Rare Diseases Projects co-funded by Office of Rare Diseases Research (ORDR) and Institutes and Centers

Project	Investigators Institute(s)/Institution
Development of antigen specific biomarkers for autoimmune uveitic disease	**NEI:** R. Caspi; M. Mattapallil; R. B. Nussenblatt; N. Sen **Benaroya Research Institute at VA:** G. T. Nepom; B. W. Kwok; E. James
Mitochondria, Telomeres, and Lifestyle in Li-Fraumeni Syndrome Outcomes	**NCI:** S. A. Savage; I. Wentzensen; P. Mai; M. Greene; R. Sinha; J. Fraumeni **NHLBI:** P. M. Hwang **Hospital A. C. Camargo - Fundação Antonio Prudente:** M. A. Achatz **International Agency for Research on Cancer:** P. Hainaut **The Hospital for Sick Children:** D. Malkin
Cyclodextrin therapy for Niemann-Pick C1 disease	**Washington University:** D. Ory **NHGRI**: J. J. Marugan **NICHD:** F. D. Porter **Albert Einstein College of Medicine**: S. U. Walkley
Amitriptyline for the Treatment of BDNF Haploinsufficiency	**NICHD:** J. Han **NIA:** B. Martin; S. Maudsley; W. Chadwick **NIMH:** S. Swedo; A. Thurm; C. Golden Williams **Medical University of South Carolina:** L. M. Luttrell
Exacerbation of HIF2alpha–dependent polycythemia by iron deficiency	**NICHD:** T. Rouault **NHLBI:** G. J. Kato **NCI:** W. M. Linehan; J. Mitchel **Howard University Medical Center:** V. Gordeuk
Biomarkers of neurodevelopment in Smith-Lemli-Opitz Syndrome	**NICHD:** F. D. Porter; A. Yergey **NHLBI:** A. Remaley **NIMH:** J. Geidd **CC:** E. Baker **Kennedy Krieger Institute/Johns Hopkins University:** E. Tierney; S. Mori; R. W. Lee

PI: **Rachel R Caspi**
Co-PI: **Mary J Mattapallil**

Project Title: **Development of antigen specific biomarkers for autoimmune uveitic disease**

Abstract:
Description of the research area:
Uveitis is the primary manifestation in ocular diseases like sympathetic ophthalmia, and birdshot retinochoroidopathy and is commonly associated with systemic diseases including sarcoidosis, Behcet's disease, and Vogt-Koyanagi-Harada disease. These types of uveitis affect the posterior part of the uveal tract where the photoreceptor cells are located, and are associated with immunological responses to antigens from the retina and uvea (e.g., retinal arrestin), indicating a possible autoimmune etiology. Susceptibility to autoimmune uveitis is strongly associated with HLA class I and class II genes. Using a 'humanized' mouse model we have identified permissive and non-permissive alleles of the HLA-DR and HLA-DQ genes and characterized allele-specific uveitogenic epitopes. The major goal of this proposal is to develop HLA allele-specific biomarkers to detect pathogenic T cells specific to retinal arrestin in the peripheral blood of uveitis patients, that are believed to have a role in driving progression of uveitis.

Significance of the proposed research (new initiative):
As of now, there are no known biomarkers available for monitoring autoimmune uveitis. Knowledge of the risk associated with the various HLA alleles and of the pathogenic epitopes that they present will permit identification and isolation of the pathogenic cells through use of HLA tetramers. Allele-specific synthetic HLA molecules could be loaded with its corresponding epitopes of retinal arrestin (HLA tetramer), to be used as a biomarker for early diagnosis of human uveitis, and to potentially aid in the development of antigen specific immunotherapies adapted to particular MHC (HLA) haplotypes.

Evidence that the project is ready for immediate implementation (Bench to Bedside):
Using a 'humanized' mouse model for experimental autoimmune uveitis (EAU) we have identified alleles of HLA class II genes that confer susceptibility or resistance to retinal arrestin-induced uveitis and have identified pathogenic epitopes that they bind. The sequences of these epitopes overlap with some of the previously identified peptides of retinal arrestin (designated as M and N), which elicit memory responses in lymphocytes of uveitis patients. HLA-DR restricted CD4+ T cells specific to peptide N could be detected in blood and draining lymph nodes of HLA-DR3 transgenic mice with uveitis, using HLA class II tetramers. Also, using this reagent, potentially pathogenic retinal arrestin specific T cells were detected in the peripheral blood of an HLA-DR3+ uveitis patient.

Preliminary list of the expected project milestones:
(1) Identify HLA restricted pathogenic epitopes of retinal arrestin by binding assays and/or by induction of uveitis in available HLA transgenic mice to develop allele-specific HLA tetramers as biomarker
(2) Recruit patients with non-infectious, autoimmune posterior uveitis from NIH/CC and do HLA typing at NIH/CC
(3) Collection of blood samples from patients typed for specific HLA alleles, in-vitro expansion of precursor cells and detection of antigen-specific T cells using HLA tetramers as biomarker.

PI: **Sharon Savage**

Project Title: **Mitochondria, Telomeres, and Lifestyle in Li-Fraumeni Syndrome Outcomes**

Abstract:
Li-Fraumeni syndrome (LFS) is an autosomal dominant cancer predisposition syndrome in which the age at cancer onset ranges from newborn to old age. Approximately 70% of individuals fitting classical diagnostic criteria have germline mutations in the p53 gene (TP53). Mutation carriers have an 80-100% chance of developing cancer prior to age 60. The p53 protein is a key component in many critical cellular functions, including apoptosis, cell cycle, senescence, DNA repair, energy metabolism and mitochondrial function. The p53 protein regulates mitochondrial oxygen utilization, reactive oxygen species (ROS) generation, and disposition of ROS. Early data suggest that mitochondrial function is abnormal in mutation carriers.

Mutations in TP53 were identified as the cause of LFS in 1990 but, despite major advances in understanding its molecular functions, clinical care for LFS patients has not improved. Small studies have tested genotype-phenotype and biomarker correlations with cancer in individuals with LFS. For example, a TP53 polymorphism in intron 3 and MDM2*SNP309 showed associations with earlier age at cancer onset in LFS. A study of copy number variation (CNV) showed TP53 mutation carriers had more CNVs than those with wild-type TP53; this effect was most pronounced in mutation-carriers with cancer. Three studies of telomere length in LFS families found shorter germline telomeres in TP53 mutation-carriers and an earlier age at cancer onset in successive generations (genetic anticipation). Studies of LFS in Southern Brazil have identified a unique cohort of patients with a founder mutation (TP53*R337H) in approximately 0.3% of newborns. This mutation was originally associated with adrenocortical cancer but subsequent studies have shown that carriers of this mutation are at risk of the complete spectrum of LFS-related cancers. Notably, there is a subset of cancer-free TP53*R337H mutation-carriers who have a “youthful” appearance, suggesting a yet-to-be identified genetic modifier with an impact on aging. LFS families with typical TP53 mutations are also present in Brazil.

The Clinical Genetics Branch, DCEG, NCI, recently conducted a very successful pilot-study of LFS patient/family accrual; 42 eligible families registered for the study in 3 months. A comprehensive clinical protocol of LFS at the NIH CC will be submitted for IRB review in early 2011. Clinical data and biospecimens will be collected to identify modifiers of the LFS phenotype. A cancer screening protocol and a chemoprevention study will also be done in collaboration with intramural and extramural investigators. The proposed study is a collaborative effort between the NCI, NHLBI, and 2 centers in Brazil which will: 1) collect common clinical data and biospecimens to permit statistically robust studies; 2) identify the contribution of germline TP53 mutations to mitochondrial function, and determine if abnormal mitochondrial function is a marker of cancer risk; (3) conduct a pilot study of diet and physical activity data collection in this high-risk setting, as a first step towards a formal intervention study; and 4) determine the role of telomere biology as a cancer risk modifier. These studies will lead to better risk stratification of patients with LFS, and advance understanding of the consequences of a germline TP53 mutation, findings which may also be broadly applicable to sporadic cancers of the type seen in LFS.

PI: **Daniel Ory**

Project Title: **Cyclodextrin therapy for Niemann-Pick C1 disease**

Abstract:
Niemann-Pick type C1 (NPC1) disease is a rare, progressive neurodegenerative disorder with an estimated incidence in Western Europeans on the order of one in 100,000. There are currently no FDA-approved therapies for this progressively fatal neurodegenerative disorder. In recent reports treatment with 2-hydroxypropyl-β-cyclodextrin (CD) has been shown to reduce both cholesterol and sphingolipid storage and prolong survival in Npc1-/- mouse and cat models. Based on the remarkable therapeutic potential of CD, it was recently granted Orphan Drug Status by the FDA. Although treatment with CD has been shown to rapidly down-regulate CNS expression of cholesterol synthetic genes, the mechanism of action for CD is uncertain and at present there are no biomarkers for evaluation of clinical efficacy. We hypothesize that peripheral blood-based biomarkers may serve as a functional biomarker for efficacy of CD in clinical trials with NPC1 subjects.

The Basic Science Aims of this proposal are (1) to develop and implement a sensitive mass spectrometry-based assay for measurement of CD in tissues and blood samples, (2) to examine in CD-treated Npc1-/- mice the pharmacokinetics of CD distribution into brain and plasma, and the effect of the CD on peripheral blood-based biomarkers, and the Clinical Science Aim of this proposal is to perform Phase I dose-escalation studies with CD in human NPC1 subjects at the NIH Clinical Center to establish dosing regimens for future clinical trials. For the Basic Science Aims, assay development will be performed in the Ory laboratory, which has extensive experience in development of mass spectrometry-based assays. The effects of CD treatment on peripheral blood-based biomarkers n in vivo will be performed collaboratively in the Ory and Walkley laboratories. Dr. Marugan at NIH, who has experience in drug development, will direct the pharmacokinetic analysis of CD distribution in vivo. The Phase I clinical studies in the Clinical Science Aim will be directed by Dr. Porter (NICHD) at the Clinical Center at NIH. Analysis of biomarker and CD levels in blood and CSF will be performed in the Ory laboratory.

The proposed studies will provide the analytical and clinical tools that are essential to establish safe and effective dosing regimens for treatment of human NPC1 subjects with CD. Since CD is already approved by the FDA for use in humans, our studies will facilitate rapid translation of CD into trials to establish the efficacy of CD in slowing disease progression in this neurodegenerative disorder.

PI: **Joan Han**

Project Title: **Amitriptyline for the Treatment of BDNF Haploinsufficiency**

Abstract:
Background: Brain-derived neurotrophic factor (BDNF) is widely expressed throughout the central nervous system (CNS), activates the TrkB receptor, and plays an important role in regulating neuronal development and survival. BDNF heterozygous KO (Bdnf+/-) mice display deficits in learning and memory, altered social behavior, anxiety-like features, altered nociception, hyperphagia and obesity. Amitriptyline (AMI), an FDA-approved tricyclic antidepressant, activates the TrkB receptor, potentiates hippocampal Bdnf gene transcription, and elevates BDNF protein levels in rodents. In human studies, increased serum BDNF has been reported after administration of AMI. BDNF in peripheral circulation is believed to reflect cerebral BDNF output; thus, AMI may act to increase BDNF production within the CNS.

Prior Mouse Studies: In our pilot studies, we observed that when orally administered to Bdnf+/- mice, AMI significantly improved learning and memory behavior, exploratory behavior, and social interactivity. AMI treatment also induced approximately a 10-15% loss of initial body weight over a 20-week period in obese 8-13 month old Bdnf+/- mice, without any significant effects in wild-type littermates. BDNF is believed to function downstream of the leptin-proopiomelanocortin signaling pathway to play a key role in the regulation of energy balance. Our findings are consistent with this, and also suggest that AMI may have different cognitive and metabolic effects depending on genetic background.

Prior Human Studies: In our studies on patients with WAGR syndrome, a rare disorder caused by heterozygous chromosome 11 deletions in the region of the BDNF gene, we have observed that BDNF haploinsufficiency is associated with lower serum BDNF, greater hyperphagia, higher prevalence of childhood obesity, diminished nociception, lower adaptive function, and greater lifetime impairment of social interaction on autism diagnostic assessment. Cognitive function also appeared to be positively correlated with serum BDNF concentrations in WAGR syndrome. In a different genetic disorder, Prader-Willi syndrome, which is also associated with obesity and cognitive impairment, we have observed lower serum BDNF concentrations compared with obese and lean controls. We have also observed that BDNF single nucleotide polymorphism (SNP) rs12291063 is associated with decreased BDNF expression in cadaveric hypothalamic tissue and higher body mass index in healthy subjects. Our findings suggest that BDNF may be important in both syndromic and common forms of obesity

Hypothesis: We hypothesize that AMI will have genotype-specific beneficial effects on cognitive function and body composition in both mice and humans with BDNF haploinsufficiency.

Proposed Bench Study (Drs. Martin, Maudsley, Chadwick, and Luttrell): We propose to characterize fully the effects of AMI in Bdnf+/- mice, including cognitive and behavioral functions (Morris Water Maze, nociception, quantitative proteomics and metabolomics on brain regions), as well as body composition (MRI), energy intake, and metabolic parameters (glucose, lipids, blood pressure). Additionally, using an in vitro cell culture approach (human neuroblastoma cells, SH-SY5Y, M-17: primary murine neurons), we will investigate the molecular mechanism of action of AMI, in comparison with BDNF, at both proteomic and genotropic levels with respect to TrkB receptor activation. We will generate AMI-induced phosphoproteomic signaling and gene regulation ‘signature’ patterns to identify key signaling mechanisms and generate an in-depth structure-activity relationship for AMI effects at the TrkB receptor to assist novel drug analog development.

Proposed Bedside Study (Drs. Han, Swedo, Thurm, and Golden Williams): We propose a randomized, double-blind, placebo-controlled, cross-over clinical trial in 25 patients with BDNF haploinsufficiency to evaluate the acute effects of AMI on serum BDNF concentration, basal metabolic rate, energy intake at a lunch buffet test meal, and cognitive function on a computerized battery of neuropsychological tests. These subjects will then be studied in an open-label, 12-month clinical trial to evaluate the long-term effects of AMI on eating behavior, body composition, cognitive and adaptive function, psychiatric symptomatology, and sensory/nociceptive thresholds.

Potential Impact: In patients with genetic defects resulting in BDNF insufficiency, targeting the TrkB receptor with AMI may show greater efficacy and unique benefits that are not observed in unselected individuals. If AMI can be shown to be safe and effective in BDNF+/- patients, it may have application in other specific genetic conditions associated with BDNF insufficiency (e.g. genetic disorders of the leptin signaling pathway, Prader-Willi syndrome, and individuals with obesity-associated SNPs of the BDNF gene).

PI: **Tracey Rouault**

Project Title: **Role of iron deficiency in pulmonary hypertension in Chuvash polycythemia**

Abstract:
In the last few years, misregulation of several of the steps in the pathway that governs renal synthesis of erythropoietin has emerged as a cause of polycythemia, pulmonary hypertension, and heart disease. Among the genetic causes of polycythemia, Chuvash polycythemia, caused by a single R200W mutation in the VHL tumor suppressor gene, has been the subject of recent studies in humans and in a mouse model in which aspects of the pathophysiology were successfully recapitulated. VHL is required to degrade HIF1 and HIF2 alpha, and HIF2 alpha promotes transcription of erythropoietin, mainly in the kidneys, but also in the liver. VHL cannot degrade the HIF proteins unless they are marked for degradation by hydroxylation of proline residues, and mutations of prolyl hydroxylase 2 are also associated with polycythemia and heart failure. Some patients with Chuvash have evidence for pulmonary hypertension. Patients with Chuvash polycythemia have been evaluated by echocardiography, but pulmonary hemodynamic studies have not been performed. We proposed to bring patients with Chuvash polycythemia to the NIH Clinical Center, to study pulmonary hemodynamics with the help of Dr. Gregory Kato and colleagues of the NHLBI Pulmonary and Vascular Medicine Branch, and to improve treatment to prevent pulmonary hypertension and death. Our treatment would consist of the following: frequent phlebotomies to remove excess red cell mass, combined with aggressive iron repletion, to repress activation of residual HIF2 alpha by iron deficiency. When we complete design of our therapeutic regimen, it will have applications for patients with abnormalities in any point in the HIF2 alpha regulatory pathway, including VHL, PHD2, HIF2 alpha, the transcript for HIF2 alpha, and erythropoietin itself.

Dr. Tracey Rouault will coordinate studies and oversee project design. Dr. Victor Gordeuk, an extramural hematologist from Howard University, Washington, D.C., will identify patients with Chuvash polycythemia who will come to the NIH for studies, Dr. Marston Linehan will accept these patients with known VHL mutations onto his clinical service, and Drs. Kato and Shoaib Alam will perform pulmonary hemodynamic studies at the NIH Clinical Center.

PI: **Forbes Porter**

Project Title: **Biomarkers of neurodevelopment in Smith-Lemli-Opitz Syndrome**

Abstract:
Smith-Lemli-Opitz syndrome (SLOS) is a rare, multiple malformation, neurodevelopmental disorder caused by impaired cholesterol synthesis. Currently there is only one very limited published study characterizing brain MRI findings in SLOS. Our retrospective analysis of previously obtained MRI data demonstrated structural abnormalities, primarily of the corpus callosum, in approximately half of SLOS patients studied. No studies have applied diffusion tensor imaging or volumetric analysis, nor have robust neurobehavioral correlations been attempted. Preliminary data obtained from baseline studies of cerebral spinal fluid (CSF) from a subset of SLOS patients enrolled in our simvastatin therapeutic trial identified significant abnormal levels of several proteins; however, MRI imaging was not performed as part of that study. This project proposes to combine proteomic analysis of cerebral spinal fluid, neurobehavioral correlations and MRI imaging to further our understanding of the pathoetiology of SLOS. Further studies applying advanced neuroimaging and molecular techniques will help to elucidate the clinical significance of these promising anatomic and biochemical disease markers. The behavioral and cognitive defects in SLOS are likely due to a combination of developmental and biochemical disturbances. The biochemical disturbances are potentially amendable to therapeutic intervention. Currently there are no established therapies for SLOS that address the behavioral and cognitive defects, and thus identification of biomarkers that correlate with MRI and neurocognitive data would be of significant value in the testing of therapeutic interventions. This intramural/extramural, multi institute collaborative study has several aims to address this issue.

Aim 1. To describe the magnetic resonance imaging (DTI, volumetric, structural, H1MRS) in SLOS compared to controls (Baker, Mori, Lee, Porter).
a. White matter tractography and volumetric processing using large deformation diffeometric mapping (Mori, Lee)
b. Structural and H1MRS analyses (Baker).

Aim 2. To utilize mass spectrometric techniques and multianalyte profiling to characterize cerebral spinal fluid (CSF) protein profiles in SLOS patients and develop Elisa based assays to quantify identified proteins for use in clinical trials (Yergey, Porter, Remaley).

Aim 3. To clinically characterize SLOS patients using cognitive, behavioral, developmental, and anthropometric testing measures and to develop an updated severity scale for SLOS that incorporates degrees of neurodevelopmental disability.

Aim 4. To correlate, across the clinical spectrum of SLOS, the above imaging and biomarkers with neurocognitive and behavioral findings.