target important sites in the female genital tract. Physical models will be developed to understand the transport processes and to guide the development of the nanoparticle delivery system.

5 R21 AI079771-02

Small-molecule inhibitors of gp41-mediated fusion as HIV-1 topical microbicides

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\$37,500

In the continuing absence of an effective vaccine, topical microbicides offer a credible alternative preventive strategy to reduce sexual transmission of HIV-1. Several viral fusion and entry inhibitors have been shown to prevent SHIV infection of rhesus macaques by the vaginal and/or rectal routes and are in preclinical and early clinical development as microbicide candidates. HIV-1 membrane fusion is mediated by a series of large-scale structural transitions in the gp41 envelope glycoprotein. Evidence indicates that a transient gp41 species known as the prehairpin intermediate is a potential target for drugs that inhibit HIV-1 entry. The long-term goal of this research plan is to use modern molecular and structural methods to identify and develop a novel small-molecule gp41 fusion inhibitor for inclusion in a topical HIV-1 microbicide. To achieve this, they will capitalize on specific surface features revealed by their recent structure determination of an autonomously folded, trimeric coiled-coil subdomain of gp41 that provides an atomic model for the putative prehairpin conformation, as well as small-molecule lead compounds developed by means of an innovative structure-based drug design technology. They propose the following specific aim for the R21 component of this project: 1) To identify and optimize two series of novel small-molecule compounds that inhibit HIV-1 membrane fusion by targeting the gp41 prehairpin intermediate. They will design and synthesize two sets of analogs of active triazinone and biphenyl compounds, characterize the equilibrium properties of interactions with the N-trimer coiled coil, and evaluate their anti-HIV-1 activity and mechanism of action. Bound inhibitors will be visualized by x-ray crystallography in order to allow refinement of binding affinity. The specific aims of the R33 phase of the project are: 2) To characterize the specificity, potency and toxicity of improved small-molecule compounds with enhanced gp41 inhibitory activity. They will conduct in vitro studies in primary cells and human cervicovaginal tissue explants to determine the virucidal activity of select small-molecule gp41 inhibitory compounds against diverse primary HIV-1 isolates, and their potentially toxic or inflammatory effects. They will also use the rabbit vaginal irritation model to evaluate the irritation potential of the fusion inhibitors. 3) To assess the in vivo potency and breadth of activity of optimized small-molecule fusion inhibitors alone and in combination with entry inhibitors targeting HIV-1 gp120 (BMS-378806) and CCR5 (CMPD167) using the NOD/SCID-hu BLT mouse vaginal transmission model. They will evaluate the protection of humanized BLT mice from vaginal challenge with multiple HIV-1 variants by small-molecule fusion inhibitors alone and in synergistic combination with BMS-378806 and CMPD167. Their emphasis is to identify a new class of potent HIV-1 fusion inhibitors suitable for development as a component of a microbicide formulation.

1 R21 AI088585-01

Plant-produced Actinohivin as a Candidate HIV Microbicide

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\$21,428

Safe, effective, and inexpensive topical microbicides are urgently needed to curb the global human immunodeficiency virus type-1 (HIV-1) epidemic. Actinohivin (AH) is an actinomycete-derived lectin. This lectin specifically binds to high-mannose clusters uniquely found on the HIV-1 envelope (Env), thereby eliciting nanomolar antiviral activity against multiple HIV strains. Preliminary analyses revealed that AH has a high safety profile in human peripheral blood mononuclear cells (PBMCs) and in the rabbit vaginal irritation assay. Meanwhile, a translational AH-AH fusion protein (recombinant dimer [rd] AH) was suggested to have stronger and broader anti-HIV-1 activity than the original monomer. Given these high potentials, they hypothesize that rAH and/or rdAH (r/rdAH) are excellent HIV-1 microbicide candidates. This project's goal is to reveal the feasibilities of r/rdAH in terms of manufacture, antiviral efficacy, and safety upon use as a vaginal microbicide. In the R21 phase, they will initially focus on developing a highly efficient, scalable production system for r/rdAH that allows for extensive efficacy and safety studies and possible global use. They will utilize recombinant plant virus-based expression systems and various molecular biological approaches for rapid and high-level expression of high-quality r/rdAH. Upon obtaining bulk r/rdAH active pharmaceutical ingredients with high purity standards, they will analyze HIV-1 neutralization effects against selected R5-type viruses in two in vitro HIV neutralization assays based on Env-pseudotyped virus-reporter gene expression and primary isolate- PBMC infection systems. Next, r/rdAH' cytotoxic, mitogenic, and inflammatory potentials will be tested in PBMCs and/or human cervicovaginal (CV) epithelial cell lines to establish the minimal safety profile. Their success criteria in the R21 phase are: (1) establishing the bulk preparation procedure; (2) demonstrating cross- clade antiviral effects to R5 viruses; and (3) demonstrating no apparent in vitro cytotoxicity, mitogenic activity, or inflammatory potential at >100 times above an average anti-HIV IC50, for plant-made r/rdAH. Upon approval of their transition to the R33 phase, they will comprehensively analyze anti-HIV-1 efficacy of r/rdAH for various modes of HIV-1 infection and transmission, using various in vitro assay systems. In addition, they will investigate potential overlap, complementation, synergy, and antagonism of anti-HIV activities between r/rdAH and other inhibitors toward potential microbicide combination strategies. Finally, they will perform extensive evaluations of r/rdAH upon vaginal application in rabbit and mouse models. They will thoroughly evaluate r/rdAH' vaginal toxicity, inflammatory potential, and stability. Upon determining the maximal tolerated dose of r/rdAH, they will examine their potential immunogenicity and toxicity after a long-term exposure. Potential toxicity to the symbiotic vaginal commensal bacteria, the *Lactobacillus* species, will be examined. In summary, the proposed studies should answer the question of whether r/rdAH is justified for advanced next-stage preclinical studies. . The proposed studies will analyze the feasibilities of the novel HIV-1-binding lectin Actinohivin and its derivative recombinant dimer, as a candidate vaginal HIV-1 microbicide. The proposed studies should generate a comprehensive data set that will reveal their large-scale producibility, anti-HIV-1 efficacy, and broad toxicity profile upon vaginal application, thereby providing criteria of whether Actinohivin and its derivative are justified for further extensive preclinical and clinical studies.

1 UH2 AI083264-01

The Microbial Ecology of Bacterial Vaginosis: A Fine Scale Resolution Metagenomic (The Human Microbiome Project)

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\$125,000

Bacterial vaginosis (BV) is the most common vaginal disease in women, and yet its cause and effective treatment remain unknown. BV is associated with many adverse health outcomes, such as preterm delivery of low birth weight babies and increased risk for infection by HIV. This research will contribute valuable information on the causes of BV, help develop improved methods for preventing and treating BV, and may help reduce major reproductive health problems associated with BV. The vaginal microbiota play an important protective role in maintaining the health of women. Disruption of the mutualistic relationship that exists between bacterial communities in the vagina and their hosts can lead to bacterial vaginosis (BV), a condition in which lactic acid producing bacteria are supplanted by a diverse array of strictly anaerobic bacteria. BV has been shown to be an independent risk factor for adverse outcomes including preterm delivery and low infant birth weight, acquisition of sexually transmitted infections and HIV, and development of pelvic inflammatory disease. National surveys indicate the prevalence of BV among U.S. women is 29.2%, and yet, despite considerable effort, the etiology of BV remains unknown. Moreover, there are no broadly effective therapies for the treatment of BV, and reoccurrence is common. In the proposed research they will test the overarching hypothesis that vaginal microbial community dynamics and activities are indicators of risk to BV. To do this, they propose to conduct a high resolution prospective study in which samples collected daily from 200 reproductive-age women over two menstrual cycles are used to capture molecular events that take place before, during, and after the spontaneous remission of BV episodes. They will use modern genomic technologies to obtain the data needed to correlate shifts in vaginal microbial community composition and function, metabolomes, and epidemiological and behavioral metadata with the occurrence of BV to better define the syndrome itself and identify patterns that are predictive of BV. The five specific aims of the research are: (1) Evaluate the association between the dynamics of vaginal microbial communities and risk to BV by characterizing the community composition of vaginal specimens archived from a vaginal douching cessation study in which 39 women self-collected vaginal swabs twice-weekly for 16 weeks; (2) Enroll 200 women in a prospective study in which self-collected vaginal swab samples and secretions are collected daily along with data on the occurrence of BV, vaginal pH, and information on time varying habits and practices; (3) Determine the gene content (metagenome) of vaginal microbial communities to assess the metabolic potential of representative vaginal communities in women before, during, and after the spontaneous remission of BV; (4) Characterize suites of expressed genes (metatranscriptome) in communities representative of vaginal community types in healthy women, as well as before, during, and after the spontaneous remission of BV; and (5) Apply model-based statistical clustering and classification approaches to associate the microbial community composition and function, with metadata and clinical diagnoses of BV. The large body of information generated will facilitate understanding of vaginal microbial community dynamics, the etiology of BV, and drive the development of better diagnostic tools for BV. Furthermore, the information will enable a more personalized and effective treatment of BV and ultimately, prevent adverse sequelae associated with this highly prevalent disruption of the vaginal microbiome.

1 R21 AI088597-01

Engineering antiviral innate immunity for safe and effective microbicides

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\$21,428

HIV infections afflict millions of people and cause tremendous health and economic burdens. One of the major risk factors for HIV-1 transmission is the pre-existing infections caused by sexually transmitted agents such as herpes simplex virus type 2 (HSV-2). Therefore, a rational prevention strategy to halt HIV spread is to target HSV-2 infection and control its spread. In the absence of vaccines against HSV-2, a more practical and effective intervention for HSV-2 is the utilization of microbicides. A promising microbicidal approach is to potentiate antiviral innate immunity effective against a broad range of viruses at the site of viral encounters. The toll-like receptor (TLR)-based innate immunity have been shown to be crucial in initiating a cascade of antiviral activities mediated by type I interferons (IFNs). Both TLR3 and TLR9 agonists, polyinosinic: polycytidylic acid (poly IC) and CpG oligonucleotides (ODNs) are effective in protection against HSV-2 infections. However, undesirable inflammatory responses and autoimmunity accompanying the non-specific stimulation of TLRs are of major concern, which could severely limit the use of TLR agonists as microbicides. Thus, the key to developing TLR agonists as microbicides is to target them to relevant cell types at the potential sites of viral exposure and to elicit IFN responses in a regulated fashion. They propose to develop localized, controlled-release, and cell-targeted delivery systems to regulate the stimulation of TLR-based innate antiviral immunity. In the R21 Phase, three aims will be accomplished: Aim 1: to design and characterize cell-targeted delivery systems based on poly (lactide-co-glycolide) (PLGA) nanoparticles to specifically and locally target pDCs and epithelial cells with TLR agonists; Aim 2: to evaluate the effectiveness against genital HSV-2 infections by locally and selectively targeting CpG ODNs and/or poly ICs to pDCs and epithelial cells with cell-targeted nanoparticles; Aim 3: to evaluate toxicity by locally and selectively targeting CpG ODNs and/or poly ICs to pDCs and epithelial cells with cell-targeted nanoparticles. Built upon the results from the R21 phase, in the R33 phase, they will accomplish: Aim 4: to design and characterize delivery systems for sustained release of TLR agonists; Aim 5: to evaluate the effectiveness against genital HSV-2 infection and toxicity by localized, sustained-release and cell-targeted nanoparticles loaded with CpG ODNs and/or poly IC; Aim 6: to evaluate the adaptive immunity against genital HSV-2 infection mediated by localized, sustained-release and cell targeted nanoparticles loaded with CpG ODNs and/or poly IC. This application will enable the translation of TLR-based antiviral innate immunity to effective and safe microbicides.

1 R21 AI088595-01

Exploring the Role of Vif Antagonists in Preventing Sexual HIV Transmission

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\$21,428

Since it has proven difficult to develop a vaccine against HIV-1, the major cause of the AIDS pandemic, the research community has shifted some of its focus to the development of topical microbicides. Since both the vaginal and rectal tract are portals of HIV-1 entry, topical microbicides suitable to protect both sites need to be developed. In this grant, they focus on a novel mechanism that has not previously been explored for HIV prevention. In 2002, it was

found that the cellular target of the HIV-1 protein Vif is APOBEC3G (A3G). A3G is an enzyme of the AID/APOBEC family, characterized by the targeted deamination of cytosine to generate uracil within DNA. APOBEC3G plays an important role in retroviral defense by acting on viral reverse transcripts and mediates numerous critical immune responses. They believe that A3G is an important innate retroviral defense mechanism in the vaginal and rectal tract. By using inhibitors of the viral protein Vif, the Vif-APOBEC3G interaction is blocked and APOBEC3G is not degraded by the proteasome. As a consequence, fatal hypermutations are introduced into the viral cDNA transcripts and HIV is rendered incompetent for replication. Their grant has four specific aims: Specific Aim 1: Explore the role of the restriction factor A3G in mucosal tissues of the vaginal and rectal tract Specific Aim 2: Examine whether RN18 and its analogs are active in microbicide cell-based assays and ex vivo explant HIV transmission models Specific Aim 3: Vaginal humanized BLT mouse model testing of promising Vif inhibitor candidates Specific Aim 4: Macaque microbicide model testing of promising Vif inhibitor candidates It is expected that these studies will define the role of A3G in the vaginal and rectal tract and whether inhibitors of the viral Vif protein can prevent sexual transmission of HIV.

5 D43 TW001035-12

Vanderbilt University-Cidrz AIDS International Training and Research Program

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Vanderbilt University, Nashville, TN

\$20,000

The Vanderbilt University (VU)- Center for Infectious Disease Research in Zambia (CIDRZ) training partnership with their international collaborators is designed to strengthen both institutional and individual biomedical and behavioral research capacities focused on HIV-related research in both prevention and care in developing countries. The VU-CIDRZ AITRP, formerly the VU-University of Alabama at Birmingham AITRP, seeks renewal of its grant, now in its tenth year due to an NIH-initiated one-year extension. They contribute research training to both institutional and individual biomedical and behavioral research capacities focused on HIV-related research in both prevention and care. The VU-CIDRZ training partnership with their international collaborators is designed to train foreign scientists and key research support staff to conduct independent research and training in their home countries, as well as perform at an internationally credible level in collaborations with local and foreign scientists. They now seek to renew their AITRP with a continued focus on Zambia (since 1998), Pakistan (since 1994), India (since 2000), China (since 2000), and their newest partnership in Mozambique (VU training partnership since 2006 and developmental AITRP engagement since 2007). They have completed their older training commitments in Mongolia, Jamaica, and Russia and will complete their training commitments for Bangladesh upon the graduation of a current doctoral training (anticipated in 2011). They have restricted their AITRP training partnerships to five focus cities in order not to dilute their impact to where they have funded overseas research and strong research training partners. At the same time, they have leveraged support in each of the five venues such that their AITRP resources will go much further than permitted by the grant's funding alone. They will continue to provide a diverse portfolio of long, medium, and short-term training options. To date 58 trainees have received graduate degrees, 97% of whom have returned to work in their home countries, 8 are currently in degree programs and over 2,000 have been trained through their in-country advanced short- courses. They believe VU remains an ideal university partner for this initiative for several significant reasons. The migration of the training

program to VU offers the opportunity for trainees to receive the absolute highest quality of graduate training and exposure to innovative HIV/AIDS/STD/TB related research, resources, and faculty mentors. The program is uniquely positioned within the infrastructure of the VU Institute for Global Health (VU IGH), directed by Dr. Vermund with its "center-without-walls" philosophy that nurtures noncompetitive partnerships among and within VU and with partner institutions around the globe. They feel that the innovative features of their renewal and their proven track record address the unmet needs in international AIDS training.

5 R21 AI082701-02

Gp340 and syndecan inhibition based microbicide for HIV

Weissman, Drew

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\$37,500

Education and microbicides active against HIV represent the best approaches to controlling the epidemic worldwide in the absence of a protective vaccine. Their research program studies the earliest events in genital tract transmission. They have identified a protein expressed by genital tract epithelial cells that could serve as a potential target for inhibition of transmission of HIV called gp-340. They have demonstrated that gp-340 is expressed on the cell surface of vaginal and cervical epithelial cells, in vivo, in vitro, and ex vivo and binds HIV envelope. Of significance to genital tract transmission, gp-340 binding of virus leads to an increase in both the infectivity and half-life of the virus. Gp-340 expressed by genital tract tissue and cell lines also mediates transcytosis of HIV, the vesicular transport of macromolecules from one side of a cell to the other. A second molecule called syndecan has been studied and shown to have similar trans-infection and transcytosis properties and is also expressed by genital tract cells. They have identified a peptide inhibitor of envelope binding to gp-340 that blocks both gp-340 mediated trans-infection and transcytosis in in vitro and ex vivo models of genital tract transmission. This peptide contains a portion of a motif that inhibits syndecan mediated transinfection, as well, and they will modify this peptide to inhibit envelope binding to both macaque gp340 and syndecan and develop it into a microbicide. This potential role of gp-340 and syndecan to act at a stage of infection after delivery to the lumen of the genital tract but prior to interaction with and infection of target cells is very attractive and novel in microbicide design. They hypothesize that interfering with this process will inhibit or block genital tract transmission. In the initial R21 portion of this proposal, they will establish in vitro macaque systems of genital tract transmission. If they demonstrate that macaque gp340 and syndecan mediate trans- infection and transcytosis and V3 loop derived peptides or improved versions block macaque gp340 and syndecan mediated transinfection and transcytosis, they will proceed with the R33 portion of the grant. The specific aims of this are: microbicide development with in vitro testing and to test the effect of blocking gp340 and syndecan-HIV Env interaction on genital tract SIV transmission in the rhesus macaque vaginal transmission model. Through these specific aims, they will develop a new type of microbicide and determine the role of genital tract gp-340 and syndecan in HIV transmission. If successful, these studies will deliver a new microbicide based on host cell interactions with HIV that promote genital tract transmission to preclinical trial studies.

5 U01 AI034994-17

Washington Metropolitan Women's Interagency HIV Study

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George Washington University

Washington, DC

\$100,000

The Washington Metropolitan WINS (WMW) Consortium has enrolled and retained a representative cohort of HIV infected and HIV uninfected women since 1993 with the purpose of investigating the consequences of HIV infection and its treatment. Although significant progress has been made in both their understanding and treatment of HIV, curative therapy is still not available and the chronically administered complex therapies used to treat HIV are not always successful. Treatment with highly active antiretrovirals (HAART) appears to be associated with a wide range of adverse effects and the impact of other co-pathogens such as HPV and HCV has yet to be fully elucidated. Additionally, the early cohort of infected women is aging, and the effects of age and changes in sex steroids both on the long term outcomes of HIV, on neurocognition and the effects of HAART treatment needs investigation. The WMW has joined with centers around the country and with sites across the metropolitan Washington region to develop a scientific plan to address these issues. A successful and flexible infrastructure has been established to allow us to accomplish these scientific aims and to assure ongoing retention of this important cohort. The WMW has successfully participated in all elements of the WIHS protocol, and has actively supported the infrastructure of the national WIHS. WMW investigators have participated in all of the major WIHS scientific initiatives. Additionally, the WMW has established both a local specimen repository and contributes to the national specimen repository. As the study has matured, an increasing number of collaborations have been established with local investigators to allow for broader access to the rich repository of WIHS specimens. Further, the WMW has expanded its local epidemiologic expertise to allow for on site data analyses. This application will describe both their accomplishments to date and the structure that they have established to 1) advance the scientific agenda as outlined in Part A, 2) to continue to expand their local collaboration in order to better define the status of women with HIV, and 3) to bring to fruition the promises of a sustainable treatment of this devastating disease.

IMMUNITY/AUTOIMMUNITY

5R21HL093181-02

Role Of 15-Lipoxygenase In Enhanced Pulmonary Vasoconstriction In Females

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Medical College Of Wisconsin

\$190,000

While relatively rare, idiopathic pulmonary arterial hypertension is a medically significant disease that occurs more frequently in young women. The disease is usually catastrophic for those afflicted. Identifying endogenous pulmonary factors that may predispose females to the development of pulmonary hypertension is timely and important considering the abundance of clinical data indicating sex differences in vascular disease. Furthermore, this work is intended to advance new concepts in women's health research and the study of sex/gender differences. Pulmonary arterial hypertension encompasses a group of diseases characterized by high

pulmonary artery pressure and pulmonary vascular resistance. Vasoconstriction, vascular remodeling and thrombosis all contribute to the increased vascular resistance. Central to the proposed studies is that while relatively rare, idiopathic pulmonary arterial hypertension is a medically significant disease that occurs more frequently in young women. The disease is usually catastrophic for those afflicted. Mechanisms to explain the sex- difference in pulmonary arterial hypertension have not been well studied. The main focus of the current proposal is to use a rabbit model to explore the role of sex in a novel signaling pathway that regulates pulmonary vascular tone. Results will lay the fundamental conceptual groundwork for future studies to understand more completely the pathogenesis of pulmonary hypertension in women. Furthermore, this work is intended to advance new concepts in women's health research and the study of sex/gender differences. Specifically, their research provided the first evidence that in pulmonary arteries obtained from female rabbits, endothelium-dependent contractions to both arachidonic acid and methacholine were enhanced when compared to responses in males. Pharmacological studies with inhibitors of arachidonic acid metabolism indicated that the factor was a lipoxygenase metabolite. They also present the first data that lipoxygenase metabolites are increased in females compared to males and the protein expression of 15-lipoxygenase is greater in female pulmonary arteries. While sex differences in vascular responses to various vasoactive agents have been documented, no studies have investigated the role of sex differences on lipoxygenase metabolism of AA in pulmonary arteries. This proposal is designed to explore the specific hypothesis that differences in AA metabolism by 15-LO contribute to the increased endothelium-dependent pulmonary vasoconstriction in females compared to males. To further develop this novel hypothesis, studies will be performed in pulmonary artery vascular preparations using chemical, biochemical, physiological and pharmacological approaches. Two specific aims will be explored: 1) To chemically identify and biologically characterize the vasoconstrictor 15- lipoxygenase metabolite(s) produced by the rabbit pulmonary artery endothelium and 2) To examine the cellular mechanisms contributing to enhanced 15- lipoxygenase expression in females compared to males. These proposed studies will not only provide new insights into the role of endogenous arachidonic acid-derived factors in the pathogenesis of pulmonary arterial hypertension but will also advance their knowledge in women's health research by identifying possible mechanisms that contribute to sex-related differences in the incidence of pulmonary arterial hypertension.

5R21AI083894-02

Role of Sex Differences In The Expression & Function Of Regulatory T Cells In Sle

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\$231,000

They propose to study regulatory T cells (CD4 and CD8) (comparing male to female SLE patients and male to female healthy individuals) for quantities, suppressive capacities and differences in gene expression. The ability of sex hormones to change Treg numbers, functions, and gene expression will be studied. Regulatory CD4+T cells and CD8+T cells have important roles in suppressing autoimmune disease in the peripheral immune system. Impaired function of regulatory/suppressor T cells contributes to development of autoimmunity. The goal of this project will be studying the quantities and functions of T regulatory cells in healthy controls and patients with SLE, comparing males to females in both groups (given the fact that lupus disease is much more frequent in females than in males). The first aim is to quantify, immunophenotype,

and perform functional analysis of the Treg cell subsets in healthy controls, and in male lupus vs female lupus. The second aim is to compare gene expression profiles of CD4+CD25+hiTreg and CD8+Ts cells in male vs female lupus patients and to compare them with healthy controls. Finally, they will test the effect of testosterone and estradiol in these cells in vitro to see their effects on cell phenotypes, gene expression, signaling and regulatory functions. The overall purpose is to understand the molecular network of these CD4+T regulatory cells and CD8+ suppressor cells in systemic autoimmunity.

5 R01 TW008151-02

Molecular Epidemiology of Drug Resistance and Population Genetic Structure of Pla

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\$50,000

This project will be of significant benefit to public health programs aimed at identifying and combating drug-resistant malaria, and has the potential to benefit the health of a substantial proportion of the world's population. The data will provide valuable information for extending the life span of individual antimalarial drugs and developing more appropriate malaria control policies in China. Malaria remains a serious public health problem in China. In the subtropical Yunnan Province and the tropical Hainan Island of China, malaria has been the most endemic with high transmission of both *Plasmodium falciparum* and *P. vivax*. However, most of the attention in terms of research and interventions has been focused in Africa and Southeast Asia, very few studies of malaria in China have been conducted. Because of extensive use, chloroquine (CQ) has now lost its efficacy due to the emergence of resistant strains in most parts of the world. Meanwhile, suspension of the use of CQ has resulted in reappearance of CQ sensitivity. However, there were differences in the evolution of CQ resistance between parasites from Yunnan and Hainan, the exact mechanism needs to be investigated. Sulfadoxine-pyrimethamine (SP) targets the *dhfr* and *dhps* genes of *P. falciparum*, and point mutations that confer resistance have been widely reported worldwide. Documenting the identity and extent of SP resistance is also critical for policy decisions regarding antimalarial drugs. In addition, *P. vivax* causes a large burden of morbidity in the world including China but traditionally has been understudied. Based on these, their long-term goal of this proposal is 1) to identify single-nucleotide polymorphism (SNP) and characterize the geographic distribution of genetic diversity, population structure, and haplotype variability at drug resistant loci of *P. falciparum* from Yunnan and Hainan, China, 2) to examine the geographic population structure, levels of genetic diversity of *P. vivax* using microsatellite and SNP, and 3) to yield valuable information for making more effective malaria control policies in China. In the past several years they have developed the molecular methods to study the genetics, population diversity, and evolution of malaria parasites, and have done some preliminary studies on malaria field isolates from Yunnan and Hainan using genetic markers, thus enabling us to study the molecular epidemiology of these important malaria parasites in this proposal. The specific aims are to: 1. Determine genetic polymorphisms associated with CQ resistance (CQR) in *P. falciparum* field isolates from Yunnan and Hainan provinces, China. 2. Determine the point mutation prevalence in the *dhfr* (pyrimethamine drug resistance) and *dhps* (sulfadoxine drug resistance) genes associated with SP resistance in *P. falciparum* field isolates from Yunnan and Hainan provinces, China. 3. Assess the changes of *P. vivax* genotypes using *pvcsp*, *pvmsp1*, *pvmsp3-1* genes, and microsatellite markers and determine the geographic

structure and specific epidemiological characteristics of *P. vivax* transmission in Yunnan and Hainan, China.

1 R21 AR058010-01A1

Mechanisms of IL-35 Protection Against Arthritis

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\$200,000

The project will evaluate the therapeutic potential of IL-35 to treat rheumatoid arthritis (RA). RA, a chronic inflammatory disease of the joints, manifests as a chronic synovitis and progressive destruction of the joints, leukocyte infiltrates, and cartilage destruction and bone erosion. It is believed this destruction is supported and perpetuated by proinflammatory cytokines contributed by autoreactive T cells. Rodent models have been developed that mimic arthritis, and one such model, collagen-induced arthritis (CIA), requires immunization with heterologous collagen II. Oral tolerance and treatments with regulatory cytokines have been suggested as possible interventions to treat arthritis. One such new therapeutic is the regulatory cytokine, IL-35; however, its mode of action has yet to be determined. IL-35 is a heterodimer cytokine composed of IL-12p35 plus IL-27 EB13 subunits. They recently expressed IL-35 as a single polypeptide using eukaryotic expression systems. The recombinant mouse IL-35 has the expected molecular weight, and it is recognized with antibodies to IL-12p35 and IL-27-EB13. Functional analysis reveals that IL-35 can completely block development of clinical symptoms of CIA. This disease suppression does appear to be IL-10-dependent, produced by heterogeneous regulatory T cell subsets, including CD25+ CD4+ T cells, as well as CD39+ CD4+ T cells. Thus, additional studies are warranted to investigate the nature of these regulatory T cells and learn which mononuclear cells are responsive to IL-35's action. Given these findings, the hypothesis to be tested in this application is that IL-35 intervention will reduce disease severity and limit disease progression of CIA by the stimulation of regulatory T cells. To test this hypothesis, two Specific Aims are proposed. Studies in Specific Aim 1 will determine if IL-35 in a paracrine/autocrine fashion stimulates endogenous IL-35 production for treatment of CIA and determine the cell types involved to facilitate IL-35's action. Studies in Specific Aim 2 will define the regulatory T cell subset induced by IL-35 that is responsible for protection against CIA and determine which inflammatory T cell subset is diminished. These collective studies will provide the foundation of whether IL-35 can be considered in RA intervention strategies and whether alternative regulatory T cells can be defined that would facilitate IL-35's therapeutic impact in humans.

2 R01 CA102667-06A1

Effects of Malaria on EBV Persistence in Children

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\$167,750

Endemic Burkitt's lymphoma (BL), the most prevalent childhood cancer in Equatorial Africa, is a rapidly growing B-cell malignancy that is ultimately fatal if untreated. The knowledge gained by this study will improve the understanding of the etiology of endemic BL which will ultimately allow for the design of programs aimed at the prevention of BL. While there is a consensus that infection with Epstein-Barr virus (EBV) and repeated infections with Plasmodium

falciparum malaria in childhood (e.g. holoendemic malaria) are essential components in the etiology of BL, the mechanisms of malaria and EBV interactions that increase the risk for endemic BL remain to be elucidated. The long-term goal of this research is to identify the events that initiate B cell oncogenesis in BL. The overall objective of this proposal is to continue this investigations of EBV and malaria interactions in Kenyan infants at risk for endemic BL. In this current R01 (CA102667), they followed a prospective cohort of children with divergent malaria exposures from 2 months through 36 months of age. They observed that children born in the malaria holoendemic region had a significantly earlier age of primary infection with EBV, with ~35% infected by 6 months of age. Importantly, children infected early in life maintained a chronic viral load. These studies support the long-held hypothesis that early age of EBV infection is a risk factor for BL. What they do not know however, is why these infants are infected early in life and how early age of infection limits control of EBV. Based on this data and the data of others, they propose a model whereby susceptibility of infants to infection with EBV by 6 months of age is linked to placental malaria. Infants infected early in life while they have under-developed immune responses will have poor immunologic control of the virus. The long term consequences of poor immunologic control is a greater number of latently infected cells which can ultimately exhaust the immune response against EBV and increase the risk for a malignant clone to emerge from the latently infected B cell. This central hypothesis is that placental malaria alters an infants ability to control primary EBV infection resulting in infection earlier in life and failure to develop effective EBV immunity. They will establish an infant cohort by enrolling pregnant women attending an antenatal clinic at Chulaimbo Hospital in Kisumu District, Kenya where malaria is holoendemic, and follow infants prospectively from birth to their second birthday. To test this hypothesis, they determine the effects of placental malaria on transfer of maternal EBV-specific neutralizing antibodies and in utero sensitization to EBV antigens; determine the factors influencing susceptibility of infants to EBV by 6 months of age; determine the effects of early age of EBV infection on the development of EBV-specific immune responses, the frequency of atypical exhausted memory B cells, and the emergence of pre-malignant B cells. If this model proves valid, the implications are that prevention of BL should focus on delaying the age of EBV infection by focusing on pregnant women with placental malaria, or on blocking transmission to infants.

1 R21 AG034523-01A1

Exploring factors influencing gender disparities in access to transplantation

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\$246,000

Although kidney transplantation is safe, effective, and life-extending for many patients, women have significantly less access to transplantation than their male counterparts, and they have shown that this disparity is widest among older women compared with older men. It is unknown whether this happens because of patient-level barriers to seeking transplantation or because of provider-level biases against referral of women compared with men. Since over 50% of dialysis patients are over the age of 65, equal access to transplantation for this subgroup is important; the goal of this project is to explore potential sources of the gender disparity in access to transplantation, and access to healthcare in general, so that interventions to minimize this disparity can be designed. In the modern era, kidney transplantation is a safe and effective treatment for many patients with kidney failure. However, choosing the right patients for kidney

transplantation is difficult, especially among older patients. Although older patients who receive transplants survive longer than if they had stayed on dialysis, still very few older patients are placed on the transplant waiting list. This is because no tools exist for determining risk in older patients undergoing transplantation, so clinical decision making has to be based on subjective perceptions of a patient's strength and reserve. Misclassification of these factors by the patient or provider likely results in decreased access to transplantation in a population that stands to greatly benefit from this treatment. Although transplant outcomes and survival benefit are similar in men and women, it has been well established that women have significantly less access to transplantation than men. They recently showed that this disparity is strongest in older patients, with older women having 30-60% less access than their male counterparts. However, it remains unclear whether patient or provider level factors contribute to this disparity. In this study they will explore differences by gender and age in factors influencing a patient's decision and ability to pursue transplantation. They will then use a new technique to explore the potential role of gender and age biases in a provider's choice to refer a patient for transplantation. Understanding the root causes of this gender/age disparity is crucial to developing interventions to improve access to transplantation, and healthcare in general, for women and older adults.

1 R21 AI090344-01

Sex Differences in Protective Immunity Against Influenza A Viruses

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\$205,000

Sex differences in the incidence and severity of influenza A virus infection as well as in response to vaccination have been documented in humans. Small animal models are critical for establishing the mechanisms mediating why males and females respond differently to influenza virus infection and vaccination. They will evaluate whether higher humoral immune responses following sublethal infection confers greater protection from challenge with pathogenic influenza A viruses in females compared with males and the extent to which these differences are mediated sex steroids, which may provide clues into why responses to pandemic influenza A viruses differ between the sexes and during pregnancy. Sex differences in the incidence and severity of influenza A virus infection have been documented in humans. Although exposure rates are often higher in men, fatality following exposure to pathogenic influenza A viruses is reportedly higher in women. Sex differences also are reported in response to influenza virus vaccines, with women consistently mounting higher antibody responses and developing more frequent and severe side effects following vaccination than men. Small animal models are critical for establishing the mechanisms mediating why males and females respond differently to influenza virus infection and vaccination. Following primary inoculation with the mouse-adapted influenza A viruses A/PR/8/34 (PR8; H1N1) or A/HK/68 (HK68; H3N2), female mice mount higher inflammatory and humoral immune responses than males. Their preliminary data further reveal that elevated immunity in females against influenza A viruses represents a delicate balance between immune responses conferring protection or causing pathology. The goal of this proposal is to develop a small animal model to test the hypothesis that protective immunity to heterosubtypic influenza A virus challenge differs between the sexes and is modulated by sex steroid hormones. In Specific Aim 1, they will establish whether neutralizing antibody responses, virus-specific T cell responses, and protection against lethal influenza A virus challenge is greater among females than males. Whether males and females differentially rely on subsets of adaptive immune cells

for protection against lethal influenza A virus infection has not been documented; thus, they also propose to compare heterosubtypic immune responses between male and female mice devoid of specific adaptive immune cell populations. If protective heterosubtypic immunity is elevated in females compared with males, then estrogens and/or progestins may enhance and androgens may suppress adaptive immunity against heterosubtypic influenza A virus challenge. In Specific Aim 2 they will test this hypothesis by manipulating sex steroid concentrations in vivo and establishing the effects on humoral and cell-mediated immunity as well as protection from lethal influenza A virus challenge. These are a series of high risk-high return experiments because there are no data to date assessing the sex-specific induction of heterosubtypic immunity in response to influenza A virus infection. Demonstrating that females mount a broadly protective immune response, however, will have important implications for dealing with annual epidemics of influenza, as this may explain why the attack rates for influenza are higher in men than in woman and influenced by pregnancy.

MENOPAUSE

5 R01 AG027702-05

Estrogen: Neuroprotection in the Perimenopause

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\$50,000

Alterations in the hypothalamic-pituitary-ovarian axis in perimenopausal women are associated with multi-organ risk factors for disease, yet the biological mechanisms underlying this increased disease risk are largely unknown. This proposal addresses unanswered questions regarding the vulnerability of the middle-aged brain to global ischemia. In young female rats, the presence of physiological levels of estradiol before and after global ischemia, as might occur during cardiac arrest, reduces hippocampal CA1 neuron loss and associated cognitive impairments. Whether estradiol retains its neuroprotective actions in middle-aged females, and whether the age-related decline in insulin-like growth factor-1 (IGF-I) increases vulnerability to ischemia-induced neurodegeneration and cognitive impairment, are unknown. This proposal aims to examine the roles of age, estrogen and IGF-I in the survival and function of hippocampal neurons in a rat model of global ischemia. The underlying hypotheses are (1) that the middle-aged brain retains its responsiveness to the neuroprotective actions of estradiol if the duration of estrogen withdrawal is brief ("critical period hypothesis") or circulating levels of IGF-I are maintained, and (2) that estrogen acts in the middle-aged brain to activate specific cell survival pathways and thereby intervenes in apoptotic cascades to prevent death of neurons otherwise "destined to die". Specific Aim 1 uses stereological cell counting and behavioral tests to evaluate the outcome of global ischemia in middle-aged female rats that are intact, ovariectomized at various intervals prior to insult, or ovariectomized and treated with estradiol at various intervals after ovariectomy. If estradiol does not preserve neurons and cognitive function in older hormone-deprived animals, we will also determine if IGF-I can reinstate estrogen protection. Specific Aim 2 examines the apoptotic death cascades triggered by global ischemia and identifies the site at which estrogen intervenes in these cascades. They will examine 1) mitogen-activated protein kinase and cAMP response element binding protein at early times after ischemia; 2) the anti-apoptotic gene Bcl-2 and activation of caspase 3 at later times after

ischemia; 3) inactivation of Akt and subsequent activation of the forkhead transcription factor FKHRL1 at early times after ischemia. These experiments will provide new information on the potential for hormone therapy instituted during the perimenopausal transition to protect the brain from damage due to global ischemia.

5 U01 AG012531-17

SWAN: Study of Women's Health Across The Nation

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\$75,000

The Study of Women's Health Across the Nation (SWAN) is a multi-center, multi-ethnic longitudinal study designed to characterize the physiological and psychosocial changes that occur during the menopausal transition and to observe their effects on subsequent health and risk factors for age-related diseases. The goals of the original RFA were to answer the following questions: How do hormones change with the menopausal transition? What factors affect the timing of the transition? What are the symptoms that accompany menopause and who is at risk? How do cardiovascular risk factors change with the transition and is there ethnic variation? What are the rates of bone loss with the transition? When does bone loss begin and what are the risk factors? What are the health consequences of menopause and who is at risk? SWAN is compiling the most comprehensive characterization to date of the health and the physiologic and psychosocial changes of women from pre- to postmenopause in community based samples. SWAN is now poised to study the effects of these menopause-related changes on subsequent healthy aging and on age-related diseases in the post-reproductive period. SWAN I was first funded in September 1994 by the National Institute on Aging (NIA), the National Institute of Nursing Research (NINR), and the Office of Research on Women's Health (ORWH) in response to RFA AG-94-002, Menopause and Health in Aging Women. The first competing continuation of SWAN (SWAN II) was funded in 1999 and the second (SWAN III) in 2004. SWAN I, II and III have been supported by a cooperative agreement mechanism, with 9 funded components: 7 clinical centers, a central reproductive hormone laboratory (CLASS), and a coordinating center. A second central laboratory (MRL) was originally funded as a subcontract to the Coordinating Center (CC). In addition, a Core Repository of serum, plasma, and urine specimens and a DNA Repository were established in June 2000 under separate funding (U01 AG 17719, PI: Dr. MaryFran Sowers). For non-study-related reasons, site operations at New Jersey Medical School stopped in April 2004. The basis of this action was allegations made by two study employees who resigned abruptly. The SWAN PI and study coordinator were subsequently exonerated from these allegations. Please see Appendix 12 for a more complete report. The grant was transferred to the Albert Einstein College of Medicine in 2005. Since that time, the New Jersey PI and project director have worked tirelessly to overcome the obstacles to re-implement the study. As of June 1, 2008, a total of 155 women have successfully completed their clinic visit and five more visits are scheduled. They project that by the end of SWAN III, data will be available for 250 women. This has been very encouraging and thus Nanette Santoro, PI of the New Jersey SWAN site has been approved by the NIA to prepare a U01 application to cover further contacts for the Hispanic women. Please note that the SWAN IV project applications pertain to the remaining six sites only. Information relative to the New Jersey site is covered in the separate application submitted by Dr. Nanette Santoro. From over 16,000 women aged 40-55 years who were screened during 1995-1997, 3302 women aged 42-52 years were enrolled in SWAN's

longitudinal cohort (approximately 450 at each of 7 clinical centers). They completed their baseline clinic visit during 1996-1997. Of the 3302 women enrolled, 1550 were Caucasian, 935 African American, 286 Hispanic, 250 Chinese, and 281 Japanese. A subset of 880 menstruating women was enrolled in the Daily Hormone Study (DHS) started in 1997, which is designed to examine cyclical daily hormone and symptom patterns during the menopausal transition.

1 R21 AG037832-01

Ovarian hormone-independent sex chromosome effects in menopause

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\$153,500

This project is designed to make new discoveries into why postmenopausal women are at increased risk for diseases like metabolic syndrome, hypertension and cardiovascular disease compared to premenopausal women. They will make these new discoveries by studying sex chromosome effects independently of the ovarian hormones using a unique animal model in which they can separate, for the first time, sex chromosome differences between males (XY) and females (XX) from the sex hormone differences (e.g., differences in estrogen and testosterone levels). By discovering new genes and pathways responsible for the increased incidence of these diseases in ovarian deficient females, new therapeutic treatments are likely to ensue for postmenopausal women and women with ovarian hormone deficiency. Postmenopausal women have a higher incidence of diseases such as metabolic syndrome, cardiovascular and renal disease than premenopausal women. To begin to uncover genes and pathways that contribute to these adverse effects of aging in the postmenopausal woman, they propose two distinct strategies for discovering novel genes and pathways that may contribute to the increased risk postmenopausal women face towards these diseases. They will take advantage of the "four core genotypes" mouse model in which sex chromosome effects can be separated from the gonadal sex thus enabling comparisons among XX and XY animals independently of whether they were born with ovaries (e.g., XX- vs. XY-females) or testes (XX- vs. XY-males). While recent microarray studies in mice have demonstrated that thousands of genes are regulated by gonadal hormones, the number of genes regulated by the sex chromosome complement independently of the gonadal hormones is far more limited. Thus, they expect to discover a handful of genes (<10) that are differentially regulated by the sex chromosome complement (SCC) in the ovarian hormone deficient female during over activity of the renin angiotensin system (RAS). Aim 1 will use a tightly focused microarray approach leveraging their ability to differentiate SCC from gonadal sex to identify genes in the kidney that are differentially regulated by the SCC in the Ang II infused E2-deficient female. Aim 2 will use a candidate gene approach to test the hypothesis that the regulation of the tissue-specific renin angiotensin system (RAS) in the kidney by ovariectomy and hypertension is sex chromosome dependent. They hypothesize that the interaction between the XX SCC with the E2-deficient state of ovariectomy tips the vasoconstrictor/vasodilator balance of the renal RAS towards vasoconstriction to a greater extent than in the XY-Female by increasing plasma and renal levels of Ang II, the ratio of the Ang II synthetic enzyme, angiotensin converting enzyme (ACE) to the catabolic enzyme, angiotensin converting enzyme 2 (ACE2) and the ratio of the type 1 angiotensin receptor (AT1R) to the vasodilator type 2 angiotensin receptor (AT2R).

1 R21 NS066147-01A1

Effects of estrogen on brain morphology and neuronal integrity in early menopause

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Mayo Clinic, Rochester, MN

\$213,812

This study will provide evidence on the neuroprotective effects of estrogens with non-invasive imaging markers of structural and functional neuronal integrity in newly menopausal women, during a hormone treatment trial. This evidence would potentially have a significant impact on women making the decision to use hormone treatments for dementia prevention as they transition into menopause. Neuroprotective effects of estrogens offer the possibility of preventing or delaying Alzheimer's disease in menopausal women. Estrogen treatment in older women who were late into menopause in the Women's Health Initiative Memory Study, did not prevent dementia. The question remains as to whether or not estrogen can preserve neurological function and decrease the risk of dementia when administered early in menopause from 6-36 months of the last menses. This project is proposed as an ancillary to the Kronos Early Estrogen Prevention Study (KEEPS), which is a nationwide, multi-center, randomized blinded study designed to provide evidence on the benefits and risks of oral and systemic estrogen treatment in recently menopausal women. Their goal is to test the neuroprotective effects of estrogen treatment in early menopause, during the 48 months of the randomized clinical trial. They will determine the rates of hemispheric atrophy on MRI, and the change in neuronal metabolite N-acetylaspartate (NAA) on proton MR spectroscopy (1H MRS) as a surrogate for the neuroprotective effects of estrogen treatment during the early postmenopausal years. In addition to the longitudinal serial measurements of whole brain, hippocampal and ischemic lesion volumes, they will use exploratory 3-dimensional voxel-based analysis of the serial MRI to determine the differences in the change in whole brain morphology in women who are taking estrogens compared to placebo. Their collaboration with the investigators of the KEEPS Cognitive and Affective Study will give us the ability to relate the change in neuronal metabolic integrity and brain morphology with the concurrent change in cognitive function in newly menopausal women. As an outcome of the proposed investigations, they expect to determine whether or not oral and transdermal estrogen treatment preserves brain structure and neuronal function during the immediate years after menopause. Several decades of follow-up are necessary to determine if estrogen treatment in newly menopausal women prevents Alzheimer's disease. This project will provide the necessary in vivo evidence on the neuroprotective effects of oral and transdermal estrogens in early menopause in the short term, for future large-scale, long term trials. The original contributions of this study to women's health research will include the demonstration of the effects of estrogens on longitudinal change in brain morphology and neuronal integrity, and the relationship between these biological changes and the concurrent change in cognitive function in recently menopausal women.

U01 AG032699-03

Ultra-low-dose Estrogen Gel for Vasomotor Symptoms in Women failing placebo or a behavioral intervention: A Randomized Trial

Lacroix, Andrea

Fred Hutchinson Cancer Research Center, Seattle, WA

\$132,000

The long-term objective of NIA's RFA-AG-08-004 entitled, "New Interventions for Menopausal Symptoms (U01) is to accelerate progress in identifying effective remedies for vasomotor symptoms (VMS) in women going through the menopausal transition. They have created a network of scientists who are highly knowledgeable about the menopausal transition and experienced in the conduct of women's health trials to fulfill this mission. This Data Coordinating Center (DCC) application is being submitted in conjunction with the network entitled, "The Menopausal Symptoms Initiative-Finding Lasting Answers to Sweats and Hot Flashes (MSI-FLASH)". Their DCC will be jointly led by Andrea LaCroix and Garnet Anderson who have served together as Co-Principal Investigators of the Women's Health Initiative Clinical Coordinating Center (Seattle) for more than a decade. The MSI-FLASH network has five clinical sites located in Boston (Lee Cohen and Hadine Joffe, PIs), Indianapolis, IN (Janet Carpenter, PI), Oakland, CA (Barbara Sternfeld and Bette Caan, PIs), Philadelphia (Ellen Freeman, PI) and Seattle (Katherine Newton and Susan Reed, PIs). This multidisciplinary investigator group proposes five randomized controlled trials testing a range of behavioral, mind-body, hormonal and pharmacologic interventions to treat hot flashes. The specific objectives of the DCC are to: 1) Provide and coordinate all necessary leadership activities to facilitate collaboration and productivity among network scientists during all phases in the lifecycle of VMS clinical trials from hypothesis formulation to publication, dissemination, and data sharing; 2) Build upon 15 years of experience and well established human and operational resources to coordinate 5 or more multi- site randomized trials including support of protocol development, recruitment, intervention, data collection and management, and statistical analysis; and 3) Create the infrastructure to involve an expanded network of scientists from the US and worldwide to facilitate the development and use of common methodologies and measurements for VMS trials inside and outside of this trial network so that emerging new treatments for hot flashes can be rapidly identified and rigorously tested for efficacy and safety with comparable results.

5 R01 AG027713-05

Menopause: Decreased Response to Increasing Inflammation

Maggi, Adriana Caterina

University of Milan, Milan, Italy

\$50,000

The long-term goal of their research is to find treatments for the prevention of the disorders associated with menopause which are safer and more efficacious than present hormone replacement therapy (HRT). The failure of present HRT to fulfill medical and women's needs has to be ascribed to an insufficient knowledge of the biology of menopause. The aim of their research is focused on the understanding the consequences of cessation of ovarian functions on the physiology of non-reproductive organs such as bone, brain, arteries and fat. In particular their studies and the studies proposed in the present project will focus on the effects of estrogen decreased production at menopause transition and after in non-reproductive organs. Given recent results demonstrating that in non-reproductive organs of fertile female mice estrogen receptors (ERs) are activated by factors other than estrogens, their Specific Aim #1 will focus on assessing the extent to which ERs are transcriptionally active during menopause transition and after. They will then try to identify the factor(s) involved in ER activation. This part of the project relates to questions which so far could be addressed only partially with the current technology. The generation of a novel model of reporter system, the ERE-Luc mouse, will enable us to precisely quantify ER activity in the organs of interest and facilitate the search of factors involved in ER

unliganded activation. Specific Aim #2 will give us the opportunity to test an original hypothesis that would explain the widespread protective effects provided by the estrogen-ER system. This hypothesis is based on numerous very recent observations made in ours and several other groups showing that estrogens and cognate receptors may exert a strong anti-inflammatory action by inhibiting the immune response of cells of the monocyte lineage. They here propose that menopause consists in a decreased response to increased inflammation. They will test this hypothesis by the direct assessment of ER relevance on macrophage activity through the generation of a novel conditional ER α K.O. mouse. Furthermore, using brain as a paradigmatic non-reproductive organ, they will measure basal and induced activity of brain inflammatory cells. Finally, the specific involvement of ER anti-inflammatory activity in the development of menopause-associated diseases will be tested with the study of the activity in menopause of another class of intracellular receptors devoted to the control of inflammation, the PPARs.

5 R01 AG027697-05

Effects of Chronic Estrogen on TIDA Neurons: Role of Cytokines and NO

Mohankumar, Puliur S.

Michigan State University, East Lansing, MI

\$50,000

Perimenopause is one of the most complex and least understood states of a woman's life. Many of the health risks associated with this state were believed to be due to decreases in estrogen levels and that estrogen could protect against health risks faced by perimenopausal women. However, estrogen fluctuates during perimenopause and if at all increases during the premenstrual and follicular phases. Recent clinical trials have shown that chronic administration of estrogenic compounds in postmenopausal women may increase the risk for several diseases. Therefore, it is important to investigate the effects of estrogen exposure on various organ systems. Studies so far indicate that estrogen's effects in the brain are beneficial. These reported effects, however, deal with non-hypothalamic regions of the brain. The effects of chronic estrogen exposure on the hypothalamus which regulates several key body functions have not been investigated. This is critical because women use estrogenic preparations on a prolonged basis and are exposed to endogenous estrogen throughout their adulthood. This proposal focuses on the effects of chronic estrogen exposure on one of the estrogen sensitive neuronal systems of the hypothalamus, namely, the tuberoinfundibular dopaminergic (TIDA) system. Dopamine (DA) released from TIDA neurons inhibits prolactin (PRL) secretion from the anterior pituitary. Age-related reductions in TIDA activity is associated with hyperprolactinemia and appearance of mammary and pituitary tumors in animal models. The mechanisms behind the loss of TIDA neuronal function is not clear. In this application, they are proposing a novel hypothesis and an interesting model to study how estrogen could affect TIDA neurons and increase PRL levels. This series of studies is important because women not only use estrogen on a long-term basis in HRT but are also exposed to environmental estrogens. Prolonged exposure to estrogen and elevated levels of PRL may promote the risk for breast cancer.

5 R01 AG027678-05

Biological Mechanisms of Arterial Stiffening with Age and Estrogen Deficiency

Moreau, Kerrie

University of Colorado, Denver, CO

\$47,579

The purpose of this R01 proposal is to determine the key functional mechanisms by which the loss of female sex hormones, particularly estradiol (E2), contribute to the age-related decrease in large artery compliance. The overall hypothesis is that basal large artery compliance will decrease in response to acute sex hormone suppression in pre- and perimenopausal women due in part to a decrease in vascular endothelial-dependent vasodilatory tone mediated, in part, to the development of vascular oxidative stress. However, E2 administration during sex hormone suppression will decrease vascular oxidative stress, improve endothelial vasodilatory tone and restore arterial compliance to basal levels. Secondary and tertiary hypotheses are that the changes in arterial compliance and vasodilatory function with sex hormone suppression and E2 will be related to unfavorable, and favorable, respectively, changes in vascular endothelial cell protein expression including oxidant (e.g., NADPH) and antioxidant (e.g., glutathione peroxidase) enzymes, vasoconstrictors (endothelin- 1), and estrogen receptor alpha (ERalpha). To test these hypotheses, healthy pre-, peri-, and postmenopausal women will be studied at before and following acute sex hormone suppression (gonadotropin releasing hormone antagonist [GnRHant]) with or without E2 add-back therapy. The GnRHant intervention will enable us to study the direct mechanisms associated with sex hormone deficiency and the E2 add-back intervention will enable us to isolate the independent effects of E2. Insight into the molecular mechanisms mediating the decrease in large artery compliance will be obtained using a novel translational research technique to determine changes in vascular endothelial cell protein expression of genes involved in the regulation of cellular and systemic adaptations to aging and sex hormone deficiency including oxidative stress, nitric oxide bioavailability, and the potent transcription factor ERalpha proteins. The results should provide new insight into the integrative biological mechanisms by which sex hormone deficiency modulates the age-related reduction in large artery compliance in women as they transition through the menopause.

2 U01 AG017719-11A2

SWAN Repository III

Sowers, Maryfran

Universtiy of Michigan, Ann Arbor,

\$100,000

This competing renewal application is to provide for ontinued maintenance of and activities associated with the SWAN Repositories of serum, plasma, urine, DNA and transformed cells generated from a 10-year study of a population of 3302 women from 5 ethnic groups who have been evaluated annually prior to, during and following the menopausal transition. These Repositories, an arm of the Study of Women's Health Across the Nation (SWAN), are meant to support, facilitate and extend the Core SWAN; additionally, the Repositories provide a mechanism for opening the resources of SWAN to the greater scientific community.

Implementing activities associated with three proposed specific aims of this competing renewal will 1) provide for the continued management of the current 1.7 million Repository specimens and the additional specimens that will accrue as a result of fielding SWAN IV in 2009 to 2014; 2) expand the DNA Repository, the most frequently requested specimen type that is uniquely renewable because of their investment in cell immortalization; 3) promote effective information interchange about the SWAN Study, its data and the Repository resources through development of a 2-level web-based "data warehouse"; 4) provide for continued administration of the application review process for specimen utilization and administrative management of specimen

distribution including Material Transfer Agreements; 5) engage in strategies to promote utilization of specimens; and, 6) expand the scope of the genetics studies associated with the SWAN study and its Repository.

5 U01 AG012553-16

Study of Women's Health Across the Nation III

Tyrrell, Kim Sutton

University of Pittsburgh, Pittsburgh, PA

\$125,000

Study of Women's Health Across the Nation (SWAN) has compiled the most comprehensive characterization to date of the health and the physiologic and psychosocial changes of women from pre- to postmenopause in community based samples. Of particular public health importance is that the continuation of SWAN will permit the study to increase understanding of the effects of these menopause-related changes on subsequent health and risk factors for age-related diseases. The SWAN is a 7-center multi-ethnic longitudinal study designed to characterize the physiological and psychosocial changes that occur during the menopausal transition. SWAN has amassed ten years of data about endocrinology of the transition and other factors relevant to midlife health and aging. As SWAN requests its fourth competing renewal, the study itself proposes to transition from a study of the menopause to a study of aging in women. The average age of participants at the beginning of the SWAN IV project will be 59 years (54 to 65) and SWAN IV will follow these women through the age range of 59 to 70. SWAN has the unprecedented capability to link the expansive biological, medical, social, behavioral, and demographic data it has collected during mid-life and the menopausal transition to the development of both positive and adverse health states in early oldage. The primary objectives of SWAN IV are to: 1) Characterize the endocrinology and symptomatology of the post-menopause (2 to 12 years after final menses); 2) Ascertain additional health outcomes (such as measured physical performance) that are relevant to the early old age range and that may be affected by the factors that they have studied in mid-life and 3) Understand the relations between the mid-life and menopausal transition experience of women and subsequent positive and negative health outcomes. To accomplish this, the investigators propose annual phone contact to closely track menopausal status, menopausal symptoms and selected health events. In addition, two in-person clinic visits are proposed to accomplish detailed physical measures of early disease. The major thematic areas of SWAN IV include 1) Physical Functioning; 2) Bone/Osteoporosis; 3) Cognitive Function/ Symptoms/ Mental Health and 4) Cardiovascular. New areas for SWAN include physical performance and osteoarthritis, history of major depression, and carotid wall thickness. SWAN will continue to monitor symptoms, cognition, cardiovascular risk factors, endocrinology, bone density and fractures. SWAN IV will advance their understanding of how modifiable risk factors related to the menopause transition are linked to sub-clinical disease measures and hard outcomes. This may lead to improved strategies for the primary prevention of disease in women.

1 R21 NR012218-01

Menopause Symptom Clusters: Refocusing Therapeutics

Woods, Nancy Fugate, Ph.D.

University of Washington, Seattle, WA

\$200,000

The results of this study will help clinicians and women, themselves, identify symptoms that cluster together and that may have different causes. Knowing which cluster of symptoms a woman has may help her clinician recommend the treatment or treatments that are most likely to work best for her. Using some of the genetic and hormone tests, as well as information about the woman's history, such as stressful experiences she has had, may help understand what causes some of her symptoms and may help her and her clinician decide on the best treatment available for her. Although women experience clusters of symptoms during the menopausal transition, most research focuses on individual symptoms such as hot flashes. The proposed study shifts the paradigm from focusing on individual symptoms to symptom clusters (SCs). Re-analyzing data on symptoms and genetic polymorphisms, endocrine biomarkers, symptom vulnerability factors and sociobehavioral risk factors from over 500 participants in the Seattle Midlife Women's Health Study (P50 NR02323, R01 NR04141, P30 NR04001, P30 ES07033) with longitudinal follow-up spanning up to 19 years will allow us to achieve these aims: 1) identify prevalent symptom clusters during the late reproductive stage, early and late menopausal transition stages, and early postmenopause using latent class analysis; 2) determine the consistency of symptom clusters as women change from one menopausal transition stage to the next; 3) test models linking genetic polymorphisms, endocrine biomarkers, symptom vulnerability factors, social-behavioral risk factors and menopause-related factors to symptom clusters, and outcomes of well-being and symptom interference; 4) conduct a systematic review of controlled clinical trials to identify symptoms as secondary treatment effects and adverse effects that will inform us about therapies for symptom clusters and 5) synthesize results of the empirical analyses and systematic review to develop novel symptom cluster management protocols to be tested in future feasibility studies. An interdisciplinary scientific advisory board including National Institute on Aging-funded MS-FLASH clinical trials investigators will provide their research team an opportunity for immediate sharing of their results in order to inform design of symptom cluster management approaches as well as their ongoing studies, including the generation of ancillary studies of symptom clusters and related mechanisms.

MENTAL HEALTH

5R21HD058989-02

Novel Approaches To Understanding Mental Disorder, Substance Abuse And Hiv-Risk A

Whitbeck, Leslie B

University Of Nebraska Lincoln

\$255,535

This R21 developmental application will set the stage for the first multi-state longitudinal diagnostic study of homeless women. It builds on more than a decade of work with hard to access homeless populations and a prior three-year longitudinal diagnostic study of homeless adolescents. This application will fund the development of innovative measures and sampling techniques specifically for this understudied population and for the piloting of measures with a sample of 200 homeless women in two Midwestern cities. This [revised] R21 application seeks two years of support to develop state-of-the-science methodologies to address four important gaps in existing research with homeless women: 1) capture the diversity of circumstances among a fluid and hard-to-access population; 2) increase their understanding of mental and substance use disorders (particularly personality disorders) across the diversity of homeless women; 3)

improve their understanding trajectories to homelessness through development of an innovative event history calendar approach; and 4) advance knowledge of homeless women's health and HIV-risk by circumstance and trajectories to homelessness. This research will provide measurement development and preliminary studies for a multi-state longitudinal R01 designed to advance their understanding of mental and substance use disorders among homeless women, their movement into and out of homelessness, the consequences of homelessness for women and minor children in their custody, and women's health, HIV-risk, and HIV testing behaviors. The planned longitudinal research will focus on a growing but poorly understood population of the nation's most vulnerable women. The specific aims of this R21 developmental application are to 1) develop and pilot a sampling plan that will better reflect the diversity of homeless women; 2) develop and pilot an innovative events history calendar for use with homeless women; 3) program and pilot Axis I (UM-CIDI) and Axis II (DIPD-IV) diagnostic interview schedules for computer-assisted personal interviews with homeless women; 4) develop and program women's health and HIV-risk measures; and 5) pilot the measures with 200 homeless women in two Midwestern cities.

5R21DA025543-02

Race And Hiv-Risk: Contextual And Neurocognitive Influences On Sex Partnerships

Floyd, Leah

Johns Hopkins University

\$246,574

Currently, there is a hidden HIV epidemic among young adult African American females with no history of substance abuse. These women are at increased risk for contracting HIV by virtue of their social/sexual networks. If successful, the proposed research project: (1) should provide insight into why African American females have higher rates of HIV than their white counterparts; (2) highlight the importance of considering the contextual influences of drugs, that is how drug markets change social structures and altered sexual norms and behaviors of entire communities; and (3) increase understanding of the processes through which neighborhood factors influence HIV risk. The primary aim of this R21 application in response to NIDA's ANSWHR Initiative (PAS-07-381) is to address gaps in literature focused on HIV risk and disparities among females. In the United States as rates have increased among females, the rate of HIV/AIDS diagnoses for African American females approaches 25 times the rate for white females. Despite the broad base of findings documenting health disparities in HIV, extant studies cannot explain why African Americans continue to be disproportionately affected. Currently, there is a hidden HIV epidemic among young adult African American females with no history of substance abuse. These women are at increased risk for contracting HIV by virtue of their social networks. The proposed study requests two years of support for a cross sectional epidemiologic examination of racial/ethnic differences in sexual partnerships among 220 females (110 Black and 110 White) residing in low socioeconomic status (SES) neighborhoods. Guided by ecosocial theory, they seek to explain why these differences exist across race/ethnicity. They will consider the extent to which neighborhood social and economic factors (e.g., drug markets) interact with race/ethnicity to produce different levels of HIV risk. They will expand drug abuse and HIV prevention research by, in addition to considering individual differences, examining the influences of neighborhood drug markets on the sexual behaviors, sexual partnerships and rates of a sexually transmitted disease among young adult females residing in disadvantaged neighborhoods. Finally, the proposed study will move beyond descriptive social epidemiology

and into identifying neurocognitive processes that mediate/moderate relationships between neighborhood factors and individual behavior. As, a small yet growing base of research suggests, to the extent that individuals are able to make decisions, solve problems and control impulses, neurocognitive functions may serve as protective factors or pathways through which external social factors influence individual behavior. Identifying social factors that influence partner selection and individual level factors that may serve to reduce the adverse effects of living in disadvantage neighborhoods will inform HIV prevention interventions for African American and underserved women. If successful, the proposed research project: (1) should provide insight into why African American females have higher rates of HIV than their white counterparts; (2) highlight the importance of considering the contextual influences of drugs, that is the influence of drug markets on social structures and sexual norms and behaviors; and (3) identify modifiable individual level factors linking neighborhood social and economic factors to individual HIV risk behaviors.

5 DP1 OD003312-04

Emotions are Emergent Events Constrained by Affective and Conceptual Processes

Barrett, Lisa

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\$391,250

Emotional states are central to mental and physical health. NIH invests tremendous resources in research on emotion, much of it devoted to animal models. Ironically, this research is guided by a scientific paradigm that is grounded in human experience. People experience fear and see it in others, so scientists assume there must be a literal (modular) neural circuit for fear in the mammalian brain. Rats freeze when they hear a tone paired with a foot shock, so they are presumed to be in a state of fear (versus surprise, anger, or even a general state of alarm) and undergoing “fear learning.” Scientists also presume that a map of the neural circuitry of freezing behavior will yield a neural mechanism for fear that is largely preserved in humans, and a decade of neuroimaging studies have focused on locating a homologous neural circuit in the human brain. In the last five years, I have traced the roots of this “natural kind” model, conducted a comprehensive review of the literature to examine its veracity, and found it wanting (Barrett, 2006a).¹ In response, I have fashioned a new systems-level model, called the Conceptual Act Model, grounded in the neuroanatomy of the human brain. My model parsimoniously incorporates neuroscience findings from rats, primates, and humans, and explains the mechanisms that produce the range and variety of behavioral and introspective instances that they call “emotion” (Barrett, b, c; Barrett, Mesquita, Ochsner, & Gross, 2007; Barrett, Ochsner, & Gross, 2007; Duncan & Barrett, 2007). The Conceptual Act Model asks different – and perhaps better – questions about what emotions are and how they function in mental and physical health. The NIH Director’s Pioneer Award will allow me the intellectual freedom and resources to continue building evidence for the Conceptual Act Model of emotion, thereby shaping a new paradigm to guide the scientific study of emotion.

MUSCULOSKELETAL SYSTEMS

1 R21 AR056404-01A2

A link between parity, trunk muscle function, and degenerative spondylolisthesis

Cholewicki, Jacek

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\$199,929

SWAN has compiled the most comprehensive characterization to date of the health and the physiologic and psychosocial changes of women from pre- to postmenopause in community based samples. Of particular public health importance is that the continuation of SWAN will permit the study to increase understanding of the effects of these menopause-related changes on subsequent health and risk factors for age-related diseases. This study will examine relationships between pregnancy, cesarean section (CS) and other abdominal surgery, trunk and abdominal muscle deficiency, and degenerative spondylolisthesis (DS) in older females. The key question is whether pregnancy and/or CS mediated trunk muscle deficiency could be a precipitating factor in the development of DS later in life. Three specific aims are to determine whether: (1) parity/CS/other abdominal surgeries are associated with DS, (2) trunk muscle deficiency is associated with DS, and (3) parity/CS/other abdominal surgeries are associated with trunk muscle deficiency. The costs associated with the treatment of degenerative low back disease make it one of the top 5 most expensive conditions in the American healthcare economy. DS is considered one of the major causes of low back pain among the older population. Women suffer from DS at a 3-9 times higher rate than men, as yet, without a clear explanation. Previous studies documented relationships between pregnancy and low back pain, and suggested abdominal muscle deficiency as an underlying cause. Of special concern is the effect of CS. The rates of CS rose three-fold over the last 3 decades and may cause significant public health problems regarding DS in coming years. They propose to conduct a case-control study of 200 DS patients and 200 age-matched (in 5 year age groups) controls, including a more detailed assessment of trunk muscle function in 80 DS and 80 matched control subjects. Group designation will be based on a DS diagnosis from a sagittal view x-ray. The 400 subjects will be administered a detailed questionnaire regarding their parity, CS, previous surgeries, and other potential covariates. A subset of 80 subjects from each group will in addition undergo a physical examination of their abdominal and trunk muscle function and quantitative assessment of motor control. Physical exam will include abdominal muscle and hip extension tests. These tests examine the ability of the abdominal and paraspinal muscles to stabilize pelvis and the lumbar spine during simple hip flexion and extension maneuvers. Motor control tests will quantify muscle reflex latencies in response to sudden trunk perturbations, and postural control while balancing on an unstable seat. Both delayed muscle reflex responses and poor postural control are associated with low back pain and constitute predisposing risk factors to future low back problems. Poor motor control could lead to spine instability, chronic problems and degenerative changes in the spine over time. All measures will be quantified (continuous or categorized) and used in the regression and chi-square analyses to test the hypotheses. Innovative aspects of this proposal comprise of quantifying muscle function objectively and documenting variables related to parity in women with and without DS, which gives a better chance of finding any relationships that might exist.

The Osteoarthritis Initiative

\$650,000

Knee osteoarthritis (OA) is the most common cause of disability in adults. The "Osteoarthritis Initiative (OAI): A Knee Health Study" is a nationwide research study that will help researchers gather more information about the physical changes that occur prior to the onset of arthritis symptoms or before OA gets worse. The purpose of this study is to examine people who have knee OA or are at high risk for knee OA; information will be used to better understand how to prevent and treat knee OA. Knee OA causes more health problems and medical expenses than any other form of arthritis. Symptoms of OA can range from stiffness and mild pain to severe joint pain and even disability. Previous research has shown that certain factors, such as knee pain, prior knee injury or knee surgery, OA of the hand, or obesity, may lead to knee OA. The OAI is a multicenter, observational study of knee OA that will collect information on potential biomarkers for OA and trends in OA onset and progression. The OAI will recruit and follow participants who have knee OA or are at high risk for developing knee OA for at least a four-year period at one of four clinical centers. Blood and urine collection, magnetic resonance imaging (MRI), and X-rays will be completed at each of four annual follow-up visits. A questionnaire and physical examination at screening will assess for risk factors for the development and progression of knee OA. Levels of knee pain and physical disability will be assessed at study start and at each of the follow-up visits by questionnaire and examination.

ORWH supported Clinical Centers in the Osteoarthritis Initiative:

N01 AR22262-12-0-1

Clinical Centers For The Osteoarthritis Initiative - Rhode Island

Eaton, Charles B.

Memorial Hospital of Rhode Island, Pawtucket, RI

N01AR22259-12-0-1

Clinical Centers For The Osteoarthritis Initiative

Hochberg, Marc

University of Maryland Baltimore, Baltimore, MD

N01 AR22261-13-0-1

Clinical Centers For The Osteoarthritis Initiative

Jackson, Rebecca

Ohio State University, Columbus, OH

N01 AR22260-13-0-1

Clinical Centers For The Osteoarthritis Initiative

Kwoh, Kent

University of Pittsburgh, Pittsburgh, PA

1 R21 HL093068-01A2

Sexual Dimorphism of Skeletal Muscle

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University of Missouri-Columbia, Columbia, MO

\$209,109

This project is to develop and validate a skeletal muscle model for the study of the primary functions of the smallest blood vessels in age-matched male and female animals of the same species, the mouse. The 2 primary functions of the microcirculation 1) blood flow to metabolizing tissue, and 2) the movement of nutrients from blood to tissue as well as the removal of wastes from tissue to blood, appear to differ between males and females in health and cardiovascular disease including hypertension and secondary to type 2 diabetes. As materials distribute themselves between blood and tissue, so too will fluids move between compartments; thus if exchange regulation differs between males and females it is likely that volume distribution will also differ. Therefore it is imperative to have access to a model to learn the differences and similarities between the sexes as the data from males, disease incidence and severity and subsequent treatment strategies will not apply equally to females. Skeletal muscle (SKM) microvasculature has been studied extensively with respect to respiratory gas and nutrient exchange, volume distribution, and blood flow control, into and within the organ, in health and disease. This R21 is in response to a PFA requesting development of models for the study of function in males and females. This is terribly important as most studies of SKM have been conducted in males (animals and humans) with the presumption that the data apply equally to both sexes. Evidence from multiple studies accumulated over the last decade is making it clear that this assumption is in error. One model used widely for in vivo study is the rodent cremaster, a thin muscle derived from the abdominal wall that raises and lowers the testes. Surprisingly, no microvascular skeletal muscle preparation of equivalent metabolic and fiber type substitutes presently for the cremaster that facilitates study of both males and females. This proposal aims to rectify this lack by validating the abdominal wall skeletal muscle preparation in males and female rodents. The hypothesis is that microvascular skeletal muscle functions do not differ between age-matched males and females of the same species. Accordingly, 3 aims will be carried out in in situ and isolated abdominal muscle microvessels from age- and strain-matched female and male mice: Aim 1 will assess whether sexual dimorphism exists with respect to blood flow regulation from measures from microvascular diameter to selected endothelium-dependent and -independent agents. Given recent data they expect to reject their hypothesis as they anticipate that a) arterioles from males will develop greater basal tone and b) the dose-response relationship for the endothelium-dependent dilation will differ between males and females. Aim 2 will assess whether sexual dimorphism exists with respect to exchange regulation from measures from measures of microvessel solute permeability (Ps). Given their data, they expect to reject their hypothesis as they anticipate that a) venules from males will be leakier than those from females, b) basal arteriole and capillary barrier properties will not differ by sex, and c) the vasoactive agents will produce a variety of exchange responses reflecting differences in sex-specific mechanisms regulating solute distribution between the vascular and tissue compartments of males and females. Aim 3 will compare the sex, age, organ and species matched diameter (Aim 1) and exchange data (Aim 2) from microvessels as they lay in the living tissue and following isolation from the tissue. This is an incredibly opportunity to make these comparisons as not all tissues are amenable to study in situ and it is assumed that the data from the isolated vessels reflect the behavior in the tissue. Data from this project will form the foundation for future

genetic, biochemical, and physiologic studies of microvascular function in males and females. It is imperative that they validate a model for study of microvascular function in both sexes to understand intelligently the sex-dependent mechanisms regulating vascular function in health and dysfunction in disease. With the knowledge the foundation, and provide rational means for prevent and treating vascular disease specific to the needs of males and females.

1 R01 AR057139-01A2

Structural, Molecular, and Functional Specialization in Osteocyte Mechanosensing

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City College of New York, New York, NY

\$200,000

Osteocytes are the cells in bone that sense mechanical loading and translate mechanical strain into biochemical signals that initiate modeling and remodeling through which bone adapts its structure to its mechanical loading environment. This ability is key to skeletal health; failure to adapt results in bone fragility. Increases and decreases in osteocyte mechanosensitivity have been implicated in regulating the bone response to anabolic agents, and conversely the bone loss resulting from estrogen loss, respectively. Thus understanding how osteocytes "perceive" and transduce mechanical signals may provide key new insights into bone physiology leading to the identification of novel therapeutic targets against bone loss due to aging and disease. Osteocytes, the cells that reside within bone matrix, are the most abundant bone cells. They function as the mechanical sensors in bone, and are critical to activation and coordination of osteoclastic and osteoblastic activities by which bone adapts to mechanical usage, maintains its health and prevents fractures. The mechanisms underlying osteocyte mechanotransduction are not well understood, though changes osteocyte mechanosensitivity have been implicated in regulating the effect of both bone anabolic agents and sex hormones. The investigators have developed engineering models which show that small whole bone strains can be amplified locally around osteocyte processes by focal attachments to the canalicular wall. Osteocyte cell bodies cannot see similar high strains as they are too compliant and lack the cellular attachments needed for local strain amplification. These mathematical models argue that the osteocyte cell process may be uniquely designed to function as a detector of small tissue strains. To test this hypothesis, they developed a broad-based multiple-PI program that combines expertise in ion channel physiology, in vivo osteocyte structure/biomechanics and bioengineering/modeling to understand how osteocytes perceive and transduce their local mechanical environment. This program will a) examine the functional polarity of osteocyte mechano- responsiveness using electrophysiological approaches on cultured osteocytes (Aim 1), b) identify the molecular components of mechanotransduction complexes in osteocytes (Aim 2), c) characterize the structure of the mechanotransduction complex in osteocytes in vivo (Aim 3) and d) build integrative mathematical models relating local hydrodynamic forces and membrane strains at osteocyte processes and cell bodies to cellular responses in vitro and in vivo (Aim 4). They have also developed a novel technology ("Stokesian" Fluid Stimulus probe) that allows us to hydrodynamically load osteocyte processes vs. cell bodies at extremely low forces (<10pN) typical of what bone cells actually experience in vivo. Expansion of this technology to interrogate mechano-responsiveness in a broad range of cell types is a developmental goal of this grant. Significance: Understanding how osteocytes "perceive" and transduce mechanical signals may provide key new insights into bone physiology leading to the identification of novel therapeutic targets against bone loss due to aging and disease.

1 R03 AR057518-01A1

Delayed pubertal development on the mechanism of bone loss at maturity

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\$75,000

Fracture risk in the elderly has its origins during growth and development. A delay in the onset of puberty results in both low bone mass and an increased incidence of stress fracture in young women. Therefore, the failure to accrue peak bone mass during the adolescent years represents a missed opportunity to optimize bone mass during one's life. Osteoporosis is "a pediatric disease with geriatric consequences". Simply stated, suboptimal skeletal development in childhood and adolescence may result in decreased bone strength and an increase in lifetime fracture incidence. A delay in the onset of puberty (primary amenorrhea) correlates with both low bone mass; and an increased incidence of stress fracture. Suboptimal bone accrual may have long term consequences. Even with current treatment options as studies that treated amenorrheic dancers for 2 years with hormone replacement therapy found no difference in bone mineral density between treated and placebo groups. The most significant factors during development may be nutritional and lifestyle factors. Therefore, their overall goal is to ascertain the affect of delayed pubertal development on the mechanism of bone loss at maturity. Density measures alone, although widely used clinically, cannot identify osteoporotic subjects who will sustain fractures, due to the large overlap in bone mass measures in individuals with fractures and those without fractures. Other factors including bone size, architecture and material properties must be considered. They have recently developed a texture analysis approach using Gabor filters, which is capable of providing insight into bone structure from localized texture information on a pixel level. The texture approach is therefore a potentially powerful tool in analyzing trabecular bone texture where orientation, shape and architecture as well as density are the fundamental components. Their previous work was analyzing 2D images but they propose to transfers this approach to 3D images. This novel approach will indicate not only bone mass changes but changes in orientation which may be very significant later in life. In Aim #1, they will test the hypothesis that the mechanism and magnitude of bone loss in a mature animal is dependent on bone development. Specifically, delayed pubertal onset will alter the architecture of bone that will affect the mechanism of bone loss at maturity. Pubertal delay will be completed by gonadotropin releasing hormone antagonist (GnRH-antagonist) injections. At 50 days of age changes in bone morphology will be evaluated using a novel 3D texture analysis. The following biomarkers will be measured to assess the response of pubertal delay on systemic changes in bone metabolism osteocalcin (a marker of bone formation) and N-telopeptide of collagen type I (NTx) (a marker of bone resorption). Serum estradiol and IGF-1 will also be assayed to confirm the hormonal response to the protocol. Flourescent histomorphometry will assess bone formation rates on trabecular bone. At maturity (150 days of age) the experimental rats will undergo ovariectomy surgery to model post menopausal bone loss. Changes in bone morphology will be evaluated using static and dynamic histomorphometry, micro-CT and texture analysis. By using a systems approach relating environmentally induced delayed puberty to bone growth, they propose to gain a new understanding of the important relationship between growth and its variability and the bone structure they become heir to during the aging process.

NEUROLOGY/NEUROSCIENCES

5R21MH084041-02

Identification And Validation Of Human Hypothalamic Nuclei In-Vivo And Ex-Vivo Us

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\$221,250

Hypothalamic nuclei will be identified in living humans using high field magnetic resonance imaging (MRI) (i.e., a 7 Tesla scanner) and in ex-vivo human hypothalamic tissue. The MRI results will be compared with postmortem human tissue to assure methodological validation. These hypothalamic nuclei are key regulators of autonomic and endocrine functions implicated in numerous psychiatric and medical disorders with known sex differences such as depression and schizophrenia. Understanding sex differences and the hypothalamic involvement in relation to neurological, psychiatric, cardiovascular, endocrine and sleep disorders is very relevant for public health in general and women's health in particular. There is increasing evidence regarding the importance of the hypothalamus for understanding women's health and sex differences in relation to neurological, psychiatric, endocrine and sleep disorders. In fact, hypothalamic nuclei, key regulators of autonomic and endocrine functions, are some of the most highly sexually dimorphic nuclei in the brain and implicated in psychiatric and medical disorders with known sex differences. They would argue that an understanding of hormonal effects on the brain and the regulation of other organs and/or systems, such as the cardiovascular and reproductive systems, are critical as downstream effects of hypothalamic activity. Thus an understanding of the neuroanatomy of hypothalamic nuclei and how they are differentially disrupted in men and women in specific disorders will contribute to elucidating sex differences in clinical medicine. However, the identification of hypothalamic nuclei in-vivo in humans has not been realized. This is important since studies have shown the association of the hypothalamus, endocrine dysfunction and sex differences in psychiatric disorders. In fact, the paraventricular hypothalamic nucleus (PVN) is enlarged in patients with major depressive disorder (MDD), in PVN neurons that are dense in corticotropin releasing hormone (CRH) and estrogen receptor (ER)¹. In their recent work in schizophrenia (SCZ) they identified structural abnormalities using MRI in the hypothalamus particularly in the PVN in women. Furthermore, in healthy women they showed, using functional MRI, regulation of brain activity in hypothalamic nuclei such as the PVN, dependent on gonadal hormone changes over the menstrual cycle. The principal focus of this study is to use a new in-vivo methodology for the assessment of the hypothalamus comparing neuroimaging data using 7 Tesla magnetic resonance imaging (MRI) and human postmortem validation. The proposed study aims to identify the PVN in-vivo and ex-vivo in the human hypothalamus using high field MRI, to investigate the relationship of the MRI methodology and the histological technique, and to establish the correlates of the histological structures with the MRI representations. In addition to the PVN, which is critical for its role within the hypothalamic-pituitary-adrenal (HPA) axis and its dysfunction in MDD and SCZ, they will identify the supraoptic nucleus (SON), which will be used as a control region. High-resolution 7 Tesla MRI will be carried out in thirty healthy subjects, and four ex-vivo human hypothalamic samples. Their overarching goal is an innovative methodological one: to identify the PVN of the human hypothalamus in healthy adult women and men in-vivo. They expect this method, once defined, to be applied clinically in subjects with MDD and SCZ.

5R21MH086731-02

Cellular And Molecular Basis Of Hippocampal Atrophy In Depressed Female Monkeys

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\$185,000

Depression is a significant health problem in the US, particularly in women, as 20% of reproductive-aged women experience clinically significant depression. Unfortunately very little research has been conducted in female animal models of depression. The use of the first primate model of adult depression in females proposed here, which has greater similarity to human neurobiology and depression than rodent models, will advance their understanding of the neurobiology of depression especially in women. Clinical and experimental studies suggest that hippocampal volumes may be smaller in individuals with depression, although the cellular mechanisms underlying this relationship are unclear. Stressful life events are associated with an increased risk of depression, and animal models, exposed to chronic stress have been used previously to investigate hippocampal shrinkage in depression. Although the data from preclinical stress models are compelling, the degree to which stress responses in animal models are relevant to human depression remains controversial, particularly since women are at two-fold greater risk of depression and the animal models are mostly male rodents. Evaluation of the causes of reduced hippocampal volume in an experimental model that more closely resembles human depression would be valuable. They have developed a primate model of depression in adult female cynomolgus monkeys which closely resembles human depression, and recently observed that depressed monkeys have relatively small anterior hippocampi. The overall goal of this proposal is to evaluate hippocampal morphologic, cellular, and molecular characteristics in depressed and nondepressed female monkeys to determine whether the smaller hippocampi of depressed female monkeys are accompanied by reductions in neurotrophins and synaptic, spinous, and dendritic integrity. They have a unique and valuable collection of fixed, frozen hippocampi derived from the population of adult female monkeys in which the behavioral and physiological characteristics of depression were studied premortem for 4 years. Using the tissue from 8 depressed and 8 nondepressed monkeys they will determine astrocyte, pyramidal, and granule neuron size and number, and protein and mRNA levels of markers of synaptic, spinous, and dendritic integrity in the cornu ammonis (CA) CA1, CA2, CA3, and DG of the anterior and posterior HC of behaviorally depressed and nondepressed monkeys. The results of this study will establish the use of the model in future investigations of the mechanisms of depression and the efficacy of interventions for depression. The research is particularly responsive to the FOA entitled 'Advancing Novel Science in Women's Health Research' (PAS-07-381). The results of the proposed study will be used in support of a competitive NIH application.

5R21NS066307-02

Sex-Specific Gene Regulation Of Neuronal Chloride Co-Transporter, Kcc2

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\$195,000

Neuronal chloride dictates nerve cells' excitability, and is reduced in chronic pathological pain as well as in certain forms of epilepsy, diseases characterized by therapeutic refractoriness and strong female preponderance. Experiments are described that will elucidate the regulation of the dominant electroneutral chloride transporter of mature neurons, KCC2. Estrogen and xenobiotic

estrogen-mimetics will be used for stimulation of primary cortical neurons in culture, which will be maintained strictly separate for male vs. female, based on a novel methodology platform described here. Neurons derived from late-pregnancy embryos of rats and mice, the latter genetically encoded to lack functional estrogen-receptors, will be subjected to assays probing function and regulation of the *kcc2* gene, namely reporter gene assays and measurement of neuronal chloride. Chronic pathological pain and certain epileptic syndromes are neuropsychiatric disorders that share an increased female prevalence and refractoriness to treatment. The latter feature is considered to be linked to pathologically increased neuronal excitability caused by increased neuronal chloride (Cl⁻), which in turn is rooted in down-regulation of the dominant neuronal Cl⁻-transporter, KCC2, which extrudes Cl⁻. Here they propose experiments to elucidate sex-specific regulation of the *kcc2* gene by estrogens, based on a hypothesis that neuronal Cl⁻ is dysregulated in response to neuronal injury in a sexually dimorphic manner, with the consequence of rendering women more susceptible to the above diseases. They have obtained exciting preliminary results (1) showing that *kcc2* transcription is regulated by the repressor REST/NRSF which binds to a novel RE1/NRSE DNA binding site in *kcc2* regulatory regions, (2) demonstrating this regulation to underlie the early developmental transformation of GABAergic transmission from excitatory to inhibitory, (3) developing a novel method to culture cortical primary neurons from individual rat E17 embryos which are being sex-typed by X- and Y-chromosome specific DNA markers. The latter method, straightforward yet possibly a groundbreaking novelty, permits strictly separate female vs. male primary cortical neuronal culture. They intend to elaborate molecular mechanisms how neuronal Cl⁻ and KCC2 are regulated sex-specifically by exposing male vs. female neurons to 17 β -estradiol and xenobiotic estrogen-mimetics. For this, they will electroporate *kcc2* reporter gene constructs, wildtype and mutated for binding sites, driving a secreted luciferase reporter, which will facilitate establishment of a time-course of *kcc2* transcription. For direct determination of Cl⁻, the fluorescent Cl⁻-indicator clomeleon will be co-transfected. Cultures will be exposed to physiologically relevant concentrations of estradiol and practically relevant concentrations of xeno-estrogens (coumestrol, bisphenol-A, dieldrin). Use of the latter compounds will allow us to address modulation of estrogen responses by these ubiquitous compounds. Any sex-specific regulation will be confirmed in primary cultures derived from gene-targeted mice (estrogen-receptor (ER)- α , - β and non-classical-ER-knockin). These experiments will be conducted in a highly collaborative environment at Duke University, involving molecular and physiology neuroscience labs, in addition molecular endocrinology and environmental toxicology input. Results can be expected to shed new light on a fundamental matter, neuronal Cl⁻-regulation, which very likely has sex-specific regulation as a basis for increased female prevalence in therapy-refractory neuropsychiatric diseases.

1 R21 NS071210-01

Sex differences in the CNS during disease

Voskuhl, Rhonda R

University of California Los Angeles, Los Angeles, CA

\$231,000

This is an exploratory (R21) grant to determine the effect of sex chromosomes and sex hormones on the central nervous system's response to an immune attack using the multiple sclerosis model, experimental autoimmune encephalomyelitis. This proposal will establish a model system to determine the effect of sex chromosomes and sex hormones on a variety of neurological diseases

characterized by a sex difference. Numerous neurological diseases are characterized by a sex difference. The neuropathology often includes infiltration of immune cells, with this immune infiltration potentially contributing to disease pathogenesis. Since it is known that sex differences exist in the immune system, this confounds investigations into sex differences in the CNS. Thus, they will use bone marrow chimeras to investigate sex differences in the CNS. By varying sex chromosomes or sex hormones in hosts reconstituted with a common immune system, one can ascertain the role of sex chromosomes and sex hormones on the brain response to injury. They will use one of the most inflammatory of all CNS disease models, the multiple sclerosis model, experimental autoimmune encephalomyelitis (EAE), to show applicability of this approach to a variety of neurological diseases. They will employ mice which differ in the complement of sex chromosomes (XX vs. XY), while having the same gonadal type, to determine the effect of sex chromosomes in the absence of confounding effects of exposure to different types of sex hormones. Specifically, in aim #1 they will determine whether the greater severity of EAE in XX, as compared to XY-, mice is due to sex chromosome effects in the CNS. In aim #2, they will determine if the sex chromosome effect in the CNS during EAE is due to the dose of X or Y genes. Finally in aim #3, they will use mice which differ in gonadal type, female vs. male, while having the same sex chromosome complement (XX vs. XX Sry) to determine whether the greater severity of EAE in female, as compared to male, mice is due to sex hormone effects in the CNS.

NUTRITION

Y1-CN-501054

National Food and Nutrient Analysis Program (NFNAP)

NCI-USDA

NCI/USDA, Bethesda, MD

\$50,000

The NFNAP is a research program that seeks to achieve sound estimates of dietary components and thus, improvements in nutrient values with particular focus on components with possible roles in human health. The project, directed by the Nutrient Data Laboratory (NDL), Agricultural Research Service, USDA, was initiated in 1997 and recently renewed in collaboration with the NIH National Cancer Institute and the Office of Dietary Supplements, ORWH, and other supporting NIH Offices, Institutes, and the FDA. The primary outcome of the program will be a body of nutrient data representative of the U.S. population intake and consumption patterns with unprecedented analytical quality. This is a collaborative, interdisciplinary project with the NFNAP. Specifically, the two leading causes of death in women in the U.S. are: (1) cardiovascular disease; and, (2) cancer. The NFNAP may prove particularly relevant to these women's health issues because the food consumption and composition databases target those foods that are major contributors of public health significance in the U.S. Specifically, the five objectives of the NFNAP are to: (1) Sample and analyze selected Key Foods; (2) Institute a monitoring program for Key Foods; (3) Develop databases for foods consumed by U.S. ethnic subpopulations; (4) Develop and update databases for bioactive food components; and (5) Develop and validate databases for dietary supplement composition. Moreover, the NFNAP may be significant to research on women's health on several different levels. Better estimates of the mean nutrient content of foods and variance

indicators will permit more accurate assessment of nutrient intakes by individuals. This will improve the ability to detect etiologic relationships, delineate biologic mechanisms, assess time trends in nutrient intake, and define populations at nutritional risk. Further, the NFNAP may provide background data supporting nutritional guidance and communications focused specifically on women.

OBESITY/OVERWEIGHT

5R21HL097252-02

Intervening On Spontaneous Physical Activity To Prevent Weight Regain In Women

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Wake Forest University Health Sciences

\$156,894

Weight loss programs using caloric restriction and regular, structured exercise can lead to a reduction in physical activity performed outside of the planned exercise sessions. This study will test whether or not women reduce their spontaneous daily physical activity more than men with weight loss, and whether this reduction can be prevented by using self-monitoring, thereby slowing or preventing weight regain. Since reductions in spontaneous physical activity could potentially negate the intent of a structured exercise program for weight loss therapy, it would be important to know whether recommendations for weight loss maintenance in women should include promotion of spontaneous physical activity, rather than structured exercise, during and following a period of intensive weight loss. Recommendations for more effective long-term weight loss strategies may need to consider the role of gender differences. If, as shown in female vs. male animal models, negative energy balance resulting in weight loss results in greater compensatory reductions in energy expenditure in women compared to men, obesity treatments may need to be tailored in women to override these reductions in total energy expenditure. Their approach focuses on a behavioral strategy (self-monitoring) to eliminate the compensatory reduction in non-exercise 'spontaneous' physical activity (SPA) seen in women who lose weight by means of a hypocaloric diet and structured exercise training. Their long-term research goal is to establish empirical evidence for innovative treatment options that are more effective in producing weight loss and preventing weight regain in women. The main goal of this pilot is to provide preliminary data and effect estimates to begin to test their overall hypothesis that prevention of weight loss-induced reductions in SPA will be more beneficial for long-term maintenance of weight loss in women than in men. They propose to conduct a pilot study using a 2-arm, 10-month design in 72 obese, older (55-70 yrs) men and women (n=36 per group). Participants will be randomized to a 5-month standardized weight loss intervention involving a hypocaloric diet and aerobic exercise (DIET+EX) or to the same weight loss intervention with addition of a behavioral component that targets self-monitoring (SM) of SPA (SM+DIET+EX), and then followed for another 5 months after weight loss. The specific aims of this R21 exploratory/developmental application are: Primary-To examine whether SPA self-monitoring results in less body weight regain in the follow-up phase in both men and women; Secondary-To examine whether: 1) women regain more weight than men in the follow-up phase; 2) SPA self-monitoring and gender have an effect on change in weight in the intensive weight loss phase; 3) SPA self-monitoring and gender have an effect on change in SPA in the intensive weight loss phase; 4) there is an association between SPA changes in the weight loss phase and weight

regain in the follow-up phase. They anticipate that the results will lead to a larger and longer trial to definitively test their hypothesis, which could potentially provide evidence against the current standard of care (i.e., exclusive prescription of structured moderate-intensity exercise) for obesity therapy in women and may lead to sex-specific treatment guidelines.

PAIN

5R21DE019267-02

Sex Differences In Acute Pain And Analgesic Responses: Psychosocial And Genetic I

Hastie, Barbara A

University Of Florida

\$181,294

Pain is one of the most costly and pervasive public health problems in the United States, and women are at increased risk for under-treatment of pain. This study will use a common acute clinical pain model to identify and characterize psychosocial, physiological and genetic factors that contribute to sex differences in pain perception, analgesia and side effects. The ultimate goal is to develop translational research that will reduce the increased burden of clinical pain in women through the development of tailored interventions designed to enhance the quality of life for women, consistent with priorities of the NIH Office of Research on Women's Health. Pain is one of the most costly and pervasive public health problems, with women and minorities facing increased risk for under-treated and mismanaged pain. Women, compared to men, report more frequent and intense pain and have increased prevalence of debilitating pain across a multitude of conditions. Women also represent the majority of the 40 million outpatient and ambulatory surgeries conducted each year. Acute post-operative pain and under-treatment of pain are well-documented and lead to prolonged recovery and potentially to development of chronic long-term pain conditions. Despite incongruent findings of sex differences in analgesic efficacy, consistent reports show that women experience between 30%-75% more adverse drug reactions (ADRs) compared to men. ADRs can lead to life-threatening complications, discontinuation of pain treatment, prolonged recovery and non-compliance. Recent pharmacogenomic studies have demonstrated that genotype may contribute to sex differences in pharmacokinetic (PK) and pharmacodynamic (PD) responses to certain drugs. Genetic and nongenetic contributions to sex differences in opioid analgesia, related side effects and treatment outcome have received limited attention in the field of pain research. This study will use a common acute clinical pain model to identify and characterize psychosocial, physiological and genetic factors that contribute to sex differences in pain perception, analgesia and side effects. Aim 1 will determine sex differences in perceptual and physiological responses to acute post-operative pain and will examine how those are related to genetic, pre-operative psychophysical and psychosocial factors. Aim 2 will determine sex differences in opioid analgesia and side effects and will examine genetic, PK, PD, and psychosocial factors that explain group differences in analgesic responses. 140 male and female patients (age range 16-45) who undergo third molar extraction will be included in this study. Preoperatively, they will assess experimental pain responses and psychosocial measures. They will monitor post-operative pain levels along with PK/PD responses to the opioid fentanyl. They will examine sex differences in post-operative pain, analgesic responses and side effects immediately and for several hours post-surgery and for 3 days post-procedure. The study is designed to build a foundation for a R01 grant proposal supporting an independent line of

clinically-relevant experimental pain research. This project will enhance understanding of translational research in pain as well as biopsychosocial factors that contribute to health disparities in pain and its treatment, particularly for women. Additionally, this study will provide insight into the complex genetic, PK/PD processes involved in post-operative pain and analgesic responses and will elucidate biopsychosocial contributions to sex differences in pain and side effects. The ultimate goal is to develop translational research that will reduce the increased burden of clinical pain in women through the development of tailored interventions designed to enhance the quality of life for women, consistent with priorities of the NIH Office of Research on Women's Health.

1 R03 AR057489-01A1

Epidemiology of Patellofemoral Pain Syndrome: Identifying Gender Specific Risk Factors
Boling, Michelle Clara

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\$72,866

Patellofemoral pain syndrome (PFP) is one of the most common chronic knee conditions affecting young adults, with an increased occurrence in females. Individuals suffering from this condition may experience symptoms lasting multiple decades, limiting their participation in physical activity, and predisposing them to chronic diseases associated with inactivity such as obesity, arthritis, coronary artery disease, diabetes, and cancer. The results from this investigation may be used to identify those at greatest risk to PFP and develop appropriate prevention programs to decrease the occurrence of this condition, particularly in females.

Patellofemoral pain syndrome (PFP) is one of the most common causes of knee pain, affecting approximately 25% of the physically active population, with females being 2-3 times more likely to develop PFP compared to their male counterparts. The overall objective of this proposal is to determine the mechanical (structural and biomechanical) and non-mechanical (demographic and psychosocial) risk factors that are associated with PFP and identify the risk factors specific to females and males. The approach will be to use a prospective cohort design to identify risk factors that are associated with incident PFP. The central hypothesis is that individuals who develop PFP will have altered movement patterns, abnormal lower extremity anatomical alignments, decreased lower extremity strength, previous history of knee injury, previous participation in a low number of athletic activities, decreased levels of hardiness, and increased number of healthcare visits. A secondary hypothesis is that females and males will have different risk factor profiles. They will utilize baseline risk factor data that has been collected on 5690 freshman (males=3482, females= 2208) during the summers of 2005- 2008 at the following military academies: United States Naval Academy, United States Military Academy, United States Air Force Academy. Baseline risk factor data was collected through a current NIH funded project (R01-AR054061001), entitled JUMP-ACL. Each participant will contribute follow up time for incident PFP until they graduate from their respective academy. Medical record reviews will be performed to identify those participants who developed PFP during their respective follow-up time. Based on the two years for the proposed investigation, follow up time will be 4 years for all participants enrolled in the JUMP-ACL investigation from 2005-2008. Poisson regression analyses will be performed to determine the risk factors for PFP. Additionally, males and females will be analyzed separately to determine gender specific risk factor profiles. The proposed project is making an efficient use of already collected risk factor data by adding analysis of a new outcome (PFP) that would not otherwise be investigated by the JUMP-ACL

project. Additionally, the proposed investigation is cost effective due to no funds being required for baseline data collection. Their rationale for the proposed investigation is that there is a crucial need for prospective studies that identify the risk factors for PFP so that more focused prevention strategies can be developed that are appropriately gender specific.

5 R21 HD053510-03

Pain and Endometriosis: Effects on Ectopic Cyst Innervation and Axons

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\$171,069

In these studies they plan to develop their recently developed model of sciatic endometriosis to make novel inquiries regarding the etiology of endometriosis-related pain. The information that this study will yield stands to improve diagnostic awareness and mechanistic understanding, and thus therapeutic approaches, of the treatment of the symptoms of endometriosis. As a result of this research, consideration and specific examination of nerves within the pelvis during ablative laparoscopic techniques may become an important additional diagnostic procedure for women with endometriosis. Women with endometriosis often have significant pain. Modern studies have implicated the neo-innervation of endometrial cysts as a primary source of this pain. However, the presence of nerve fibers does not necessarily specify their function and cannot determine whether, or in which situations, they are active. There has been no investigation to functionally characterize the effect of endometrial lesions on nerves or on axons. Their laboratory has focused on the effects of inflammation on axons. They have shown that nerve inflammation induces ectopic mechanical sensitivity of nociceptor axons, which are not normally sensitive. Their data also indicate that nerve inflammation induces ongoing activity that arises from both the inflamed site and / or the cell body, and that sympathetic neuronal activity is decreased during nerve inflammation. Recently they adapted the model of rat endometriosis to involve the sciatic nerve. This model is very similar to the rat endometriosis model where a section of uterus is transplanted to an intraperitoneal site. In preliminary data, a uterine section was transplanted to the sciatic nerve. Three complimentary electrophysiological methods are proposed to determine the characteristics of the effect of uterus, endometrium, and myometrium on the sciatic nerve. First, the proportion of through-conducting axons will be determined. Then, teased fiber recordings will be made from the dorsal roots in some experiments and from the distal end of peripheral nerves in other experiments. This combination of methods offers the advantage that sensory and sympathetic axons that pass through the cyst, as well as those innervating the cyst, can be studied. They will determine if the axons passing in close proximity to the cyst or directly innervating the cyst develop ongoing activity and / or mechanical sensitivity. Recordings will be made 3 months post surgically, after the cysts become stable, and the results compared to myometrium or fat transplant, and unoperated nerves. Because preliminary results revealed the presence of intraneural immune cells, they will determine whether the lesions of endometriosis damage axons. Importantly, they will use not only full thickness uterus, but also isolated endometrium and myometrium, and evaluate the viability of these specific tissues to form cysts. Using immunohistological methods, they will determine the extent of neutrophil and macrophage invasion of the nerve-uterus complex. They will also determine if axons are damaged using ninjurin and fluoro-jade, assessing the presence in both axons and dorsal root ganglion cells. These studies will determine the function and thus the importance of the ectopic innervation of endometrial cysts, as well as the effects of the lesions on

through-conducting axons. The results of this study will impact the understanding of endometriosis pain and seed further research into the pain mechanisms of endometriosis.

1 K23 NS070891-01

Functional Networks in Migraine

Schwedt, Todd

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\$163,523

Migraine is an exceedingly common disease, affecting 12% of the population. Migraine results in substantial disability due to headache pain, hypersensitivities to environmental stimuli, and skin sensitization. Unfortunately, migraine is poorly understood and can be difficult to treat. This study will employ advanced neuroimaging techniques to investigate functional networks in the brain that may explain the relationship between migraine headache and hypersensitivities, as well as how environmental stimuli trigger a migraine attack. Description of these functional networks will allow for future investigations into methods to normalize or block activation of these networks, methods that may reduce migraine symptoms and improve the lives of millions of migraine sufferers. Migraine attacks are characterized by moderate to severe pain, nausea, vomiting, increased sensitivity to lights, sounds and odors, and cutaneous allodynia. Migraine, which afflicts 36 million Americans, causes substantial individual and societal burden. Although individual migraine attacks last for several hours to a few days, there is often persistence of hypersensitivities such as photophobia, phonophobia and osmophobia and cutaneous sensitization between migraine attacks. Furthermore, migraine attacks can be triggered by light, noise and odors. The mechanisms for interictal persistence of these symptoms and the mechanisms by which environmental stimuli trigger migraine attacks are unknown. In this set of experiments they address these associations by using functional magnetic resonance imaging to investigate functional networks in episodic and chronic migraine subjects. The specific aims will test the following hypotheses: 1) stimulus-induced deactivation of the default mode network is less in migraine subjects compared to non-migraine controls; 2) migraineurs have stronger functional connectivity among regions of the brain responsible for pain processing and between these pain processing regions and those responsible for processing of auditory, visual and olfactory stimuli; 3) abnormal default mode network deactivation and stronger functional connectivity among pain regions and between pain regions and regions of the auditory, visual and olfactory networks are positively associated with greater migraine burden. It is necessary to establish and assess functional networks in migraine so that future studies can investigate methods to normalize potentially aberrant networks and block activation of these networks, actions that may prevent and alleviate migraine symptoms. The candidate is an Assistant Professor of Neurology and Anesthesiology and Director of the Washington University Headache Center in St. Louis, Missouri. The candidate's short-term goal is to enhance his functional neuroimaging skills so that he can transition from mentored to independent patient-oriented research employing functional imaging to investigate the pain and associated symptoms (hypersensitivities to and triggering of migraine by sound, light and odors and cutaneous sensitization) of migraine. Once the relationships between migraine headache and these associated symptoms are described, the longer-term goal is to explore mechanisms by which to normalize these relationships or block activation of functional networks that lead to migraine symptoms. The multidisciplinary team of world-class mentors and the extensive intellectual and

physical resources available at Washington University will optimize the candidate's training experience and likelihood for successful transition to independent research.

REPRODUCTIVE HEALTH/DEVELOPMENTAL BIOLOGY

5R21AI083954-02

Advancing Research On The Sexually Transmitted Female 'Nuisance' Pathogen *Trichomonas vaginalis*
Carlton, Jane

New York University School Of Medicine

\$211,250

Trichomoniasis, caused by the eukaryotic parasite *Trichomonas vaginalis*, is the most common, non-viral, sexually transmitted infection worldwide, but long considered a female 'nuisance' disease. The goal of this project is to determine the genetic diversity of the parasite in women attending STD clinics in New York City, and to use these extant isolates in the development of a model system for the study of colonization of the vagina. *Trichomoniasis* is the most common non-viral STD, estimated to cause ~174 million infections world-wide each year. The *Trichomonas vaginalis* parasite resides in the urogenital tract of both sexes and can cause vaginitis in women and urethritis and prostatitis in men. However, the disease is known more as a female 'nuisance' condition, which has resulted in a lack of scientific and medical attention and scant interest by public health officials in developing *trichomoniasis* control programs. Acute infections among women are associated with pelvic inflammatory disease and adverse pregnancy outcomes. Most alarming is the recognition that *T. vaginalis* infection appears to increase women's susceptibility to HIV-1 infection. Because of the association between *T. vaginalis* and risk for HIV-1 acquisition, interventions to reduce *T. vaginalis* infection and transmission would likely result in fewer HIV-1 infections. Completion of the *T. vaginalis* genome sequence in 2007 has significantly increased their knowledge concerning the biology and mechanisms of pathogenesis of the parasite, but significant gaps remain. In particular, the genetic diversity of the parasite is not known, i.e. whether the parasite is maintained as a clonal population, or whether genetic exchange occurs between parasites in the urogenital tract. The extent of genetic diversity has implications for the control of the disease, for example it determines how virulent parasites spread or how they may evade a vaccine. The focus of this R21 proposal is to examine the genetic diversity of *T. vaginalis* infecting women attending eight New York City Bureau of STD clinics in inner city areas, and to use some of these isolates to develop a standardized and accessible in vitro model system for the study of colonization of the vagina by the parasite. A panel of polymorphic genetic markers - microsatellites and single copy genes - will be developed using the *T. vaginalis* genome sequence, and used to genotype *T. vaginalis* isolates identified in vaginal swabs taken from women attending the clinics. Knowledge of the genetic diversity and colonization characteristics of the parasite will provide important data points for subsequent studies, for example determining associations between *T. vaginalis* genotypes and the commensal microbes that make up the vaginal 'microbiome'.

3R21HD059015-02S1

The Role Of Gpr54 Signaling In Pubertal Disorders

Bianco, Suzy Drumond Carvalho

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\$65,912

The long term goal of this project is to identify factors that regulate the timing of pubertal onset and reproductive maturation. The identification of GPR54, a G-protein coupled receptor, and its ligand, kisspeptin, as upstream regulators of GnRH secretion has led to intense research to elucidate their roles in the regulation of the reproductive axis. Inactivating mutations in GPR54 cause failure to undergo puberty and infertility. In contrast, early stimulation of this receptor triggers precocious puberty in mice. Their preliminary results indicate that GPR54 is desensitized and internalized in response to continuous kisspeptin stimulation, and that a GPR54 amino acid substitution identified in a female patient with central precocious puberty (a disorder with disproportionately high female incidence) increases GPR54 responsiveness by delaying the desensitization of the receptor. Thus, they hypothesize that the timing of signaling and desensitization of GPR54 is critical for its role in controlling puberty and reproduction, and that amino acid substitutions in GPR54 may affect its responsiveness by interfering with signaling or desensitization, thereby contributing to the clinical presentation. Although G-protein coupled receptor desensitization is generally strongly regulated, no data have been published on GPR54 desensitization. The short term goal of this project is to define the mechanisms underlying GPR54 desensitization, in order to understand how genetic mutations of this receptor affect these mechanisms and hence the timing of pubertal onset and sexual maturation. Specifically, the aims of this proposal are to: (1) Define the mechanisms of GPR54 desensitization, focusing on the roles of phosphorylation and arrestin recruitment; (2) Define the mechanisms of GPR54 internalization, focusing on the roles of arrestin, dynamin, and clathrin; and (3) Define the fate of the internalized GPR54, to determine whether the receptor is directed to lysosomal degradation or recycled back to the plasma membrane. In each case, the effects of two mutations in GPR54, one identified in a patient with precocious puberty, and the other in a patient with hypogonadotropic hypogonadism, on these pathways will be determined. A thorough understanding of the mechanisms underlying GPR54 signaling may uncover the basis of gender differences in normal and abnormal pubertal development, as well as reveal a new array of potential targets of pharmacological manipulation for the treatment and prevention of abnormal pubertal development and possibly other reproductive disorders.

Role of GPR54 Signaling in Pubertal Disorders Narrative

The goal of this project is to define the mechanisms underlying the regulation of GPR54 receptor signaling and desensitization, in order to understand how genetic mutations of this receptor affect these mechanisms and hence the timing of pubertal onset and sexual maturation. These studies are expected to offer important contributions to their understanding of the mechanisms underlying the reproductive disorders in the patients carrying the mutations. These insights, in turn, may contribute to future development of therapies designed to modulate the timing of puberty by manipulating the kisspeptin-GPR54 signaling system.

5R21HD061644-02

Physiological Reactivity To Acute Stress During Pregnancy

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Ohio State University

\$221,012

This study will fill important gaps in their knowledge regarding physiological adaptation during pregnancy and effects of race on such adaptation. Information gained from this study will provide the groundwork for the following: 1) identification of women at greater risk of negative perinatal outcomes; 2) describing physiological mechanisms underlying the link between stress and risk of preterm delivery; and 3) providing interventions designed to reduce the effects of stress and promote healthy pregnancy and fetal development. Preterm delivery, an increasingly frequent occurrence in the United States, is associated with significant family burden and an estimated societal cost of at least \$26 billion per year. In the U.S., the preterm birth rate is 12-13% as compared to 5-9% in other developed countries. Persistent racial disparities contribute to this discrepancy. Psychosocial stress and related physiological sequelae may contribute to preterm birth overall, as well as to racial disparities in preterm birth. The experience of chronic stress, such as that conferred by racial minority status, may sensitize physiological stress responses. Indeed, as compared to Caucasians, African-Americans exhibit greater cardiovascular reactivity to a variety of acute stressors. Importantly, blood pressure, glucocorticoid, and catecholamine responses to acute stress are attenuated during healthy pregnancy as compared to nonpregnancy. This adaptation may protect the mother and fetus from potentially detrimental effects of maternal physiological activation. Thus, women who exhibit greater and more extended physiological reactions to everyday stressors may be at increased risk for negative perinatal outcomes. Notably, no studies of acute stress during pregnancy have examined inflammatory immune responses or mechanisms underlying blood pressure change (i.e., cardiac output, total peripheral resistance). Moreover, limited information is available regarding effects of race on physiological adaptation to pregnancy. The current study will address important gaps in the literature by examining cardiovascular, endocrine, and immune reactivity to acute stress among 40 healthy pregnant women (20 Caucasian, 20 African-American) and 40 demographically matched nonpregnant control women. This research is designed to ultimately lead to the identification of women at greater risk for negative perinatal outcomes and elucidation of mechanisms underlying increased risk, providing a basis for individualized health care services. Specific Aim #1: To utilize more comprehensive and advanced methodology to assess physiological reactivity during pregnancy versus nonpregnancy, including measures of inflammation, impedance cardiography, and glucocorticoid receptor function. Hypothesis #1: Pregnant women will show attenuated physiological responses to acute stress as compared to nonpregnant women. Specific Aim #2: To examine racial differences in physiological reactivity during pregnancy versus nonpregnancy. Hypothesis #2: As compared to Caucasian women, African-American women will exhibit greater physiological reactivity to stress during pregnancy and nonpregnancy. Specific Aim #3: To examine psychosocial correlates of physiological reactivity during pregnancy and nonpregnancy. Hypothesis #3: Women reporting greater distress will exhibit greater physiological reactivity during pregnancy and nonpregnancy. Specific Aim #4: To examine associations between physiological reactivity and length of gestation. Hypothesis #4: Greater physiological reactivity to acute stress will predict shorter gestational length.

1R03HD061853-01A1

Upstream Regulation Of Kiss1 Cells

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University Of Washington

\$78,000

Ovulation depends on a surge in the release of luteinizing hormone (LH), which in turn depends on a surge of gonadotropin-releasing hormone (GnRH). In recent years, kisspeptin (KISS) has emerged as the most potent stimulator of GnRH and a key regulator of reproductive development and health in vertebrates, including humans. In females, KISS signaling to GnRH cells is critical for the induction of the LH surge. Despite the central role of KISS in reproduction and specifically in female reproductive development and fertility, little is known about the upstream regulators of neurons expressing Kiss1, the gene coding for KISS. Here they present preliminary results that indicate that Kiss1 expression and the expression of c-fos within Kiss1 neurons in female mice is under circadian regulation, and this regulation is dependent on the presence of high ovarian estrogen levels. The overall goal of this proposal is to determine the pathways by which the circadian system may regulate the activity of Kiss1 neurons. Their laboratory has developed a rat model of circadian desynchronization in which independent circadian outputs are associated with the desynchronized activity of anatomically identifiable subregions of the hypothalamic suprachiasmatic nucleus (SCN), the site of the mammalian master circadian pacemaker. Their preliminary data on this forced desynchronized rat model indicates that the gating of the luteinizing hormone (LH) surge is associated with the activity of the dorsomedial (dm) SCN irrespective of the activity of the ventrolateral (vl) SCN. Because the dmSCN is the main source of vasopressinergic efferent fibers, their hypothesis is that vasopressin (VP) release is a critical SCN signal to induce the LH surge and therefore to activate Kiss1 neurons in a circadian pattern. They propose experiments that test specific predictions of this hypothesis. They will test the prediction that SCN vasopressinergic fibers innervate Kiss1-expressing cells and that innervation of the Kiss1 neuronal network by SCN efferent fibers is critical to sustain the circadian regulation of Kiss1 and of c-fos expression within Kiss1 neurons, which are concomitant with the LH surge. They will use unilateral lesions of the SCN to ipsilaterally deplete the anteroventral periventricular nucleus of SCN efferent fibers. In these animals they will assess the level of asymmetric VP innervation of Kiss1 neurons as well as the asymmetry in the circadian regulation of Kiss1 expression and c-fos expression within Kiss1 cells. Their proposed studies will characterize the pathways and mechanisms by which the activity of the Kiss1 neuronal network is regulated. Specifically, they will determine how a critical component of the mechanisms leading to ovulation such as the circadian system regulates gene expression within Kiss1 cells. Because the activity of these neurons and the release of KISS are essential for normal ovulation, understanding the upstream regulators of Kiss1 neurons will be key to developing therapies to treat disorders of the hypothalamo-pituitary-ovarian axis.

5R21HD059074-02

A Study Of The Factors Influencing Women's Decision About Childbirth

Regan, Mary J

University Of Maryland Baltimore

\$187,063

This project is focused on building knowledge about what women want from their birthing experience and what informs their choices about mode of birth. This knowledge is essential if

they are to understand the role of maternal demand in use of CS. Their data will inform public health policy concerned with both supporting maternal choice and ensuring long term maternal-child health by reducing the risks associated with childbirth. This study is part of a systematic program of research dedicated to improving women's health and satisfaction with their birthing experience. Cesarean section (CS) is currently used at over twice the rate recommended by the World Health Organization (CDC, 2006); use of the procedure has almost doubled in the last two decades for reasons that are as yet poorly understood. Overutilization results in avoidable morbidity and mortality and higher health costs related to childbirth. Many causes for the increased use of CS have been suggested, including growth in the number of 'maternal requests' - healthy women asking for CS in the absence of medical indications. An NIH expert panel explored maternal request CS and concluded that at this time there is insufficient evidence to warrant CS on maternal demand without medical indications and recommended 'increased research devoted to strategies to predict and influence the likelihood of successful vaginal birth' (NIH, 2006, p. 20). Using the same data, the American College of Obstetrics and Gynecologists (ACOG) concluded that there is no reason to deny a surgical birth to a healthy mother as long as she is well-informed (ACOG, 2003). The divergence between these positions points to a critical gap in knowledge about the factors that drive CS rates, including the influence of maternal demand on the use of CS. Despite this recent focus on maternal demand, there is scant research on what women want from their birthing experience, including their reasons for choosing one mode of childbirth over another. The purpose of their proposed research is to answer the question: what factors influence women's decisions about how their babies will be born? Women's hopes and desires for their first birth experience are influenced by what they know - both consciously and unconsciously. Because people are only partly aware of the attitudes and beliefs that inform their hopes and desires, this proposal will use three methods of data collection. The first is a projective method commonly used in the social sciences to access knowledge that exists outside of consciousness. The second is a focus group method that provides a venue for birthing women to articulate the conscious basis for their ideas about childbirth and allow participants to compare their ideas with others. Third, all women will be interviewed after the baby is born to build understanding about how their experiences influence future birthing choices the women make. Participants will be 50 primigravid women with uncomplicated pregnancies aged 21 or older. This proposal builds on the researchers' previous work related to the use of CS. It is one step in a defined program of research directed towards improving the health of mothers and their children by optimizing care during pregnancy, labor and birth.

5R21ES016846-02

Modulation Of Pah Ovarian Toxicity By Biotransformation Enzyme Polymorphisms

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\$189,823

The primary and long-term goal of this research is to understand how toxicants cause ovarian dysfunction so that they can prevent it. These studies will provide insights that will help us to understand why some women are more sensitive to ovarian toxicants than other women. In so doing, they will also lay the groundwork for possible interventions to protect against ovarian dysfunction. Infertility or impaired fecundity affects 12% of American women. Ovarian dysfunction, including premature ovarian failure is a major cause of infertility. It is likely that

exposure to environmental toxicants is responsible for many more cases of impaired ovarian function than is currently appreciated. Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants, which are known to impair ovarian function and cause ovarian failure in rodents and are probable ovarian toxicants in women. Tobacco smoke, foods, and air pollution are among the sources of exposure to PAHs. The mechanistic basis for interindividual variation in susceptibility to PAH ovarian toxicity is not understood, but polymorphisms in enzymes that metabolize PAHs likely play an important role. The work outlined in this proposal will demonstrate the feasibility of a larger study to test the hypothesis that genetic variations in Phase 1 and Phase 2 biotransformation enzymes involved in metabolizing PAHs modulate the ovarian toxicity of PAHs in women. Specific Aim 1: To test the feasibility of prospectively measuring time to pregnancy and PAH exposure and of using genomewide genotyping methods to determine PAH biotransformation enzyme polymorphisms for a study analyzing the associations between PAH exposure and biotransformation enzyme polymorphisms and fecundability (time to pregnancy). Specific Aim 2: To test the feasibility of using microelectronic dipstick monitors to measure daily urinary reproductive hormone concentrations over multiple menstrual cycles for study of the associations between PAH metabolizing enzyme polymorphisms and PAH exposure and menstrual cycle abnormalities. Specific Aim 3: To pilot test serum anti-Müllerian hormone, follicle stimulating hormone, and inhibin B concentrations as markers of ovarian reserve for study of the associations between PAH exposure and diminished ovarian reserve.

5R03NS063233-02

Neuroactive Steroids And Seizure Control During Pregnancy In Women With Epilepsy

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Brigham And Women's Hospital

\$101,800

Treating women with epilepsy during pregnancy requires a precarious balance of controlling the mother's seizures without exposing the developing fetus to more anticonvulsant medication than necessary. During pregnancy, the rising sex steroid hormones and their metabolic byproducts may directly influence seizure control; this study will analyze blood samples already obtained in women with epilepsy during pregnancy to examine whether women with increased seizures have alterations in neuroactive steroid levels. These findings could lead to the development of novel treatment strategies for women with epilepsy during pregnancy, such as use of supplemental progesterone, to improve mother and child health outcomes. Epilepsy is a common disorder, affecting approximately 1.3 million women of child-bearing age in the United States. Seizures during pregnancy can cause increased risks to both the mother and fetus. These risks have to be balanced against the known teratogenic effects of antiepileptic drugs (AEDs). During pregnancy, the sex steroid hormones estradiol and progesterone increase dramatically. Sex steroid hormones and the metabolic byproducts that are capable of modifying neural activity are classified as neuroactive steroids (NAS). Animal models demonstrate modulation of seizure activity by the NAS 17 β -estradiol (EST), progesterone (PROG), and allopregnanolone (ALLO). In women, fluctuations in these NAS have been implicated in seizure control in the non-pregnant state, with worsening seizures at certain times of the menstrual cycle (catamenial epilepsy). Human studies have demonstrated an increase in seizure frequency with elevated EST/PROG ratios and with declining or low PROG levels. This has not been studied during pregnancy in women with epilepsy. This proposed study will utilize serum samples (n=810 samples) already collected from

135 women with epilepsy during different stages of pregnancy during a Specialized Center of Research in Women and Gender Issues program project grant. These women were enrolled prospectively with tracking of seizures and medications. Collection of plasma samples occurred at multiple points in each trimester. Based on variable points of enrollment (< 20 weeks gestation), they have increased observations/samples in the later trimesters of pregnancy. Seizure frequency will be analyzed during the second and third trimesters of pregnancy and compared to the nonpregnant baseline for each subject. Consistent with the R03 mechanism, the current application will extend the analysis of these existing data/samples via measurement of the neuroactive steroids EST, PROG, and ALLO. The working hypotheses are 1) during pregnancy, changing concentrations of EST and PROG influence seizure control; 2) the progesterone metabolite, ALLO, mediates the seizure-reducing effect of PROG. The following will be analyzed in relationship to change in seizure frequency during pregnancy: EST/PROG ratio, the rate of rise of PROG, and the rate of rise of ALLO. Additionally, given that labor and delivery is a particularly vulnerable time for increased seizures; ALLO and PROG levels will be compared between women who had peripartum seizures and those who did not. This study can ultimately lead to a better understanding of the NAS regulation of seizure control during pregnancy. Insights gained from this study could provide the impetus for further development of NAS analogs, with treatment benefits extending to both genders and across all ages. During pregnancy, treatment with supplemental progesterone could allow for decreased levels of fetal exposure to AEDs in utero, with improved seizure control and reduced anatomical and neurodevelopmental teratogenicity.

1 R01 HD064398-01

Genetic Determinants of Uterine Fibroids in African-American and Caucasian Women

Aissani, Brahim

University of Alabama, Birmingham, AL

\$83,333

Currently, the only effective and non-invasive therapy to treat uterine fibroids is a hormone (gonadotropin releasing hormone) - based therapy with serious side effects. The knowledge to be gained from this study could, at some point in the near future, lead to the development of the first genetic counseling protocol for fibroids and ultimately to a more appropriate therapy. Uterine leiomyomas (ULs) are the most common pelvic tumors in women of reproductive age, accounting for over 600,000 hysterectomies annually in the United States. Several lines of evidence support a genetic liability in the pathogenesis of ULs, yet no susceptibility gene is known. Advances in research on the genetics of ULs (fibroids) have so far been limited by the paucity of genetic epidemiologic studies and infrastructure to conduct them. The goal of this epidemiologic study is to evaluate the contribution of a region of Chr.1q43 that predisposes to uterine fibroids but remains inadequately investigated. Genetic predisposition to ULs has been studied primarily in the context of two rare inherited autosomal-dominant conditions, the hereditary leiomyomatosis and renal cell cancer (HLRCC) and the multiple cutaneous and uterine leiomyomatosis (MCUL1) syndromes, where germline mutations were found in the gene on Chr. 1q43 encoding the tricarboxylic acid cycle (Krebs cycle) fumarate hydratase (FH) enzyme. However, a direct role of this important metabolic housekeeping gene in tumorigenesis remains to be proven. Inactivating FH mutations have rarely (< 1-2% of the tumors analyzed) been observed in nonsyndromic (common) ULs; however, loss of FH appears to be a significant event in the pathogenesis of a subset of these tumors. Furthermore, several observations support

the existence of an alternative or additional candidate gene on Chr.1q43 acting alone or interacting with FH to increase the risk of ULs in susceptible individuals: 1) the absence of FH genotype-phenotype correlations, 2) the marked genetic heterogeneity in ULs, and 3) the failure to observe ULs or multiple leiomyomatosis in siblings or parents of cases with fumarase deficiency, a severe recessive disorder. Taken together, these observations underscore the importance of exploring an extended FH region in a population-based study of ULs. To this end, they will generate a high-density single nucleotide polymorphism genotyping data across a 2-Mb region spanning FH in subsets of African American (n=582) and Caucasian (n=455) women enrolled in the NIEHS-Uterine Fibroids Study. This is a well designed cross-sectional study of ULs that includes data on most potential confounders. Their study is not intended to shift any paradigm about the origins of ULs; rather it will extensively investigate the role of FH in nonsyndromic ULs, dissect the intricate genetic correlates of Chr.1q43 markers in the expression of the disease phenotype and evaluate their effects in two populations with a marked difference in disease risk. Recent updates in the genome databases have revealed new potential candidate genes for tumor growth and important structural variations including a large (~ 308 Kb) copy number variation in the vicinity of FH; these new findings further justify a study with the proposed depth and extent of genetic coverage. This study will likely open new avenues for research and may ultimately redirect current preventive and therapeutic approaches or enhance their efficacy.

5 U10 HD054215-05

The Cleveland Clinic Clinical Site

Barber, Matthew

Cleveland Clinic, Cleveland, OH

\$25,000

Pelvic floor disorders (PFD) including urinary incontinence, pelvic organ prolapse (POP), and fecal incontinence affect a substantial proportion of women in the U.S. PFD result in significant psychosocial costs to an individual and their aggregate social and economic costs to society are enormous. Despite their substantial health impact, the quality of the evidence supporting most of the commonly used treatments, especially surgical interventions, is limited by the lack of standardization of diagnostic and therapeutic interventions, use of non-standardized and non-validated outcome measures, poor quality research designs, and inadequate power to detect clinically meaningful differences. The long-term objective of the Pelvic Floor Disorders Network (PFDN) is to identify optimum diagnosis and management strategies for women with PFD using the highest quality research methods available. The specific aims of this application are: 1) to demonstrate that the Cleveland Clinic Foundation (CCF) possesses the personnel, patient, clinical, and administrative resources needed for successful participation as a Clinical Site in the PFDN; and that their participation would be advantageous to the successful attainment of the Network's scientific goals and 2) to present a concept application for potential conduct by the PFDN. The broad, long-term objectives of their concept application are 1) to compare sacrospinous ligament fixation (SSLF) to uterosacral vaginal vault fixation (USWS) and 2) to assess the role of perioperative pelvic floor physiotherapy (PFPT) in women undergoing transvaginal surgery for apical or uterine POP. Their Specific aims are to: 1) compare the anatomic outcomes of SSLF to USWS in women undergoing transvaginal surgery for Stage 2-4 POP involving the vaginal apex or uterus 3 years after surgery; 2) compare functional, sexual, and health-related quality of life (HRQOL) outcomes of SSLF to USWS in the same women 3

years after surgery; 3) assess whether short-term functional, sexual, and HRQOL outcomes improve in women receiving PFPT perioperatively compared to those who receive surgery alone; 4) assess whether perioperative PFPT improves anatomic, functional, sexual and HRQOL outcomes 3 years after surgery (long-term) compared to surgery alone and 5) determine the incremental cost-effectiveness of perioperative PFPT at the time of transvaginal surgery for POP. They present a collaborative multi-centered randomized trial comparing SSLF to USSVS with or without perioperative PFPT using a 2x2 factorial study design. A standardized common protocol for enrollment, treatment and data collection will be employed by 6-8 Clinical Sites within the PFDN coordinated by the data coordinating center.

5 K24 HD060687-02

Midcareer investigator award in patient oriented research

Barnhart, Kurt T

University of Pennsylvania, Philadelphia, PA

\$187,466

The recruitment and retention of productive junior investigators is one of the more critical priorities of academic medical Institutions and the research community in general. The purpose of this Midcareer Investigator Award In Patient-Oriented Research is to provide support for Kurt Barnhart MD, MSCE, a reproductive endocrinologist and epidemiologist at the University of Pennsylvania. Dr. Barnhart is an accomplished clinical investigator with continuous NIH support since he has joined the faculty at Penn in 1996. He has also been recognized as an outstanding mentor. The candidate's immediate and long-term career goals center on his desire and intention to continue to evolve and mature as a patient-oriented researcher, teacher, and mentor. In doing so, he needs to be able to enhance and focus his efforts on conducting patient-oriented research (POR), and building a clear training and mentoring path for those interested in POR in women's health. This award will be essential to allow him to achieve these goals by protecting 50% of his time by reducing his clinical and administrative duties. He will also reduce effort on some of his funded projects while concomitantly increasing the effort of junior faculty he currently mentors. Mentoring: Dr. Barnhart will focus his mentoring on scholars enrolled in the Masters of Science in Clinical Epidemiology (MSCE) via the NIH supported T32 Reproductive Epidemiology training grant. Candidates for this program include fellows in sub-specialties in women's health, family medicine and/or pediatrics. Other mentees will include junior faculty, and fellows in Reproductive Endocrinology and Infertility. He plans to serve as primary thesis mentor for some, a research mentor for others, and will transition to become PI of the T32 training grant. Research Plan: New science proposed in this application will evaluate the short term and long term consequences of assisted reproductive technology (ART), a priority area of research for the NIH. In a series of three specific aims he will investigate the association of ART with short term perinatal morbidity and childhood development. These three aims were chosen for the ability to adequately design and conduct the study in a reasonable period of time, each with a specific hypothesis that would lead to important information. Complementary, diverse, and sophisticated research methods have been proposed to address this important research area, with focus on overcoming inherent limitations in imperfect datasets and potential bias in nonrandomized studies. SA#1 will use the national SART database to test if a fresh embryo transfer is associated with increased adverse outcome compared to frozen embryo transfer. Aim #2 is use a three arm cohort study assessing childhood development in children conceived with IVF, superovulation or without medical assistance. SA #3 will use a large administrative dataset to link mothers and

children and assess for autistic spectrum disorder in true population setting. These aims are designed to advance the skills of the PI, enhance multidisciplinary research and provide optimal opportunity for mentorship. Finally these aims will likely provide evidence to be used to design larger trials, hopefully using the growing cadre of reproductive epidemiologists and POR researchers in women's health nationwide, many of whom will have been mentored by Dr Barnhart.

5 U10 HD041250-10

The Pelvic Floor Disorders Network

Brubaker, Linda

Loyola University, Chicago, IL

\$25,000

Loyola is a productive, innovative clinical research institution that has contributed to the first cycle of the Pelvic Floor Disorders Network and they are eager to build on the PFDN's excellent start. Their application documents: I. The Qualifications and Commitment of Institution and Key Personnel at Loyola A qualified and committed institution with a multidisciplinary faculty with experience in clinical trials design and conduct. A highly qualified and committed research team Lead by the same PI, Dr. Brubaker, this research team contains urogynecologists and urologists. Two of the faculty members received Master's Degrees in Clinical Research Design and Statistical Analysis and one is currently in this degree program. A cadre of study coordinators are cross-trained to meet the needs of the PFDN study roster. The team has a excellent collaborations within the Loyola faculty. II. Loyola's Participation in PFDN Protocols and Procedures High quality participation in PFDN protocols with excellent and consistent recruitment. They also demonstrate their consistent contributions in PFDN work, including dissemination of PFDN scientific findings. Loyola has been productive and has worked well with the PFDN team. Their first cycle application proposed the essence of the CARE trial, which was completed ahead of schedule and is under consideration for publication. III. A Feasible, Scientifically Relevant Concept Protocol (Randomized Surgical Trial): They believe they have demonstrated their ability to design and conduct high quality clinical trials. This application also describes a randomized surgical trial for women who select vaginal apical reconstruction. A comparison of the two most common techniques may inform a future study which seeks to determine which route of surgery (abdominal vs. vaginal) is best suited for an individual woman. This trial is a feasible, scientifically relevant randomized surgical trial. The draft protocol is suitable for PFDN Steering Committee discussion and revision, prior to implementation.

5 P01 HD057877-02

UTERINE Leiomyoma Res Ctr

Bulun, Serdar E.

Northwestern University, Chicago, IL

\$250,000

Symptomatic uterine leiomyomata affect millions of US women and cause irregular uterine bleeding, anemia, recurrent pregnancy loss leading to more than 200,000 hysterectomies per year. Available treatments are limited due in large part to the fact that the mechanisms regulating the development and growth of these tumors are unclear. They propose integrated molecular, cellular and translational studies that should lead to a better understanding and future development of novel therapeutics for uterine leiomyomata. Uterine leiomyomata (fibroids)

represent the most prevalent benign gynecologic disorder in the US. The cellular and molecular mechanisms regulating the development and growth of leiomyoma are not well understood. Their multidisciplinary team has designed 3 well-integrated projects focusing on Interactions between biologically critical hormonal pathways in uterine leiomyoma involving the transcription factors progesterone receptor (PR) and FOXO, the signaling pathway PI3K/AKT and the pro-fibrotic factor TGF-beta. Project I (Bulun) will be pursued to understand the mechanisms as to how anti-progestins such as RU486 reduce tumor size. They hypothesize that progesterone regulates a number of critical genes, that favors increased proliferation and decreased apoptosis of leiomyoma smooth muscle cells, whereas anti-progestins reverse this effect by enhancing apoptosis and decreasing proliferation. Project II (Kim/Chakravarti) will determine the role of the PI3K/AKT/FOXO signaling pathway regulating leiomyoma cell growth and survival in response to progesterone. They hypothesize that progesterone Induces proliferation of leiomyoma cells through activation of the PI3K/AKT/FOXO signaling pathway and that Inhibitors of the AKT pathway should override the proliferative effects of progesterone and promote apoptosis. Project III (Nowak) will define the mechanisms as to how antifibrotic drugs regulate leiomyoma growth. They hypothesize that the increased proliferation exhibited by leiomyoma smooth muscle cells Is due to a major shift in the extracellular matrix environment caused by increased synthesis of new, monomeric collagen type I by these cells. They will determine whether antifibrotic drugs may be an effective new treatment for leiomyomas. These projects are supported by an Administrative Core (Bulun) and Tissue Procurement and Cell Culture Core (Kurita). Overall, as part of their long range goal, all projects investigate local hormonal signaling regulating apoptosis and proliferation as biologic endpoints and test existing and upcoming pharmaceutical compounds that target these pathways in uterine leiomyomata.

5 R01 HD061821-02

Identification of Genes Predisposing to Pelvic Floor Disorders

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\$66,667

This research has a major potential to affect public health in the prevention of PFDs: they may be able to identify high risk populations who can be identified at a young age, studied and possibly targeted for prevention; and at a later stage in the development of PFDs, special interventions can be studied and possibly implemented in women at risk for recurrence of their condition. Someday, identification of these high risk populations may be as general as familial risk, or as specific as specific gene screening. The investigators propose a unique and powerful collaboration between basic and clinical scientists in Utah to identify genes affecting predisposition to pelvic organ prolapse (POP). The co-PIs both have significant experience, Dr. Norton in Pelvic Floor Disorder (PFD) genetics and Dr. Cannon-Albright in predisposition gene identification. The investigators will access the Utah Population Database, a computerized genealogy of Utah combined with decades of medical data from the two largest healthcare systems in Utah (serving 90% of the state), to identify and recruit surgically treated cases of POP (1,250 cases in 5 years). All POP cases sampled will be genotyped with the Illumina 610Q SNP marker set. The PIs will apply multiple different genetic analyses to this resource of genotyped POP cases to aid in the identification of predisposition genes. The record linkage of medical procedure codes (identifying surgeries performed on each patient) to individual genealogy data allows us to identify all genetic relationships among the POP cases. They will perform genome-

wide association analysis, using software they have developed which allows inclusion of both independent and related cases. They will identify all genetic relationships between the sampled POP cases and perform linkage analysis in informative, high-risk POP pedigrees. They will identify chromosomal regions shared Identical by Descent (IBD) in very distantly related cases in these pedigrees, and they will identify IBD sharing within the small subset of POP cases (2%) who are inbred. Initial collaborative analysis of data obtained by Dr. Norton's NIH funded study of affected PFD sib-ships has already provided significant evidence for a predisposition gene localization on chromosome arm 9q, and suggestive evidence for at least one other locus on chromosome 1. In summary, they will create a population-based resource of surgically treated POP cases, they will pursue established and new methods to identify and localize predisposition genes affecting POP, and they will begin a detailed search for the chromosome 9 gene they have localized.

1 R01 HD061541-01A1

Cellular Mechanisms of Amniotic Fluid Volume Regulation

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Oregon Health and Science University, Portland, OR

\$200,000

The proposed studies will generate a new level of understanding for the mechanisms of amniotic fluid volume regulation. This knowledge is crucial in the development of therapies for the treatment of pregnancy complications due to abnormalities in amniotic fluid volume. The studies will ultimately lead to improvement in the clinical management of oligohydramnios and polyhydramnios, thereby reducing perinatal and neonatal morbidity. A normal volume of amniotic fluid (AF) is essential for normal fetal development with favorable perinatal outcome. However the mechanisms that regulate AF volume and the factors that maintain the volume within the physiological range are not well understood. The current understanding suggests that the transfer of AF water and solutes across the fetal membranes into fetal blood vessels that vascularize the surface of the placenta is the pathway where regulation occurs. This intramembranous (IM) pathway for AF absorption is constituted by an active bulk transport component and a passive diffusional component. In addition, the active process is regulated by stimulatory and inhibitory factors in the AF and amniotic membrane. Although the existence of these regulatory factors has been proposed, their identity and mechanisms of action are not known. In this application, they propose to elucidate the cellular pathways of IM transport and decipher the factors that regulate these pathways. These studies will be carried out in ovine amnion cells in vitro and chronically catheterized ovine fetuses in vivo. In Specific Aim 1, they will identify the cellular pathway for transport of solutes across amnion cells and test the hypothesis that AF transport is a vesicular transcytotic process via caveolae. They will investigate the role of VEGF165 as a stimulator and VEGF165b as an inhibitor of caveolar transport, as well as the effect of the soluble VEGF receptor 1 (sVEGFR-1) in antagonizing VEGF bioactivity. Specific Aim 2 will determine VEGF165 and VEGF165b mRNA and protein levels in amnion cells and amniotic membranes under conditions of normal, increased or decreased IM absorption rates. The correlation of VEGF165 levels with sVEGFR-1 will be determined. In Specific Aim 3, they will examine the VEGF165 activation of caveolar transcytosis by induction of VEGF receptor 2 to initiate a c-Src signaling pathway leading to downstream activation of cavelin-1 and dynamin-2 as required for caveolar endocytosis and transcytosis. The involvement of other signaling proteins including protein kinase C and

phosphatidylinositol 3-kinase will be explored. Specific Aim 4 will investigate the expression of the water channel proteins aquaporin 1, 3 and 9 in amnion cells and determine their effects on passive and active transport across amnion cells. In Specific Aim 5, they will evaluate the in vivo function of the stimulator VEGF165 and the inhibitors VEGF165b and sVEGFR-1 in modulating IM absorption rate in ovine fetuses under conditions of normal, increased or decreased AF volume. They anticipate the in vivo results to support the in vitro findings that VEGF165 is an important determinant of IM absorption and that its stimulatory effect is antagonized by VEGF165b and sVEGFR-1. Overall these studies will elucidate the transcellular vesicular pathway for AF transport and determine the stimulatory and inhibitory regulatory factors that modulate this pathway in amnion cells. Further, the signal transduction cascades that mediate these transport events will be investigated. The findings will lead to an improved understanding of the etiology of amniotic fluid volume abnormalities.

1 S06 GM092238-01

Oklahoma Native American Research Centers for Health (ONARCH VI)

Grim, Gloria Ann

Cherokee Nation, Tahlequah, OK

\$100,000

The purposes of this project are: to encourage competitive research linked to reducing health disparities; to increase the capacity of the Tribes and University of Oklahoma to work in partnership to reduce distrust by the Native American communities and peoples toward research; and to develop a cadre of Native American scientists and health professionals engaged in biomedical, clinical and behavioral research that is competitive for NIH funding. The sixth Oklahoma Native American Research Center for Health (ONARCH6) continues the productive research and training partnership with the University of Oklahoma Health Sciences Center (OUHSC) by the Tribes, especially the Chickasaw, Creek, Choctaw and Cherokee Nations. Population served consists of 42,749 Chickasaws and 121,680 Cherokees, 49,714 Choctaws and 30,181 Creeks for a total of 244,324 in North East and South Central Oklahoma. The research will include 1) the impact of infections on maternal and child health in Native Americans, 2) research to develop better diagnostic and prognostic tests for rheumatic disease in Oklahoma tribal members, and to examine the potential roles of environmental triggers for autoimmunity focusing on vitamin D levels, tobacco smoke exposure (through serum cotinine levels) and abnormal immune responses to common viruses, 3) research to prevent excessive gestational weight gain in otherwise healthy but overweight Native American women and consequently decrease the proportion of women who gain in excess of the guidelines has the potential to decrease the risk and costs of obstetric complications associated with excessive weight gain, and 4) to develop methods to understand attitudes, beliefs, and perceived barriers or motivators to organ/tissue donation among American Indians living off-reservation.

5 S06 GM087165-02

Research to Improve Preconception Health of Adolescent Women

Jumping Eagle, Sara

Oglala Lakota Oyate, Pine Ridge, SD

\$128,436

The Oglala Sioux Tribe, in partnership with Stanford Research/University of South Dakota School of Medicine and the Oglala Lakota College, will be addressing priority health issues

identified by the tribe and to support and expand the research capacity and infrastructure that will build on the research foundation that has been developed within the tribe over the past decade.

1 R01 HD064402-01

Xenograft Study on Growth-Control of Human Uterine Leiomyomata

Kurita, Takeshi

Northwestern University, Chicago, IL

\$83,333

A better understanding of how uterine leiomyomata grow is essential to developing novel therapies for this tumor. While the dependency of uterine leiomyoma on ovarian steroids is well established, the relative importance and function of 17 β -estradiol versus progesterone are yet to be clarified. Recently, they developed a method of growing human uterine leiomyoma tumors in immunodeficient mice. The xenografts of human uterine leiomyoma faithfully preserved the phenotype and hormone responsiveness of original human tumors in situ, and their growth was totally dependent on progesterone and 17 β -estradiol. Using this novel xenograft model in combination with viral gene transduction, they will elucidate the cellular and molecular mechanisms of human uterine leiomyoma growth. The ultimate goal of this study is to elucidate the molecular mechanisms of uterine leiomyoma (UL) formation and growth, and identify potential targets for novel therapeutic and preventive treatments of this disease. UL is a benign tumor of the myometrium that affects millions of reproductive-age women. Surgical removal of the entire uterus (hysterectomy) is the primary treatment option, and management of UL puts an enormous burden on the healthcare system. Therefore, finding a new therapeutic treatment replacing surgery is of great interest to the public. Due to the absence of a proper research model system reflecting characteristics of the original tumors, the biological nature and the causes of UL are poorly understood. Although growth dependency of UL on ovarian steroids (17 β -estradiol and progesterone) is well established, the relative importance and function of 17 β -estradiol and progesterone are yet to be clarified. In spite of accumulating evidence for the essential role of progesterone in UL growth, no research model has clearly demonstrated a growth-promoting effect of progesterone on UL. To elucidate the function of ovarian steroids in UL, they have established a novel xenograft model in which tissue fragments of human leiomyoma were grafted beneath the renal capsule of immunodeficient mice. The size of the leiomyoma xenografts increased in response to 17 β -estradiol and progesterone as demonstrated by cell proliferation and accumulation of extra-cellular matrix. In contrast, xenograft growth induced by 17 β -estradiol and progesterone was blocked by the anti-progestin RU486, indicating the essential role of progesterone and progesterone receptor (PR) in leiomyoma tumor growth. Previously, 17 β -estradiol has been thought to be the primary stimulus for UL growth. Surprisingly, 17 β -estradiol by itself neither increased nor maintained tumor size. Likewise, progesterone alone did not affect UL growth in this model. Although not mitogenic, 17 β -estradiol was required for expression of PR, and was essential for progesterone to act on UL xenografts. Their study clearly demonstrates the pivotal role of progesterone in growth and maintenance of UL. The results of their xenograft model agree with clinical observations, yet radically change the paradigm of steroid hormone-regulated human UL growth by emphasizing the importance of progesterone instead of 17 β -estradiol. Using the novel xenograft model, they will elucidate the cellular and molecular mechanisms of human UL tumor growth controlled by 17 β -estradiol and progesterone.

5 R01 HD061811-02

Comprehensive Evaluation of Prolapse Meshes by an Interdisciplinary Research Team
Moalli, Pamela A.

Magee-Womens Research Institute, Pittsburgh, PA

\$66,667

Prolapse (i.e., abnormal descent) of the pelvic organs is a common costly condition that negatively impacts the lives of millions of women world-wide. Biologic and synthetic meshes are often used in the surgical repair of prolapse due to improved anatomical outcomes over native tissue repairs; but with little scientific data on which to base the selection of a particular product. Unfortunately, the complications associated with certain meshes cause unacceptably high rates of morbidity including infection, tissue contraction, vaginal discharge, and pain. In this proposal, they aim to establish a comprehensive mesh testing center in which previously or newly marketed prolapse meshes can be objectively tested and the next generation of prolapse meshes can be developed based on specific scientific criteria. Each year roughly 200,000 U.S. women undergo a surgery to repair pelvic organ prolapse. Biologic and synthetic meshes are widely used in prolapse repairs to improve anatomical outcomes over native tissue repairs which currently have a failure rate of over 30%. To date, however, there is little scientific data to guide surgeons in the selection of a particular product. As a result, meshes are used based on the recommendations of a local vendor and consequently, are placed in women on a trial and error basis. There is growing evidence, however, that the complications associated with prolapse meshes cause unacceptably high rates of morbidity including infection, mesh shrinkage, mesh erosion, mesh exposure, pelvic, rectal and bladder pain and dyspareunia. Such complications have become significant enough for the FDA to recently release a warning about mesh use, especially when it is placed transvaginally. In this proposal, they therefore, aim to establish an interdisciplinary team of scientists dedicated to the comprehensive testing of previously or newly marketed prolapse meshes and for the development of the next generation of graft materials based on specific scientific criteria. In the first phase of the study, they determine how biochemical and structural changes in the prolapsed vagina impact passive and active mechanical behavior so as to develop a mesh in which these deficiencies are repaired or compensated for, allowing us to restore the prolapsed vagina to the nonprolapsed condition. In the second phase, they hypothesize that the shortcoming of current prolapse meshes is that they are too stiff. While this results in a repair with increased tensile strength, it occurs at the expense of tissue function with accelerated tissue contraction, decreased elasticity and compliance, and deterioration of smooth muscle function. To test their hypothesis, they implant commonly used synthetic prolapse meshes into the vagina of nonhuman primates with prolapse using the gold standard surgical procedure (the abdominal sacrocolpopexy) and then define the cellular, biochemical and biomechanical impact on the vagina at 6 months post implantation. Eventually, they will implant meshes transvaginally to characterize the distinct host response to this surgical approach. In the third phase, they explore the development of future grafts for prolapse surgery. They hypothesize that because of its bioinductive effects, a combined biologic/synthetic mesh will be superior to a synthetic mesh alone in restoring vaginal structure and function. They propose that a key yet poorly developed component of prolapse repairs is the re-establishment of smooth muscle reactivity and therefore, test the use of a temporary biologically active scaffold in achieving this process. In this way, this grant proposal provides a mechanism to establish the first team of scientists dedicated to the comprehensive unbiased evaluation of prolapse meshes as a means of educating both current and future prolapse surgeons, and the public regarding potential problems

associated with certain materials. Indeed, the development of such a group is imperative for protecting the health of women.

1 R01 HD060530-01A1

Genetic Studies of Uterine Leiomyomata

Morton, Cynthia Casson

Brigham and Women's Hospital, Boston, MA

\$83,333

The importance of this research is to further their understanding of the biology of uterine leiomyomata. Uterine leiomyomata, or fibroids, are the most common pelvic tumors in females and occur in a minimum of 20-25% of women of reproductive age. Uterine leiomyomata may serve as an important model system to study the genetic events that distinguish benign and malignant neoplasms. A more complete understanding of the genes involved in the pathogenesis and pathobiology of uterine leiomyomata will provide a foundation for future diagnosis, management and treatment of uterine fibroids. Uterine leiomyomata, or fibroids, are the most common pelvic tumors in females and occur in a minimum of 20- 25% of women of reproductive age. Although benign neoplasms, they constitute a major public health problem as 25-50% of affected women experience debilitating symptoms including excessive menstrual bleeding and pelvic discomfort as well as reproductive failure. Fibroids are the major indication for hysterectomy accounting for over 200,000 procedures annually in the United States. It is highly likely that there is a genetic liability to develop fibroids; they are at least three times more frequent in African American than Caucasian women (representing a serious health disparity) and twin-pair correlations for hysterectomy in monozygotic twins are about twice that observed in dizygous twins. Despite these findings and enhanced research in this area in recent years, much remains to be known about this racial predisposition and specific genes involved in the pathogenesis of fibroids. Also of particular interest and of unknown molecular mechanism, fibroids rarely proceed to their malignant counterpart, uterine leiomyosarcoma. Thus, it follows that uterine leiomyomata may serve as an important model system to study the genetic events that distinguish benign and malignant neoplasms. Consistent chromosome aberrations have been observed in fibroids indicating the location of genes involved in these tumors. A number of cytogenetic subgroups have been identified and they have been successful in using positional candidate gene approaches in determining that two high mobility protein genes, HMGA2 and HMGA1, located on chromosomes 12 and 6, respectively, participate in the pathobiology of uterine leiomyomata, in addition to MYST4, located on chromosome 10. The major goal of this proposed application is to further their understanding of the biology of uterine leiomyomata. Experiments are focused on continuing to develop and use a uterine leiomyomata tissue bank and database for gene discovery, gene expression studies, and genotype-phenotype correlations. A variety of molecular and cytogenetic approaches will be used in the identification, isolation and characterization of genes involved in the pathogenesis and pathobiology of uterine leiomyomata. Chromosomal rearrangements in tumor cells will provide biological landmarks for positional cloning experiments. Transcriptional profiling offers a powerful approach to discriminate genes that differentiate fibroids of different cytogenetic subgroups as well as fibroids of variant histologies from their normal smooth muscle counterpart, the myometrium, or their malignant counterpart, uterine leiomyosarcoma. Lastly, the potential role of sequence variants in HMGA2 will be explored by a variety of mechanistic experiments to assess their role in uterine leiomyomata.

5 U10 HD054214-05

Pelvic Floor Disorders Network

Nager, Charles William

University of California San Diego, La Jolla, CA

\$25,000

Memory is a critical element of human existence. Consequently, memory loss through neurological disease, such as Alzheimer's, or head trauma, severely impacts quality of life. Their work will provide a fundamental understanding of the cellular, molecular and neural circuit processes of memory providing potential avenues for mnemonic therapy in humans. Memory is a fundamental element of human life yet it remains one of the greatest mysteries of modern biological research. Memory loss through neurological disease, such as Alzheimer's, or head trauma, has a devastating impact on the quality of life. Understanding the molecular process of memory would therefore provide potential avenues for mnemonic therapy. The long-term goal of this proposal is to understand how memories are formed, consolidated and retrieved at the molecular, cellular and neural network level. They use the fruit fly *Drosophila* as their model system because it can learn, it has a relatively simple brain and it is amenable to a sophisticated genetic approach. They will use the most up-to-date technology available with a new appetitive long-term memory assay to investigate how conserved signaling molecules function within the context of defined neural circuits to encode memory. They expect that these studies will have a major impact on strategies for human mnemonic therapy.

5 R01 HD061787-02

Wireless Remote Abdominal Pressure System: Developing a More Comprehensive Unders

Nygaard, Ingrid E.

University of Utah, Salt Lake City, UT

\$66,667

The effect of strenuous physical activity on new or recurrent pelvic floor disorders is unknown. They developed an intravaginal pressure sensor to measure intraabdominal pressure. They will perfect the wireless technology needed to use the sensor remotely so that they can understand how different activities done during real world settings affect intraabdominal pressures and pelvic floor disorders. Pelvic floor disorders affect one in four American women. Few modifiable risk factors have been identified that might reduce the incidence or progression of pelvic floor disorders. Popular wisdom and scant clinical data suggest that strenuous activity causes or promotes pelvic floor disorders. Given the health benefits of activity, women should be encouraged to be maximally active unless there is scientific evidence to the contrary. Existing physical activity instruments are largely designed to assess cardiovascular exertion and are validated using activity diaries, accelerometers, and step counters. Such measures may not accurately measure activities that increase loading on the pelvic floor (such as lifting). After researching available technologies, they concluded that a tool to understand how physical activities impact abdominal pressure in the real world does not exist. Over the past 18 months, their interdisciplinary team of bioengineers, urogynecologists, electrical engineers, and exercise scientists developed and validated the performance of a prototype for an intravaginal abdominal pressure sensor that accurately measures pressure in the upper vagina, an easily accessible space that records pressures similar to the true intraabdominal pressure. In this proposal, they plan first to further develop an integrated system (the "WRAPS", Wireless Remote Abdominal Pressure System) to monitor intraabdominal pressure outside of the clinical setting. This system will

consist of three key elements: an intravaginal pressure sensor with wireless data transmission capability, a small portable data monitoring and storage unit, and computer based data translation software for downloading and managing the pressure data. In a controlled exercise laboratory setting, they will then use intraabdominal pressure data generated by the WRAPS to determine the reproducibility of intraabdominal pressures measured during specific types of physical activity and will finalize development of a valid questionnaire that categorizes the magnitude of intraabdominal pressures during activities. Finally, in a real-world setting in which participants wear the intravaginal sensor during waking hours for four 1-week periods over the course of a year, they will characterize intraabdominal pressures experienced by women of varying degrees of habitual physical activity and, using WRAPS data as the gold standard, determine whether activity can be appropriately categorized in terms of pelvic loading by means of self-administered questionnaires, the current standard. Obtaining future evidence about the impact of physical stressors on pelvic floor disorders relies on their ability to measure the risk factor in question. This innovative translational collaboration will remove a critical barrier to progress in understanding the etiology of pelvic floor disorders in women.

5 U10 HD054136-05

Utah Pelvic Floor Disorders Network

Nygaard, Ingrid E.

University of Utah, Salt Lake City, UT

\$25,000

Pelvic floor disorders are common, bothersome, and inadequately treated. The overarching aim of the investigators from the proposed University of Utah Pelvic Floor Disorders Clinical Site is to improve women's health in the area of pelvic floor dysfunction. To this end, site specific aims include: 1) Identifying priority areas of research, 2) Developing assessment tools, 3) Developing and implementing PFDN protocols, 4) Recruiting and enrolling subjects in PFDN protocols, 5) Achieving on-target recruitment goals and high subject retention, 6) Ensuring high-quality data, 7) Transmitting data accurately to the Data Coordinating Center, 8) Participating in data analysis, 9) Disseminating results to the research community, and 10) Producing high-quality publications. The broad scientific aim for the randomized clinical trial outlined in this proposal is to evaluate whether post-operative pelvic floor muscle training following surgery for pelvic organ prolapse and/or stress urinary incontinence improves post-operative outcomes (anatomic, symptomatic and quality of life outcomes) at 3 months, 1 year and 2 years post-operatively.

1 U01 HD063036-01

Racial disparity in adverse pregnancy outcomes: Ancillary study to Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-Be (NuMoM2b)

Parker, Corette B

Research Triangle Institute, Research Triangle Park, NC

\$100,000

The incidence of preterm birth is unequally distributed among races and ethnic groups. For example, African-Americans have the highest rate of preterm birth, followed by Mexican-Americans, Asians, and Caucasians. Strikingly, a substantial health disparity exists between African-Americans and Caucasians, with African-Americans being 1.6 times more likely to deliver preterm infants than Caucasians. In addition, the United States' high infant mortality rate compared to that of Europe is mainly due to the higher frequency of preterm births in the United

States. The NuMoM2b project is a prospective cohort study of a racially/ethnically/geographically diverse population of 10,000 nulliparous women with singleton gestations. The women will undergo intensive research assessments during the course of their pregnancies to study the mechanisms for and prediction of adverse pregnancy outcomes such as preterm birth, preeclampsia, and fetal growth restriction in their first pregnancy. With funding from ORWH, biomarkers reflecting the maternal-placental-fetal endocrine milieu related to stress will be analyzed from the bio-specimens being collected from 10,000 women. As part of the parent study, various questionnaires assessing psychosocial status during pregnancy are being administered during pregnancy. The combination of race/ethnic-specific profiles of genetic variation, perceived stress and resilience factors (psychosocial environment) as well as biomarker differences will likely account, in part, for racial/ethnic disparities in the risk of preterm birth.

1 U01 HD063036-01

The role of maternal nutrition in adverse pregnancy outcomes: Ancillary study to Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-Be (NuMoM2b)

Parker, Corette B

Research Triangle Institute, Research Triangle Park, NC

\$100,000

Preterm birth affects 1 in 8 women in the US, with half a million preterm births each year. Excluding congenital malformations, preterm birth accounts for approximately 70 percent of all neonatal deaths and nearly 50 percent of long-term neurological problems. Despite decades of research, there has been little progress in developing effective interventions to prevent preterm birth in the first pregnancy. The NuMoM2b project is a prospective cohort study of a racially/ethnically/geographically diverse population of 10,000 nulliparous women with singleton gestations. Nutrition is an understudied area with relation to preterm birth, although several nutritional factors have been related to preterm birth. There is biologic plausibility for the relationship of folate and vitamin B12 with preterm birth, as both may be important regulators of pro-inflammatory cytokine and DNA synthesis. Vitamin D is another example of a nutritional factor that, if deficient, has been linked to adverse pregnancy outcomes, including preterm birth, preeclampsia, and fetal growth restriction. With funding from ORWH, the role of nutrition in adverse pregnancy outcomes will be studied by administering the Modified Block 2005 Food Frequency Questionnaire in all 10,000 women in the cohort. A full assessment of usual intake of over 51 nutrients and 7 food groups in the 3 months before conception will be conducted by 14 weeks of gestation using a semi-quantitative modified Block 2005 food frequency questionnaire and findings will be correlated to clinical data and biospecimen collection as part of the parent study to determine the role of maternal nutrient status in adverse pregnancy outcomes.

5 U10 HD041261-10

Perioperative Pelvic Floor Rehab: A Randomized Trial

Richter, Holly E

University of Alabama At Birmingham, Birmingham, AL

\$25,000

Surgical techniques for the treatment of stress incontinence (SUI) have significantly evolved over the last 100 years. The gold standard Burch urethropexy and pubovaginal sling procedures are now being performed less frequently, with the increased use of the newer minimally invasive

mid-urethral sling procedures, the most common being the tension-free vaginal tape procedure (TVT). The TVT procedure is comparable in efficacy to the open Burch procedure with low morbidity and fewer complications. Because the sling is placed at the level of the mid-urethra under no tension, it was thought that the TVT would yield fewer postoperative lower urinary tract symptoms. However, a review of the literature has not borne this out, with postoperative storage symptoms reported in up to 42% of women. The primary purpose of the proposed randomized clinical trial is to test whether a perioperative behavioral/pelvic floor muscle training program can reduce the occurrence of these postoperative storage symptoms and voiding dysfunction in women undergoing a TVT procedure for SUI. Behavioral interventions are known to be effective for treating urge incontinence and voiding dysfunction unrelated to surgery, but have not been tested as a preventive adjunctive strategy. Approximately 400 subjects will be randomized to a perioperative behavioral program or usual care. The intervention will be implemented 2 weeks preoperatively, and reinforced before leaving the hospital and two weeks postoperatively. The primary outcome will be complaints of urgency, frequency, nocturia and urge incontinence using the overactive bladder questionnaire (OABq). Evaluations will be performed at 2 and 6 weeks, 3, 6, and 12 months postop, and will include the OABq, questionnaire for urinary diagnosis (QUID), urogenital distress inventory (UDI), pelvic organ prolapse/urinary incontinence sexual function questionnaire (PISQ), patient global impression of severity (PGI-S) and SF-36. Subjects will also complete a 7-day bladder diary to assess frequency of storage symptoms. Secondary aims are to determine whether this intervention reduces time to voiding and symptoms of voiding dysfunction, whether it impacts on patient satisfaction and quality of life, and to identify predictors of postoperative storage symptoms and voiding dysfunction symptoms. This type of information will allow physicians to more effectively counsel and treat their incontinent female patients to further enhance long-term quality of life.

5 U10 HD054241-05

Nichd Pelvic Floor Disorders Network

Schaffer, Joseph I

University of Texas Southwest Medical Center, Dallas, TX

\$25,000

This application describes the qualifications and experience of the urogynecology and urology faculty and research teams at the University of Texas Southwestern (UT Southwestern) Medical Center and Parkland Hospital and the facilities and patient population available to carry out clinical protocols sponsored by the Pelvic Floor Disorders Network. In 2004, there were more than 2,100 women with pelvic floor disorders seen in their clinics and 617 women underwent surgical procedures for correction of pelvic floor disorders. The Departments of Obstetrics and Gynecology and Urology have increasingly collaborated since 1997 to offer comprehensive care of women with pelvic floor disorders. In addition to urogynecology and urology, collaboration includes faculty from colorectal surgery, radiology, physical therapy, and maternal-fetal medicine. The clinical research teams described in this application have successful prior as well as on-going experience in NIH sponsored national multi-center trials. Centerpieces in this application are two existing research clinics, one targeted at private patients (operated by the Urology Department) and the other focused on medically indigent patients (operated by the Obstetrics and Gynecology Department). Also included in this application is a concept application for a randomized trial designed to assess the efficacy of end-to-end versus

overlapping repair of the external anal sphincter lacerated during childbirth. The primary outcome is anal incontinence which is a significant consequence of such lacerations. This trial would permit accurate evaluation of the outcome of specific surgical procedures which is one of the prime areas of interest leading to creation of the Pelvic Floor Disorders Network. They are of the view that along with strategies for prevention of anal sphincter laceration during childbirth, optimal management of the torn sphincter should also be studied since more than 200,000 women sustain such pelvic floor injuries each year in the United States.

Z01 HD008737-10

ORWH-NICHD Leiomyoma Tissue Bank

Segars, James

NICHD Intramural program, Bethesda, MD

\$50,000

The health of 30-50% of women in the U.S. is adversely affected by uterine leiomyoma (fibroids). Uterine fibroids are a health disparity issue that disproportionately affects African American women. Research into causes and treatment has lagged behind other disciplines, in part due to lack of available tissues, since surgical samples are often not made available to scientists. To address the problem of tissue availability, and promote research on this condition, this project proposes to establish a fibroid tissue bank as an initiative in the intramural program of NICHD. This tissue bank will provide samples to NIH-funded investigators and DoD-funded investigators to support work on this condition. The Leiomyoma Tissue Bank (LTB) will be physically located in space assigned to Dr. Segars of NICHD. The LTB will be structured after RStAR-banks for endometrium and ovary established by the Specialized Cooperative Program in Reproductive Research. Computerization of sample inventory will be performed with software provided by NICHD.

5 U01 HD041249-10

Pelvic Floor Disorders Network-Data Coordinating Center

Spino, Catherine A

University of Michigan, Ann Arbor, MI

\$25,000

Pelvic floor disorders, such as urinary incontinence, pelvic organ prolapse, and fecal incontinence, are common and significant health-related problems for women in the United States. Outcomes following surgical and non-surgical intervention for pelvic floor disorders have not been adequately evaluated. As a result, data necessary to fully inform patients and to make important policy decisions are unavailable. The long-term objective of the Pelvic Floor Disorders Network (PFDN) is to systematically evaluate these outcomes. This application to be the Data Coordinating Center (DCC) for the pelvic floor disorders network brings together experienced investigators from biostatistics, urogynecology, urology, quality of life and health services research to prospectively assess the outcomes from various surgical interventions for female pelvic floor disorders. The specific aims of the DCC are to: 1. Assist in protocol development by providing expertise in the design, conduct and analysis of clinical trials conducted by the PFDN. 2. Provide expertise in measurement of quality of life and in the selection of the appropriate instruments to assess treatment outcomes and, when appropriate, to perform the interviews. 3. Coordinate the implementation of the study protocols approved by the Steering Committee, including design of the case report forms and interviewing protocols, development of a manual

of operations, centralized database management with either centralized or remote data entry, submission of an IND to the FDA when necessary, and by organizing training and certification sessions, as needed. 4. Establish a database for each study conducted by the PFDN. 5. Implement either centralized or web-based data entry and verification. 6. Monitor the clinical sites with respect to data quality. 7. Provide infrastructure for monitoring adverse events and regulatory oversight for the network. 8. Provide logistical support for the Steering Committee, Advisory Board and DSMB, for both face-to-face meetings and teleconferences. 9. Maintain a website for the PFDN that includes web pages with content for the public, and a password-protected site with all study documentation and databases. 10. Manage and distribute protocol funds to the Clinical Centers. To illustrate the work of the DCC, a randomized clinical trial is proposed to compare surgical procedures for pelvic organ prolapse using a vaginal approach.

Adrenal hyperplasia among adolescent patients polycystic ovarian syndrome (Bench To Bedside Program)

Stratakis, Constantine A.

NICHD Intramural, Bethesda, MD

\$200,000

Adolescent and young women of reproductive age and clinical picture of polycystic ovarian syndrome (PCOS) have increased adrenal androgens. Anecdotal reports from patients and animal studies have also suggested that this phenotype is also associated with adrenal hyperplasia. Finally, certain minority populations (i.e. Hispanics, African-Americans) are particularly susceptible to PCOS and related complications (e.g. diabetes, infertility). The common link between the adrenal and ovarian pathology has been postulated to be insulin resistance, but this remains unclear. Dr. Ten runs a large clinic in down-town New York where a lot of these patients are being seen. In this proposal, they plan to bring a subset of these patients, those that have elevated adrenal steroid hormones, to the NIH Clinical Research Center (NIH-CRC) and study their metabolic profile, adrenal imaging and steroidogenesis before and after treatment with metformin, an insulin sensitizer, and genetic parameters. This study will clearly lead to a new clinical protocol and offer the opportunity to study a new disorder for the pediatric endocrine clinical and program.

5 U10 HD041267-11

Pelvic Floor Disorders Network

Visco, Anthony G

Duke University, Durham, NC

\$25,000

Women's health research at the University of North Carolina (UNC) is sophisticated and widespread with many committed investigators addressing issues of fundamental importance to women. UNC has a tradition of excellence in clinical care, training and research in pelvic floor disorders and includes one of the nation's first accredited fellowship programs in the Division of Urogynecology and Reconstructive Pelvic Surgery. They offer comprehensive evaluation and treatment options in a high-volume care setting that serves as a tertiary referral center for women from across the state. Women sought consultation or treatment for more than 2700 pelvic floor disorders by Urogynecologists at UNC in the previous two years. Seventy-eight percent of the women were Caucasian and 15% were African American, predominantly from rural and suburban communities with stable care and follow-up patterns. Approximately 427 women had

multi-channel urodynamic studies annually. UNC providers have extensive expertise in both surgical and non-surgical management of urinary incontinence, pelvic organ prolapse and defecatory dysfunction. The Division of Urogynecology performs an average of 106 surgical procedures for the primary indication of urinary incontinence, 300 for prolapse and provides medical management for over 1,464 women with these conditions each year. The UNC Pelvic Floor Disorders Research Collaborative, led by the Division of Urogynecology is a multidisciplinary team of outstanding investigators in Urogynecology, urology, gastroenterology, radiology, maternal-fetal medicine and clinical research methodology. They have a history of strong clinical ties and dedication to interdisciplinary research. Diagnostic resources include multi-channel urodynamic testing, cystoscopy, defecography, pelvic MRI, 360-degree endoanal ultrasound, anal manometry and needle electromyography. Clinical services include surgical treatment of complex pelvic floor disorders and a wide range of non-surgical options. As an active PFDN clinical network site, UNC has an established research infrastructure with the proven ability to support large-scale, multi-centered clinical research. The collaborative is well-equipped and uniquely qualified to continue as a valuable member of the Pelvic Floor Disorders Network. Given the exceptional quality of the research opportunities and resources available at UNC, the stable and diverse patient population, the strength of the investigator pool, their proven high-level recruitment and the commitment of the institution to the stated goals of this RFA, they look forward to continuing to make substantial contributions to advancing women's health related to pelvic floor disorders.

1 R01 HD65029-01A1

The Genetics of Polycystic Ovary Syndrome

Welt, Corrine, M.D.

Massachusetts General Hospital, Boston, MA

\$200,000

Polycystic ovary syndrome (PCOS) is a disorder of irregular menses and elevated androgens that carries a high risk for diabetes, hypertension and elevated lipids. The investigators have now discovered a genetic variant that is associated with polycystic ovary syndrome in a genome-wide association study and will try to determine the causal variant and gene it marks. Discovering the variants and/or gene(s) that predispose to PCOS will determine an etiology and will provide a novel target to develop new treatments for the 1 in 10 reproductive age women it affects.

Polycystic ovary syndrome is the most common endocrine disorder in reproductive age women, yet its etiology is poorly understood. The disorder is defined by its cardinal features: irregular menstrual cycles, hyperandrogenism and a polycystic ovary pattern on ultrasound. In addition, women with PCOS have increased risk for infertility, endometrial cancer, type 2 diabetes and cardiovascular risk factors. They completed a genome-wide association study in collaboration with deCODE in Iceland. The study identified a variant on chromosome 4 reaching genomewide significance in an Icelandic case control cohort and replicating in an identically phenotyped Boston cohort. The broad goal of this proposal is to identify the causal variant that this risk variant marks through fine mapping. They will also examine the functional effects of the causal variant using expression studies and/or assays of protein function. Finally, they will examine the phenotypic features defined by the genotype. Specific Aim 1 will examine the region around the chromosome 4 variant to identify the causal variant that affects protein production or gene expression. Fine mapping will be performed using common single nucleotide polymorphisms (SNPs) in the HapMap and 1000 genomes projects. In addition, the exons and promoter regions

of genes in linkage disequilibrium with the associated variant will be sequenced in large numbers to identify rare variants that may affect protein production or gene expression. Specific Aim 2 will dissect the phenotype conferred by the genotype in PCOS, controls, males and postmenopausal women using an extensive database assembled by the PI over the past 6 years. Specific Aim 3 will examine expression of two candidate genes in LD with the chromosome 4 variant in carriers and non-carriers to determine the gene of interest. When a causal variant is identified, expression will also be examined to identify a functional effect of variant(s) in a lymphoblastoid cell line database and in adipose, theca and peripheral white blood cells in vitro using quantitative PCR. Coding sequence causal variants and rare variants will be assessed using signaling assays and overexpression or knock-down of the variants in cell systems and animal models. These studies will uncover the causal variant and gene that is marked by the first known variant identified in a genome-wide case control association study of PCOS. The proposal has the potential to illuminate the etiology of PCOS. Such information has been long in coming and is essential to provide better diagnostic and treatment information for this very common disorder with its adverse health consequences.