2010 DERC P&F Grants AWARDED
Pilot and Feasibility Projects in Endocrinology & Diabetes
Pilot & Feasibility Program, Director: Pinchas Cohen

On behalf of the UCSD/UCLA Diabetes & Endocrinology Research Center Pilot and Feasibility Grant Committee, the UCSD/UCLA DERC Center is delighted to announce that we have awarded seven outstanding projects for seed funding in 2010 out of an unprecedented 27 superb applications. This large number of applications is clear evidence for the remarkable scientific environment that exists in our universities for supporting diabetes research especially among promising young scientists. As part of the ARRA Funds awarded to our UCSD/UCLA DERC grant, the Pilot and Feasibility grant program has been provided with an additional $150,000 for the 2010 awards. Thus, the UCSD/UCLA DERC has awarded 7 grantees at approximately $40,000-$60,000 per year for 2010, double the amount normally available. Thus, the UCSD/UCLA DERC 2010 competition has awarded $300,000 in awards for P&F.

THE UCSD/UCLA DERC is Proud to Announce the 2010 P&F AWARDEES:

Da Young Oh, PhD, from UCSD, leads this year’s awardees, and is our center’s inaugural Junior Faculty Developmental Award winner for her project studying GPR120 as an Omega-3 Fatty Acid Receptor Mediating Potent Anti-Inflammatory and Insulin Sensitizing Effects.

The other six P&F grants were awarded to:

Ning-Ai Liu, MD, PhD, from Cedars-Sinai-UCLA, for her proposal to study Glucocorticoid-induced insulin resistance in zebrafish.

Renata Pereira, PhD, from UCLA, for studying the role of FGF-23 in skeletal mineralization.

Daniel DeUgarte, MD, from UCLA, to investigate the Impact of Bariatric Surgery on Metabolic Phenotype, Gene Expression, and Epigenome.

Alexander Sasha Kaufman, PhD, from UCSD, for investigating Neural Circuits Regulating and Timing Puberty in Normal and Overweight Mice.

WuQiang Fan, MD, PhD, from UCSD, for a project to study FOXO1 and Insulin resistance.

Jane Kim, MD, from UCSD, for her work on Sialic Acids in Obesity and Metabolism.

Please join us all in congratulating these promising young investigators and we all look forward to seeing the fruits of their research in the literature and in future DERC meetings.

Pinchas Cohen, M.D., Professor and Chief of Diabetes & Endocrinology, Mattel Children’s Hospital at UCLA & the David Geffen School of Medicine at UCLA. Co-Director, UCSD/UCLA Diabetes/Endocrinology Research Center, and Director of the Pilot and Feasibility Program.

Final report and presentation at the annual retreat
A report on each pilot and feasibility study conducted will be provided at the end of the study period and an update will be provided yearly for four years after the completion of the award. These brief reports will contain professional career status at the time of the award and at the time of the report; an overview of the project including its significance and salient results; a list of resulting publications; and peer-reviewed subsequent funding in the same or related areas. Funded P&F investigators will attend the annual DERC retreat as well as a meeting of Regional P&F awardees, and present the results of their work in the year immediately following their award. Travel to these meetings will be charged to the individual P&F awards.

ALL PAPERS MUST CITE P30 DK063491
Listserv for DERC Members

Send announcements, communications, requests, etc., to your DERC colleagues:

DERC-L@UCSD.EDU

If you are receiving this newsletter directly, you are already subscribed. If you would like to subscribe, please email mellonadmin@ucsd.edu. This is a moderated listserv, so messages will be prescreened such that only relevant and important messages will reach you.

NEW WEBSITE
http://DERC.UCSD.EDU

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Summer Medical Student Research Program in Diabetes and Endocrinology

The Medical Student Research Program in Diabetes is sponsored by the National Institutes of Health through the NIDDK and allows medical students to conduct research under the direction of an established scientist in the areas of diabetes, hormone action, physiology, islet cell biology or obesity at an institution with one of 17 NIDDK-funded Diabetes and Endocrinology Research Centers (DERC) or Diabetes Research and Training Centers (DRTC) during the summer between the first and second year or second and third year of medical school. The UCSD/UCLA DERC center has supported 4 medical student trainees for summer research in each of the summers of 2009 and 2010: two students at UCLA/Cedars Sinai and two at UCSD/Salk each summer, for 8 students total. This program will be continued through the summer of 2011 and likely beyond.

The goals of the Program are to encourage medical students to consider research in diabetes and its complications as a career and to educate students about diabetes and endocrinology. The diabetes-related and obesity-related research opportunities are quite broad and range from basic laboratory studies to clinical studies in humans. Program Consultants will assist students in selecting an appropriate research project and preceptor. The preceptor and the medical student jointly design a research project that is then conducted over the course of the summer. Prior research experience is not required. Students must be U.S. Citizens and/or permanent residents to participate in this Program. Students spend 2-3 months working on their research project at a DERC or DRTC of their choice and receive a weekly stipend. In addition to working on his/her own research project, each student attends a web-cast series of seminars addressing various clinical and research aspects of diabetes mellitus and its complications and all students present a poster in Nashville, TN at a meeting in early August. Commencement dates and conclusion for the program are reasonably flexible. More information can be found at http://medicalstudentdiabetesresearch.org/

If you have interest in hosting one of these outstanding medical students for summer research project, please contact Dr. Pamela Mellon pmellon@ucsd.edu or Dr. Hassy Cohen hassy@mednet.ucla.edu
Mouse Phenotyping CORE: Rajendra Tangirala, PhD
Core Director
Pinchas Cohen, MD
Core Co-Director
Andrea Hevener, PhD
Core Co-Director
David Hwang, PhD
Core Co-Director
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Genechip CORE: Leslie J. Raffel, M.D. Leslie.Raffel@cshs.org
Core Director
Genetic statistical analysis assistance
Genotyping services including chip methodology, the lab recommends single nucleotide polymorphism (SNP) testing of candidate genes either using Illumina or Taqman methodology
Genotyping Laboratory: Kent D. Taylor, Ph.D. Kent.Taylor@cshs.org

Inflammation CORE: Peter Tontonoz, M.D., Ph.D. Core Director
Rajendra Tangirala, PhD
Core Co-Director
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DERC Human Genetics Core

CORE Directors: Jerome I. Rotter, M.D. & Leslie J. Raffel, M.D.
The goal of the Human Genetics Core is to offer expertise to DERC investigators conducting studies into the genetics of diabetes, its complications and related endocrine disorders. The Core provides access to both the expertise and facilities necessary for such genetic research in human populations.

Core Services:
- Establishment and maintenance of EBV-transformed lymphoblastoid cell lines and generation of nonviable cell pellets for DNA/RNA isolation
- Access to anonymized lymphoblastoid cell lines from subjects well characterized for diabetes and/or insulin sensitivity for such purposes as searching for variations and/or mutations in susceptible genes (in development)
- Genotyping services including chip-based genome wide association and linkage scans, and SNP testing of candidate genes either using Illumina or Taqman methodology
- Assistance with genetic study design
- Genetic statistical analysis assistance
- Training for DERC investigators and their staffs to perform many of these procedures themselves, with ongoing consultative support from Core staff

CONTACT INFORMATION
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Statistical Genetic Analysis: Xiuqing Guo, Ph.D. Xiuqing.Guo@cshs.org

Main Core Contact: Cynthia Hernandez, R.N., Genetics Study Coordinator, Division of Medical Genetics, Cedars-Sinai Medical Center, 8700 Beverly Blvd. PACT4, Los Angeles, CA 90048, Tel. (310) 423-1457; Fax (310) 423-0237; Email Cynthia.Hernandez@cshs.org

GENOTYPING IN THE GWAS AND POST-GWAS ERA
The technological developments that have occurred in the field of genetics and genomics in recent years have been dramatic, making it difficult for the average researcher to keep track of which is the most appropriate approach to testing for genetic involvement in disease development, complications or response to therapy. In deciding what genotyping approach makes most sense for a given project, the first question to ask is whether you have hypotheses that a specific gene or group of genes is involved in the condition under study (Candidate gene approach) or if you are looking more broadly for the involvement of any gene or locus in the disease of interest (Genome-wide approach).

Genotyping Approaches for Candidate Gene Studies
Candidate gene approaches generally involve testing a limited number of genes, although the number can vary from one to two genes, up to several hundred. If there are functional variants known to produce an abnormal protein or alter gene expression, you may only wish to test for those specific variants, even one single nucleotide polymorphism (SNP). It this is the case, then use of individual PCR assays for genotyping may be the best way to go. In most cases, however, functional variants will not be known or if known, may not be thought to explain all of the effect a gene may have on a disease. In this situation, it is better to plan on genotyping a large number SNPs across the gene, to interrogate the gene in detail. The number of SNPs needed will depend on the size of the gene and the number of haplotype blocks (regions of the gene that demonstrate a large amount of linkage disequilibrium and thus tend to be inherited without undergoing recombination). For a small gene, 5-10 SNPs may be sufficient but for very large genes, 50-100 or even more SNPs may need to be genotyped to fully evaluate genetic associations.
If a moderate number of SNPs (~10-100) is going to be genotyped, the appropriate technology to use is TaqMan® SNP Genotyping, available to DERC investigators in the Human Genetics Core Laboratory. If your study will involve large numbers of SNPs (~350-thousands), then a more efficient and cost-effective approach will be the use of Illumina bead technology, which is also available in the DERC Human Genetics Core Laboratory. This genotyping technology utilizes hybridization capture by coded glass beads followed by dye-labelled base extension (Gunderson,2006) (Illumina Infinium assay). While some standard genotyping chips are available (for example an MHC panel that might be of interest to someone studying the HLA region in Type 1 diabetes), typically the investigator will need to purchase a custom SNP chip designed to include the particular SNPs appropriate for his/her study. The Core Genotyping Lab staff will assist you in selecting the appropriate SNPs, using several software programs specifically designed to perform this function. Because not all SNPs will genotype efficiently using beadchip methodology, the lab recommends selecting about 10% of the SNPs to provide redundancy. Unless your study population is ethnically very homogeneous, you will be recommended to include a number of ancestry informative markers (AIMS) as this will allow estimation of principal components that can be used to avoid spurious associations resulting from population stratification. Once SNP selection has been completed, the chips are ordered and received in the lab, the actual genotyping can be
completed in a matter of weeks. The laboratory has robotic stations for high throughput and extensive experience with genotyping. Several quality control (QC) measurements are incorporated within the Illumina bead-chip design and the GenomeStudio software immediately provides metrics of the success of the various molecular biology steps (amplification, fragmentation, hybridization, and enzymatic single base extension) and chip processing steps (immunohistochemical staining & washing by robotic work station).

**Genome wide approaches**

As advances have made genotyping of hundreds of thousands of SNPs both technologically feasible and financially realistic, many investigators have abandoned candidate gene based studies in favor of genome wide association studies (GWAS). In this type of study design, extremely large numbers of SNPs spanning the entire genome are genotyped, with the goal of identifying associations in particular regions that warrant further exploration. The number of SNPs keeps increasing; a year or two ago, roughly 350,000 SNPs was standard but today at least 700,000 SNPs is considered appropriate. While the cost per SNP is very small, the chips for GWAS genotyping this many SNPs are not inexpensive; the genotyping costs will be in the range of $300-500/subject and one must plan on at least several hundred samples being genotyped in order to have sufficient statistical power to detect any associations. If you are thinking about a GWAS study, we recommend you contact Dr. Rotter, Dr. Raffel or Dr. Taylor to discuss your study population, sample size, etc. to make sure that this approach is likely to produce meaningful data for you.

More challenging in many ways than the actual genotyping, statistical analysis of the massive amounts of data generated by GWAS often takes more time than the laboratory studies themselves. Dr. Xiuqing Guo heads the DERC Human Genetics Core Statistical Analysis group and is available for consultation as you are designing your study. A number of computer programs have been developed for GWAS analysis and the group is familiar with their use. In addition, one of our junior scientists, SooIn Kwon PhD, has developed a novel approach to statistical analysis that is proving to be very useful. One of the real challenges of GWAS analysis is dealing with the high volume of SNPs per subject; most analytic techniques assume that the number of subjects will exceed the number of statistical comparisons being performed but the reverse situation (the number of statistical comparisons far exceeds the number of subjects) is what occurs in GWAS studies. As a result, the analysis time is substantial and the methods employed only allow analysis of each SNP individually. Dr. Kwon’s method, called Bayesian multivariate regression with singular value decomposition (BMRSVD), both improve computing speed and enable simultaneous analysis of multiple markers to identify gene-gene interactions.

**Future Directions in Genotyping and Mutation Identification**

While GWAS studies have identified a large number of genes that play a role in susceptibility to common disorders, including type 1 and type 2 diabetes, the proportion of the genetic contribution to disease accounted for by the genes that have been identified has been smaller than was anticipated. There may well be a number of reasons that this is the case, but one that is thought to be quite important is the likelihood that rare variants have a greater role in disease susceptibility than was previously thought. GWAS studies are effective in identifying common variants that are involved in disease susceptibility in a relatively large proportion of affected individuals, but have little ability to identify rare mutations that are contributing to disease in only a handful of people. The approach that is being taken to try and find such rare variants is large-scale sequencing. Technologically, it is now possible to perform exon sequencing of all genes and a number of studies of this type are currently underway. In addition, the goal is to be able to perform whole genome sequencing in the foreseeable future, as it is expected that disease associated variants will be found in other regions of the genome as well as in protein coding regions. Currently, the DERC Genetics Core does not offer large scale sequencing services, but the DERC Genomics Core can provide such services.
**Recent Major Findings by Our Membership**

DERC faculty members at UCSF/Salk and UCLA/Cedars represent a large, highly productive and diverse group of scientists, four projects highlighted here represent different areas of scientific endeavor, which reflect emerging issues in the overall fields.

The first advance is from *Peter Tontonoz’s laboratory*. This study started with the premise that dysregulated lipid metabolism is an important component of human metabolic diseases. The Liver X Receptors (LXRs) are nuclear b receptors that play central roles in the transcriptional control of lipid metabolism. LXRs function as nuclear “cholesterol sensors” that are activated in response to elevated intracellular cholesterol levels in multiple cell types. Once activated, LXRs induce the expression of an array of genes involved in cholesterol absorption, efflux, transport and excretion. In their recent paper, they demonstrated that *LXRs inhibit cholesterol uptake by inducing the ubiquitination and degradation of the LDL receptor* (LDLR). LXR inhibits the LDLR pathway through transcriptional induction of Idol (inducible degrader of the LDLR), an E3 ubiquitin ligase that triggers ubiquitination of the LDLR on its cytoplasmic domain, thereby targeting it for degradation. LXR ligand reduces, whereas LXR knockout increases, LDLR protein levels in vivo in a tissue selective manner. Idol knockdown in hepatocytes increases LDLR protein levels and promotes LDL uptake. Conversely, adenosine-mediated expression of Idol in mouse liver promotes LDLR degradation and elevates plasma LDL levels. The LXR-Idol-LDLR axis defines a complementary pathway to sterol response element–binding proteins for sterol regulation of cholesterol uptake. The ability of LXRs to integrate metabolic and inflammatory signaling makes them potentially attractive targets for intervention in human metabolic disease.

The second paper is from *Dr. Seth Field*. Phosphoinositides play many different important roles in biology but the full breadth and detailed mechanisms of function are not understood. Dr. Field’s lab has taken several innovative approaches to systematically elucidate the functions of the phosphoinositides and the biological processes and disease states that they control. They published a recent Cell paper that provides a particularly good example of the strength of their approach. Using an unbiased proteomic screen of a large portion of the Drosophila proteome they identified a novel protein called GOLPH3 that binds to PI4P. PI4P has been well-known to be enriched at the Golgi and required for Golgi function in all organisms from budding yeast to humans. They showed that the yeast, fly, and human GOLPH3 proteins localize to the Golgi by binding to PI4P and are required for Golgi function. The big surprise came when they figured out how GOLPH3 works. His lab discovered that GOLPH3 also binds to myosin18A, and this myosin can apply a stretching tensile force to the Golgi membrane via GOLPH3/PI4P. This stretching force is used to pull on the Golgi membrane to help vesicles bud from the Golgi, and therefore is a key component of the mechanism of vesicle trafficking. Moreover, a side effect of the tensile force pulling on the Golgi is to flatten the Golgi stacks, and to stretch the Golgi ribbon around the nucleus. In short, they have discovered a key component of the mechanism of vesicle trafficking from the Golgi, and have shown that the Golgi's unique morphology is a consequence of the mechanism of trafficking.

*Marc Montminy’s laboratory* continues to explore the key relationship between *TORC2* (CRTC2) and *hepatic glucose metabolism*. He has studied the interlocking connections between ER stress and hepatic glucose production. It is well known that obesity leads to a state of hepatic insulin resistance and that ER stress in the liver might be an important etiology in these processes. In these studies, the focus was on TORC2, and they found that activation of ER stress stimulates TORC2 dephosphorylation, which facilitates its nuclear entry and activation. Surprisingly, this did not lead to increased gluconeogenesis, which is the usual role of TORC2 in the liver. This led them to identify a novel mechanism in which the ER stress response factor Activating Transcription Factor 6a (ATF6a) physically binds to TORC2 and this complex drives transcription of a set of ER quality control genes as part of the unfolded protein response. They then went on to show that this interaction between ATF6a and TORC2, which recruits TORC2 to ER stress inducible promoters, decreases the ability of TORC2 to interact with CREB on gluconeogenic genes. This provides a new mechanism whereby TORC2 can sense ER stress to help establish the ER stress response and at the same time can sense nutrient signals such as fasting, to regulate hepatic gluconeogenesis.

*Dr. Pinchas Cohen* recently discovered a novel family of mitochondrial-derived peptides that regulate cell survival and metabolism (for which he was awarded a transformative RO1 grant). One of these peptides, known as humanin, was shown to be a potent, centrally acting, systemic insulin sensitizer. The main effect of humanin, acting via a hypothalamic stat-3 pathway, is to potentiate suppress hepatic glucose production, although it has additional effects on insulin sensitization and insulin secretion. Remarkably, a single IV dose of the ultra-potent humanin analogue HNG6F6A, completely normalized blood sugar in a severely diabetic Zucker rat model. Humanin and other novel mitochondrial-derived peptides are emerging as potential treatments for diabetes and other metabolic diseases.