Description of Research and Training Opportunities for MRDD Trainees

Basic Research Training in MRDD:

Robert J. Desnick, PhD, MD; Program Director
Inborn Errors of Metabolism, Genomics and Molecular-Based Therapeutics

Dr. Desnick's laboratory is involved in several areas of genetic research involving MRDD. Active research programs include the molecular genetics of inborn errors of the lysosome and of heme biosynthesis, gene discovery and genomic medicine, and gene therapy. In each of these research themes, emphasis is directed on the basic science (e.g., generation of knockout mice to study disease pathogenesis, crystallization of proteins to determine structure/function, etc.) as well as translational research to develop and evaluate new diagnostics and novel therapies. Highlights of these research projects, which provide opportunities for predoctoral and postdoctoral fellows, are described briefly below.

Project 1. Molecular Genetics and Treatment of Lysosomal Storage Diseases:
For the past two decades, studies of the lysosome and lysosomal storage diseases have been a major research theme of this laboratory. Among the various lysosomal storage diseases, Fabry and Schindler diseases have provided model systems for the application of biochemical, molecular, and cellular techniques to understand lysosomal disease pathogenesis, improve diagnosis, and to develop novel therapies. For example, in Fabry disease (α-galactosidase A (α-Gal A) deficiency), our group purified the enzyme, isolated the gene, developed novel methods to produce and purify large amounts of the normal human enzyme, and evaluated the effectiveness of various enzyme glycoforms in the Fabry "knockout" mouse model we established. These basic science studies provided the rationale for the clinical trials of enzyme therapy that have proved effective in this disease. Current research efforts in Fabry disease are directed to: 1) crystallize α-Gal A to understand its basic structure and reaction mechanism, 2) characterize the mutations which cause the classical and variant phenotypes of the disease and identify correlations useful for patient and family management, 3) identify and characterize α-Gal A gene cis-acting regulatory elements using Luciferase constructs and transgenic mice with α-Gal A promoter deletions as the transgene, and 3) develop gene replacement for Fabry disease.

Schindler disease is an inherited neuroaxonal dystrophy due to the deficient activity of α-*N*-acetygalactosaminidase (or α-Gal B). The inherited neuroaxonal dystrophies constitute a family of neurodegenerative diseases with common neuropathology, the presence of swellings or "spheroids" in the terminal endings and/or the distal portions of axons in the central nervous system. It is presumed that these pathologic findings result from abnormalities in axonal transport, the process by which molecules and organelles that are synthesized and assembled in the soma of the neuron are incorporated into vesicles and transported along a microtubular system down the axon to the synapse and then back to the soma for degradation. Until recently, the metabolic defects causing the neuroaxonal dystrophies remained an enigma. Insight into to the etiology of these disorders was provided by the discovery that Schindler disease was due to the deficient activity of α-Gal B. Patients with this disease excrete large amounts of glycopeptides which have *O*-glycosidically linked α-*N*-acetylgalactosaminyl moieties. This discovery represents the first metabolic defect identified in a neuroaxonal dystrophy and provides the clue for the elucidation of the metabolic defects in the other neuroaxonal dystrophies.

Accomplishments by our laboratory include: 1) purification of human α-gal B to homogeneity, 2) isolation and characterization of the full-length cDNA and entire genomic sequence, 3) determination of the molecular lesions in Schindler disease, 4) identification of a milder, adult-onset form of the disorder and detection of the causative mutation, and 5) construction of a murine "knock-out" model of Schindler disease by homologous recombination in ES cells. Current efforts are directed to: 1) overexpress and purify the recombinant enzyme for physical/kinetic characterization and crystallization, 2) characterize the α-Gal B deficient knock-out mouse which has neuroaxonal as well as visceral pathology, and 3) use the mouse model to elucidate the role of α-N-acetylgalactosaminidase in neuroaxonal transport. In addition, the knock-out model will be used to develop neural gene therapy for lysosomal and other diseases with global neuronal involvement.

Project 2. Molecular Genetics of the Inherited Porphyrias:
Of the metabolic pathways in man, only a few are known to be tightly regulated (e.g., cholesterol and heme biosynthesis). In man, heme biosynthesis requires eight enzymatic steps to convert succinyl-CoA and glycine to the final product, heme. All eight enzymes are encoded by nuclear genes and four of the reactions occur in the cytosol, while four take place in the mitochondrion. The focus of this research opportunity is the investigation of the inborn errors of heme biosynthesis, the inherited porphyrias. Each of these disorders results from the deficient activity of a particular heme biosynthetic enzyme. The inborn errors of heme biosynthesis provide the opportunity to investigate the effects of dominant mutations and the action of environmental factors, since most of these diseases are latent until exacerbated by an environmental or pharmacologic stress. Our laboratory has a long and successful record of basic and applied research in heme biosynthesis and the porphyrias. We have: 1) developed specific assays, 2) purified to homogeneity and characterized the first five enzymes in the pathway, 3) isolated and characterized the cDNAs and/or genomic sequences encoding the first five enzymes, 4) studied the tissue-specific regulation of these genes encoding the first, second and fourth enzymes in the pathway, and 5) identified the molecular lesions causing five of the porphyrias. Current efforts are focused at developing enzyme and gene therapies for the porphyrias using "knock-in" mice under development in our laboratory. These studies involve sophisticated genetic and biochemical techniques to make the recombinant enzymes and mouse models to evaluate novel enzyme and gene therapies. The two disease targets are acute intermittent porphyria, an acute hepatic porphyria, and congenital erythropoietic porphyria.

Project 3. Gene Discovery, Functional Genomics and Pharmacogenomics:
i) Gene Discovery: In collaboration with Departmental faculty, efforts are directed to exploit the Human Genome Project to: 1) identify the genes underlying various Mendelian and complex diseases that cause MRDD, 2) identify genes by functional genomics, and 3) determine the pharmacogenomic traits in man as a means of studying human variation. Using linkage analysis and positional cloning strategies, our previous efforts have resulted in the identification of several genes causing Mendelian disorders. Current research is focused on several Mendelian disorders and two complex traits, one being Crohn disease, a common inflammatory bowel disease. This research affords predoctoral and postdoctoral fellows the opportunity to use genomic techniques to identify new genes causing rare and common diseases.

ii) Functional Genomics: A major effort is underway to determine the functional genomics of the lysosome. Using high-resolution mass spectroscopy, the genes involved in human and/or murine lysosomal biogenesis and function are being identified. Novel methods have been developed to isolate lysosomes, perform 2-D gel electrophoresis to separate their membrane and soluble proteins, and then to identify each of the proteins using mass spectroscopy to sequence each of

these proteins. In this way, novel proteins involved in lysosomal degradation and membrane biogenesis will be identified.

iii) Pharmacogenomics: These studies involve the identification of variations in human genes responsible for the metabolism of drugs. These variations cause the adverse drug responses that are common and often life-threatening. Examples of the known genes with varying pharmacogenetic responses are the P450 genes. By identifying the key genetic variations in an individual's genome that alter the activation, metabolism, transport, distribution and clearance of a given drug, a person's pharmacogenetic profile can be determined, permitting personalized drug selection and dosage. Using single nucleotide polymorphisms (SNPs), candidate genes for a given drug are interrogated for informative haplotypes which are then tested in a given population of individuals experiencing adverse affects of the drug. In addition, variations that alter drug metabolism can be tested in individuals taking the drug.

Project 4. Molecular-Based Therapy of Gene to Diseases

These studies are designed to develop and evaluate various strategies for the treatment of inherited metabolic diseases. For certain disorders resulting from the deficiency of a specific enzyme, the cDNA encoding the normal enzyme is isolated and used to produce large amounts of the recombinant enzyme for animal, and then human trials of enzyme replacement therapy. For other diseases in which gene replacement is feasible, efforts are being directed to insert the cDNA into various vectors for gene transfer studies. The availability of animal models (mice, cats, and dogs) with certain lysosomal storage diseases and porphyrias provide the unique opportunity to develop and evaluate new methods of gene therapy, including the use of neurotropic vectors for the treatment of neurologic disease.

Edward H. Schuchman, PhD; Associate Program Director
Gene Based Therapy for Inborn Errors of Metabolism

Project 1. Niemann-Pick Disease: Genotype/Phenotype Analyses and Molecular-Based Therapy. Types A and B Niemann-Pick disease (NPD) are storage disorders resulting from the deficient activity of acid sphingomyelinase (ASM). Patients with ASM deficiency exhibit a phenotypic spectrum ranging from a severe neurodegenerative course which usually leads to death by three years of age (classical Type A) to little or no neurologic involvement and survival into adulthood (classical Type B). Despite the fact that this disorder was described over seventy years ago, no reliable biochemical tests have been developed to predict the phenotypic outcome of newly diagnosed individuals and no treatment is available for affected patients. Thus, the specific aims of this proposal are to: 1) characterize the natural history of Types A and B disease, identify causative ASM mutations, and identify predictive genotype/phenotype correlations and 2) develop specific therapies for Type B patients including a) drug and dietary strategies to reduce the hyperlipidemia, b) enzyme replacement, c) bone marrow transplantation, and d) hematopoietic stem cell-mediated gene therapy. Towards these goals, we have: 1) isolated and characterized the full-length cDNA and genomic sequences encoding human and murine ASM, 2) identified and expressed disease-causing mutations, 3) stably overexpressed human ASM in Chinese hamster ovary (CHO) cells, 4) constructed ASM retroviral vectors and demonstrated metabolic correction of transduced NPD cells, and 5) generated ASM deficient “knock-out” mice.

The proposed clinical studies will document the clinical, biochemical and molecular pathology of a large series of patients. To determine the cause of the hyperlipidemia and hypercholesterolemia that occurs in NPD, cholesterol and triglyceride transport and metabolism will be investigated. Recombinant human ASM will be overexpressed in CHO cells and, following preclinical studies

in ASM "knock-out" mice, enzyme replacement trials will be undertaken. Allogeneic bone marrow transplantation in selected patients will be evaluated prior to efforts to cure Type B disease by *ex vivo* gene transfer of hematopoietic stem cells transfected with ASM retroviral constructs. The effectiveness of each of these therapeutic endeavors will be evaluated by serial assessment of selected clinical and biochemical endpoints.

Project 2. Mucopolysaccharidosis Type VI: Animal Models and Gene Therapy. Mucopolysaccharidosis type VI (MPS VI) is a lysosomal storage disease resulting from the deficient activity of arylsulfatase B (ASB). In addition to man, MPS VI has been described in cats and rats, two important animal models for which breeding colonies are available. We have been investigating the molecular genetics of MPS VI and developing gene therapy for this disorder using these animal model systems. Full-length human, cat and rat ASB cDNAs have been isolated and transiently expressed, facilitating the identification of six new human mutations, as well as the lesions causing the murine and feline disorders. To develop gene therapy for MPS VI, the full-length ASB cDNAs have also been inserted into retroviral vectors and used to transduce cultured fibroblasts from MPS VI patients and animals. In each case, the retroviral vectors expressed high levels of enzymatic activity and, importantly, diseased cells that had been transduced with these vectors secreted high levels of the enzyme, facilitating cross-correction of non-transduced cells. The retroviral vectors have also been used to transduce bone marrow cells and the MPS VI animal models are being used to evaluate the *in vivo* efficacy of bone marrow-mediated gene therapy and for the construction of overexpressing organoids. To facilitate these gene therapy studies, a novel selection method is under development to directly select retrovirally-transduced cells based on the uptake and catabolism of fluorescently-labeled dermatan sulfate. Preliminary studies indicate that the fluorescent substrate can be internalized by cells and, following its degradation, it serves as a useful marker of enzyme-expressing cells.

Aneel Aggarwal, PhD
Structural Analysis of DNA Binding Proteins

The research in this laboratory attempts to understand how proteins bind DNA, leading to specific DNA cleavage and regulation of transcription. Efforts are concentrated on two categories of DNA binding proteins, restriction enzymes and transcription factors. The primary techniques used are X-ray crystallography combined with biochemical and other biophysical methods. These techniques have permitted the determination of the structures of restriction endonuclease BamHI with and without DNA, and the structure of multimiodular endonuclease FokI with DNA. The BamHI structures have changed the view of the conformational transitions that can occur when proteins bind to DNA. The FokI structure reveals an unusual architecture that provides a basis for creating artificial enzyme with novel specificities. In addition to extending the above systems, future goals include the study of intron encoded endonucleases that propagate through the insertion of introns into intron-less alleles. The research on transcription factors is focused on uncovering the interactions that determine the decision to initiate transcription in eukaryotes. They have determined the structure of Even-skipped homeodomain bound to a tandemly repeated DNA site, and the structure of pituitary specific factor Pit-1 POU domain bound to DNA as a homodimer. Both structures reveal novel modes of DNA binding that have broad implications for the mechanisms by which transcription factors select DNA sites. An increasing emphasis of this work is towards the study of multiprotein complexes that characterize eukaryotic transcription. Systems under study include the homeodomain Extradenticle/Labial complex, Pit 1-cofactor complexes, and complexes between interferon regulatory factors and coactivators. Such studies are critical for understanding how transcriptional complexes are established in eukaryotic organisms, leading to specific cells and response to extracellular signals. Pre- and Postdoctoral trainees receive training in all aspects of structural biology and molecular modeling.

George F. Atweh, MD
Gene Therapy of Sickle Cell Disease and ß-Thalassemia

The major aim of our research is the cure of sickle cell disease and ß-thalassemia by gene therapy. Previous attempts to develop retroviral gene transfer vectors to transduce normal globin genes into the hematopoietic stem cells have had limited success. The "first generation" retroviral vectors included a human ßglobin gene with its immediate flanking region. When these retroviruses were used to transduce mouse bone marrow cells, the level of expression of the transduced human ß-globin in the differentiated red blood cells was too low to be clinically useful. In the "second generation" retroviral vectors, a powerful distant regulatory element from the ß-globin gene complex known as locus control region (LCR) was included in the retroviral vectors. The human ß-globin gene transduced by these retroviruses was expressed at levels comparable to the mouse $ß^{maj}$ globin gene. Unfortunately, the inclusion of the highly recombinogenic ß-globin LCR element or one of its four subunits in the retroviruses made those vectors extremely unstable and the titers of the retroviral stocks very low. Our strategy for developing a "third generation" retroviral vector makes use of the major regulatory element of the ß-globin gene known as HS-40, or ? -LCR, instead of the ß-LCR to enhance the expression of transduced ß-globin genes. We have recently started evaluating new gene transfer vectors *in vivo* in the mouse system. We found that these retroviruses can infect primary hematopoietic progenitors efficiently as seen in methylcellulose assays and in CFU-S assays. Long-term bone marrow reconstitution experiments are underway to determine the efficiency of transduction of the hematopoietic stem cells. We will also examine the ability of these retroviruses to cure mice with sickle cell disease and ß-thalassemia. We hope to be able to extrapolate these studies in the near future to the gene therapy of these disorders in humans. Trainees learn how to conduct hematopoietic stem cell gene therapy studies in mouse models.

Margaret Baron, MD, PhD
Stem Cell Biology and Gene Therapy for Hematopoietic Disorders

Dr. Baron's laboratory is using a variety of molecular, cellular, and genetic approaches to study the induction and morphogenesis of the hematopoietic and vascular systems in the mouse embryo. They have developed a novel explant culture assay for signaling interactions required for embryonic hematopoiesis and vasculogenesis in mouse embryos. Using this assay, they have identified signaling interactions between primitive (visceral) endoderm (VE) and mesoderm that are required for hematopoietic and vascular induction in the early mouse embryo, and that are required only during a specific window of time during development. They have now shown that a member of the Hedgehog family of signaling molecules, Indian hedgehog (Ihh), is secreted by the VE layer of the embryo and alone is sufficient to induce formation of hematopoietic and endothelial cells. Remarkably, like VE tissue itself, Ihh can also respecify prospective neural ectoderm (anterior epiblast) along hematopoietic and endothelial (posterior) lineages. Downstream targets of the Hh signaling pathway (*Ptch, Smo, Gli1*) are upregulated in anterior epiblasts cultured in the presence of Ihh protein. Dispersed cells from IHH-treated anterior epiblasts form primitive or definitive hematopoietic colonies in secondary cultures in the presence of appropriate cytokines, indicating that functional hematopoietic stem/progenitor cells are produced. Blocking Ihh function in the endoderm inhibits activation of hematopoiesis and vasculogenesis in the adjacent epiblast, suggesting that Ihh is an endogenous signal that plays a key role in the development of the earliest hemato-vascular system. *Hedgehog* genes and protein are expressed by adult mouse and human bone marrow stromal cells and *Ptch* and *Smo* are expressed in hematopoietic stem/progenitor as well as endothelial cells. Ihh treatment also results

in upregulation of *Bmp4*, which may mediate the effects of Ihh. Indeed, BMP-4 protein can substitute for IHH protein in the explant culture assay, and a homologue of the frog *Mix* genes is upregulated in posterior mesoderm. Based on their mRNA and protein expression studies and experiments in frog embryos, there is evidence to suggest that this gene may play a role both in primitive hematopoiesis/vascular development and in endoderm formation and would, therefore, likely function downstream of Ihh and Bmp4 *in vivo*. Trainees receive exposure to many aspects of developmental biology and comprehensive training in molecular genetics and gene therapy.

Deanna Benson, PhD
Targeting and Assembly of Synapses

Dr. Deanna Benson's laboratory is exploring the mechanisms underlying synapse targeting and assembly during development, during long-term potentiation, and in response to injury. She has been investigating both general properties of synapse formation like timing and inductive influences, as well as the molecules involved. Cell adhesion molecules in particular are good candidates for both generating junctions and encoding specificity, and her lab has been focusing on the cadherin and immunoglobulin superfamilies of cell adhesion molecules. Cadherins are numerous, diverse and clustered at synapses while immunoglobulin superfamily members are more broadly distributed with apparently more general roles like axon bundling or fasciculation. Both families play integral roles in synaptogenesis. Work on the function of cell adhesion molecules has also generated research on how the molecules are targeted and retained at particular locations along the plasma membrane. Questions are addressed using cell biological, imaging, anatomical and recombinant DNA techniques. Pre- and postdoctoral students receive broad training in vertebrate, developmental neurobiology.

Gary Benson, PhD
Detection and Analysis of Tandem Repeats in DNA

Dr. Gary Benson's research is focused on the development of algorithms and programs to analyze DNA sequences for various repeats and functional patterns. He is interested in theoretical issues in algorithm design as well as the biology of the repeats. His research encompasses several areas of computational biology, including pattern detection, sequence alignment, statistical and probabilistic analysis, and data clustering methods. His current work involves the detection and analysis of tandem repeats in DNA. He has developed algorithms for detecting tandem repeats, aligning sequences containing tandem repeats and exploring the characteristics of duplication events. He is currently developing algorithms for comparing and clustering repeats into families in order to construct a multi-genome database of tandem repeats. He is also is working on methods to detect low-copy repeats, which occur as non-adjacent copies, and composition repeats which occur as variations in DNA composition rather than as `word' patterns. The former contribute to many types of disease and the latter may have regulatory and/or structural effects

Andrew Bergemann, PhD
Axon Guidance and Developmental Neurobiology

The overall interest of this laboratory is in developmental neurobiology. Currently, the lab is focused on two projects. The first is to determine molecular cues guiding the early development of tissues derived from the dorso-medial roof of the third ventricle. This small region of the developing brain encompasses the primordial of five organs: the subfornical organ, the lamina terminalis, the choroid plexus of the third ventricle, the pineal gland and the subcommissural organ. In the adult vetebrate, each of these organs act as a rapid exchange point for macromolecules between the brain and either the blood or the cerebro-spinal fluid. The second

project seeks to biochemically characterize the mechanisms by which the interactions of the eph receptor tyrosine kinases with their ligands, the ephrins, control many aspects of axon guidance in the developing vetebrate embryo. In particular, we are very interested in mechanisms mediating, and regulating, the oligomerization of the ephrin molecule since the monomeric forms of ephrin can readlty bind to, but not activate, the receptors. Trainees will be exposed to many contemporary research approaches to studying veterbrate neurodevelopment, including morphological, biochemical and molecular analyses. Trainees are exposed to many aspects of developmental neurobiology.

James Bieker, PhD
Regulation of Gene Expression During Erythropoiesis

The molecular events that confer the ability to express lineage-specific genes upon an initially uncommitted, pluripotent hematopoietic stem cell remain a major question in cell differentiation. Use of an immortalized erythroid cell line as a means to isolate genes that may be important for erythroid function allowed this group to identify a novel, erythroid-specific gene, which was named EKLF (erythroid Krüppel-like factor). EKLF binds to and activates transcription from the CACCC element, one of a trio of localized promoter and enhancer DNA binding sites known to be crucial for transcription of globin and other erythroid cell-specific genes. Biological analyses reveal that murine EKLF is expressed in primitive erythroid cells by embryonic day 7.5, and in definitive erythroid cells within the hepatic primordia by embryonic day 9.5. However, its ability to preferentially activate a β-globin promoter over a linked γ-globin promoter led to the proposal that EKLF may be an important factor for γ- to β-globin gene switching. This was verified by studies showing that EKLF is absolutely required for normal red cell development, since its genetic disruption leads to death by embryonic day 14-16 (precisely the time of the switch in mice) due to a deficiency of mature, definitive red cells. EKLF-deficient mice exhibit drastically low β-globin expression at the transcriptional level, i.e., a severe β-thalassemia phenotype, and contain an altered chromatin structure at the β-globin locus. These molecular and biological studies have established that EKLF is an essential component required for globin switching and completion of the definitive erythroid program. We are vigorously continuing its study using a number of approaches, including biochemical and structure/function analyses of the EKLF protein, identification of its protein partners, and monitoring how EKLF expression itself is so precisely regulated during development. Pre- and postdoctoral trainees receive training in many aspects of molecular genetics and biochemistry.

David F. Bishop, PhD
Molecular Genetics and Regulation of Heme Biosynthesis and Disorders of Heme Metabolism

The research interests of Dr. Bishop's laboratory are directed to an understanding of the molecular genetics of heme biosynthesis and, in particular, its regulation and control by the rate-limiting enzyme of the pathway, 5-aminolevulinate synthase (ALAS). His laboratory's previous demonstration of the existence of erythroid and non-erythroid isozymes of human ALAS was confirmed by the cloning and sequencing of the human housekeeping gene, ALAS1, and the erythroid tissue-specific gene, ALAS2. While these genes share greater than 50% amino acid identity over most of their coding sequences, they were localized to two different chromosomes (i.e. 3 and X, respectively, indicating that they arose from gene duplication and divergence presumably directed by their different functional requirements). This laboratory's studies of the erythroid-specific gene, ALAS2, are focused on its role in erythroid differentiation and genetic disorders. It is being characterized with respect to its control by erythroid transcription factors

including GATA-1 and its coordinated regulation during erythroid differentiation in human erythroleukemic cell lines such as K562. Recently, this laboratory identified ALAS2 as the defective gene in patients with X-linked sideroblastic anemia and identified a new late-onset variant of this disorder. A high-level prokaryotic expression system is being employed to generate normal and mutant enzyme for biochemical characterization and eventual structural analysis by X-ray crystallography. Structure-function correlations are being analyzed with the goals of diagnostic and therapeutic advances in the treatment of X-linked sideroblastic anemia. In liver, the housekeeping gene, ALAS1, is the rate-limiting enzyme of heme biosynthesis and its activity is markedly elevated in the acute hepatic porphyrias, including acute intermittent porphyria and coproporphyria. The regulation of heme biosynthesis by ALAS1 is unique in that it is one of the few mammalian pathways controlled by negative feedback repression by its end product, heme. This group is studying the repression of ALAS1 transcription by heme as well as its heme-inhibited transport to the mitochondrion. Knock-out and knock-in mice are being generated by homologous recombination in embryonic stem cells in order to characterize the negative feedback mechanisms and the effects of heme deprivation.

Andrea Branch, PhD
RNA-Based Gene Therapy for Neurological Diseases

Research in this laboratory is devoted to the development of RNA-based gene therapy for neurological diseases. In particular, we are developing novel ribozymes that can be used to destroy pathogenic cytokines expressed in the brains of individuals with MRDD, and antisense based approaches to down regulate abnormally expressed genes. Since RNAs can readily cross the blood brain barrier, we anticipate that these approaches will be particularly useful for neurological diseases. Several potential RNA therpeutics are currently being tested in animal model systems. Pre and postdoctoral trainees will have the opportunity to learn how to develop RNA-based gene therapy and evaluate efficacy in animal models of neurological disease.

Jonathan Bromberg, MD, PhD
Carrier Molecules for Gene Therapy

Gene transfer and gene therapy have the ability to treat a variety of disease process including transplantation by the transfer of immunosuppressive agents into grafts with their subsequent expression and prevention of allograft rejection. The laboratory is investigating the use of a variety of plasmid and viral vectors to transfer the immunosuppressive cytokines viral IL-10 and TGFB1 into cardiac allografts to prolong allograft survival and attempt to achieve definite graft survival and antigen specific transplantation tolerance. Specific projects focus on optimization of various carrier molecules such as defined peptides, dendrimer, or lipids for enhanced transfection of plasmid DNA into target cells in organs. Another area of focus deals with regulation of the promoters linked to the transfected and transduced vector DNA. Lastly, there is also a focus on the types of immunologic mechanisms that are induced by gene transfer of immunosuppressive cytokines. The specific expression of immunosuppressive cytokines within an allograft may also elucidate how the immune system determines and channels immune reponses away from reactivity and towards suppression, anergy, and tolerance.

Dieter Brömme, PhD
Studies of Cysteine Proteases in Health and Disease

Proteases represent approximately 2% of the gene products in the human genome and are responsible for the general turnover as well as for the highly specific processing of most of the

remaining 98% of the genome. Proteolytic enzymes are critically involved in such diverse functions as the terminal breakdown of protein nutrients and intracellular proteins for energy consumption and *de novo* protein biosynthesis, and the regulation of apoptosis, immune responses and development. Moreover, they are directly responsible for the tissue-destructive processes in diseases as diverse as arthritis, Alzheimer's diseases, cancer, and infections of all kind. We know five different protease classes whose classification is based on their catalytic mechanism: Serine, cysteine, aspartate, threonine and metallo proteases. Our laboratory is focused on lysosomal proteases. In past years, we have identified and characterized novel cysteine protease genes critically involved in various degenerative diseases and inflammatory immune responses. For example, cathepsin K is the enzyme defect in the genetic disorder, pycnodisostois. In both processes, proteases are pivotal enzyme activities responsible for the destruction of the extracellular matrix and for the regulation of the disease progress. Here, it is of particular interest that some of those proteases are expressed in a highly tissue-specific manner which makes them attractive drug targets. The research is focused on the understanding of protease activities in the etiology of osteoporosis and rheumatoid arthritis as well as of accompanied inflammatory mechanisms involving natural killer cells and the MHC class II pathway. Methods in our laboratory include molecular biology, enzymology, protein purification, histochemistry, cell biology, and animal models.

Joseph Buxbaum, PhD
Gene Discovery for Autism and other Behavioral Disorders

The laboratory of molecular neuropsychiatry is focused on genetic studies of neurodegenerative diseases, including Alzheimer's disease, autism and schizophrenia. Population-based gene mapping studies are carried out to identify candidate causative or susceptibility genes, which are then analyzed using state-of-the-art biochemical, molecular and cell biological techniques. RNA profiling and other genome-based techniques are also used to identify changes in gene and protein expression in the brains of individuals with these disorders. Trainees have the opportunity to join these genome based projects and participate in the genetic analysis of these common neurological diseases.

Luz Claudio, PhD
In Vitro Models of the Blood Brain Barrier

Research in this laboratory centers on the cellular and molecular mechanisms by which environmental factors may cause or aggravate neurological disease an affect brain development. Dr. Claudio's laboratory specifically focuses on developing in vitro models of the human blood-brain barrier that can be used to study how environmental chemicals enter the brain and disrupt neurological function and to use as a testing model for new neurotrophic drugs. Dr. Claudio also directs the Community Outreach and Education program within the Department of Enviornmental Medicine. In that capacity, she has developed educational activities for students at all academic levels.

George Diaz, MD, PhD
Genomic Approaches to Gene Identification

The focus of activity in this laboratory is the application of genomic approaches for the identification and characterization of genes that, when mutated, lead to inherited diseases in humans. Current projects focus on traits in which the disease manifestations suggest that identification of the affected gene products might allow fundamental insights into the relevant cellular pathways. For example, a positional cloning approach was successfully applied in the

cloning of the disease gene for thiamine-responsive megaloblastic anemia (Rogers syndrome), which is characterized clinically by anemia, deafness and diabetes mellitus. The disease gene, *SLC19A2*, encodes the first identified mammalian thiamin transporter and represented the second human disease gene identified completely *in silico* from high throughput genomic sequence. A murine model system is now being developed, which should yield further insights into disease pathogenesis. An effort is also underway to identify the disease gene responsible for autosomal recessive Kenny-Caffey syndrome (KCS), a skeletal dysplasia associated with congenital hypoparathyroidism, mental retardation and immune dysfunction. The widespread phenotypic manifestations of KCS suggest that the disease gene will play an important developmental role in a variety of organ systems. Physical mapping of the region has been completed and the critical interval has recently been genetically refined to ~230 kb. The remaining positional candidate genes are currently being screened. A third trait under study is an immunodeficiency condition, WHIM syndrome, which leads to neutropenia and hypogammagloublinemia with an unusual susceptibility to human papillomavirus (HPV). Linkage mapping resulted in localization of the disease gene to a broad interval on chromosome 2q21. An analysis of genes in the interval identified an excellent positional candidate, *CXCR4*, a medically important gene because of its role as a co-receptor for HIV. Two truncation mutations, one recurrent and one private, were found in affected individuals. Stimulation of affected cells with the chemokine SDF-1 revealed that calcium flux is substantially greater in the mutant cells, consistent with a gain-of-function mutation. The identification of *CXCR4* as the causative gene represents the first instance of a chemokine or its receptor causing a human disease. This discovery may allow insight into the mechanisms by which the immune system combats infection by HPV, which can be a cause of significant morbidity.

Dan P. Felsenfeld, PhD
Adhesion Receptor Function in Axon Guidance

This laboratory studies axon growth and guidance at the cellular level, focused primarily on the function of the adhesion receptor L1, a neuronal immunoglobulin family member. L1 spans the cytoplasmic membrane, interacting with both the cytoskeleton, where the forces needed for axon outgrowth are generated, and substrate-bound ligands in the extracellular environment. L1 has been implicated in the guidance of neurons in the veterbrate central nervous system, suggesting that its function is regulated to permit direct growth. Trainees will employ a combination of video microscopy and molecular genetics to characterize the regulation of the L1-cytoskeleton link. Specifically, they will use an otpical gradient laser trap or "laser tweezer" to place and manipulate microscopic beads on the neuronal surface. These beads when coated with an antibody or ligand for L1, allows us to detect L1-cytoskeleton attachment by monitoring the position and movement of L1 on the surface of live neurons. By combining this approach with molecular genetics, we can identify the domains of L1 that are required for the regulation of L1 cytoskeleton interaction in growing neurons.

Douglas Forrest, PhD
Transcription Factor Mutations and Disease: Thyroid Hormone Receptor as a Model System

This laboratory investigates several areas of research related to the genetics of "Mental Retardation and Developmental Disabilities". In particular, we have a long-standing interest in the role of the thyroid hormone receptor genes in the development of the nervous system. It is well known that thyroid hormone is essential for the correct maturation of brain functions. Children born with inadequate thyroid hormone are at risk for severe mental retardation and in some cases deafness. This research has focused on the molecular biology and genetics of the

different thyroid hormone receptor isotypes, which act as hormone-dependent transcription factors. These receptors are expressed from the two related genes *THRA* and *THRB* (equivalent to *Thra* and *Thrb* in mice). Making extensive use of gene targeting approaches to determine the physiological roles of the receptor isotypes, unique insights into the receptor networks that underlie thyroid hormone action in development have been made. Targeted mutations in the mouse *Thrb* gene have also provided a model for the inherited human syndrome of resistance to thyroid hormone. It has been found that these receptor genes play an unexpectedly critical role in the development of the sensory systems for hearing and color vision. These present novel systems in which to investigate the molecular, cellular, and physiologic functions of the thyroid receptor genes. These studies aim to elucidate the processes involved in development of these sensory systems, which is also likely to be relevant to understanding the pathogenesis of inherited diseases associated with deafness and blindness or retinal degeneration.

Bruce Gelb, MD
Positional Cloning of Mendelian and Complex Traits: Char Syndrome, Noonan Syndrome and Related Disorders

Char syndrome is an autosomal dominant disorder with features that include abnormalities of craniofacial and hand development and patent ductus arteriosus (PDA). Using a positional cloning/candidacy strategy the trait was linked to chromosome 6p12-p21.1 and mutations were identified in the transcription factor gene, *AP-2b*. Expression of mutant AP-2b proteins *in vitro* and in cell culture established that the mutations engendered dominant-negative effects. Current efforts are directed at cloning and characterizing addition members of the *AP-2* family, creating a mouse model of Char syndrome through transgenesis, and studying the effects of the loss of Ap-2b in the developing cardiovascular system of the mouse. Noonan syndrome is a relatively common autosomal dominant disorder with pleiomorphic features that include short stature, dysmorphism, mental retardation, and heart disease. This disorder is the most common non-chromosomal syndrome with heart defects, which include pulmonic stenosis and hypertrophic cardiomyopathy. The disorder had been mapped to chromosome 12q24, but has also been shown to be genetically heterogeneous. Very recently, our group documented that mutations in a non-membrane phosphatase that plays an important role in several receptor tyrosine kinase signaling pathways cause Noonan syndrome. Current efforts are directed at established the range of molecular defects that underlie Noonan syndrome, to exploring the effects of the mutant phosphatase on signal transduction, particularly during development, and to establishing the disease genes for other genetic forms of Noonan syndrome and related disorders (cardiofaciocutaneous and Costello syndromes).

Mitchell Goldfarb, PhD
Signal Transduction in the Developing Vertebrate Nervous System

This laboratory studies the signaling pathways that promote neuronal differentiation. Our studies have identified a family of related receptor-associated signaling adaptors termed SNTs which may play a central role in triggering differentiation. We have characterized the molecular basis of SNT recognition by neurotrophins and FGFs, and in collaboration with one of Mount Sinai's structural biologists (Ming-ming Zhou), determined the structural basis for receptor/SNT interactions. Trainees will be comprehensively trained in signal transduction methodologies as they relates to vertebrate CNS development.

Jon W. Gordon, MD, PhD
Transgenic Mouse Models of Human Disease and Gene Therapy

Transgenic animals represent an experimentally idealized model for evaluating gene therapy strategies since 100% of the target cells are stably transformed with an efficiently expressed vector. Specific projects under investigation utilizing these models include: 1) Gene Therapy of Diabetes . In these experiments, attempts are being made to overcome insulin deficiency by expressing insulin from the liver. The goal is to design constructs for hepatic expression that would be regulated in a manner similar to pancreatic insulin. The construct currently under study is pyruvate kinase (PK) linked to the human insulin gene. PK is lowered by glucagon expression and augmented in response to increases in blood glucose. These transgenic mouse studies permit the preclinical evaluation of gene therapies strategies and provide the rationale for future human trials; 2) Transgenic Models of Amyotrophic Lateral Sclerosis. Our laboratory has recently produced a transgenic model of amyotrophic lateral sclerosis by over expressing an altered form of the murine Cu/Zn superoxide dismutase gene. These mice exhibit classic phenotypic and histopathological features of ALS in humans. Experiments are underway to analyze the mechanism by which altered gene expression produces this abnormality, to characterize the defect histopathologically, and to test possible therapies; 3) Gene Therapy of Hepatocellular Carcinoma. In these experiments, transgenic mice have been engineered to express the Herpes thymidine kinase (TK) gene product from the ? -fetoprotein (AFP) promoter. Transgenic mice then turn on the transgene when hepatic malignancies develop, since these malignancies express AFP. Because TK is a conditional toxin, killing dividing cells in the presence of various analogs (e.g., ganciclovir), the model allows us to test the efficacy of this form of gene therapy in the treatment of this malignancy, and to study the biology of tumor cells that survive TK ablation *in vivo*.

Andrea Gore, PhD
Regulation of the Gonadotropin-Releasing Hormone Neurosecretory System

This laboratory studies the regulation of the gonadotropin-releasing hormone (GnRH) neurosecretory system. GnRH is the key hormone controlling reproduction. In all vetebrate organisms, the GnbRH peptide is released ina lulsatile manner from neuroterminals in the median eminence into the portal circulation, leading to the anterior pituitary gland, where it regulates the synthesis and release of the gonadoropins, leteinizing hormone and follicle-stimulating horomone. Our laboratory using multidisciplinary approaches to study the mechanisms of hormone release from peripheral nerves, and studies gene regulation related to reproductive neuroendocrinology.

Kurt Hirschhorn, MD
Cytogenetics and Molecular Cytogenetics

In the Cytogenetics laboratory, molecular and cytogenetic techniques, such as in situ hybridization, and/or FISH are being used for refined diagnosis and mapping of genetic loci. The use of chromosome elongation (late prophase preparations), newer banding techniques, and FISH also are employed to characterize structural alterations in chromosomes of parents with congenital malformation syndromes and in couples with infertility/recurrent miscarriages. This laboratory utilizes traditional and molecular cytogenetic techniques (fluorescent *in situ* hybridization and comparative genomic hybridization) for the identification and characterization of structural chromosomal abnormalities, aneuploidy, chromosome microdeletions and marker chromosomes. Clinical applications of newer molecular technology include the use of interphase cytogenetics on prenatal specimens for rapid sex determination and aneuploidy screening and the development of novel technologies for prenatal diagnosis including isolation and characterization of fetal cells from maternal circulation and single cell genetic diagnosis (pre-implantation diagnosis).

George Huntley, PhD

Development and Plasticity of Cerebral Cortex Structure and Function

Dr. Huntley's research is on the development of cellular and synaptic organization of cerebral cortex and mechanisms of synaptic plasticity through which cortical function is modified by experience. His non-human primate model system is primarily the sensory and motor areas of the cerebral neocortex, which contain representational "maps" of the body's sensory receptors or muscles. Such maps can be revealed both by anatomical methods, as well as by neurophysiological and functional imaging methods. Some of the fundamental questions addressed by this research include: 1) how are the different body parts of the cortical maps interconnected, and how do these interconnections contribute to map reorganization during skill learning or as a result of a traumatic injury to a limb or spinal cord? 2) how do the various anatomical connections of these areas develop, and what are the factors during early life which influence their organization and function? 3) what are the molecular components of the synapse which govern the formation, maintenance and subsequent plasticity of synapses in the brain? 4) what are the guidance and targeting molecules which orchestrate connection formation during development? 5) what is the role of adhesion molecules in regulating synaptic plasticity in the mature brain? To answer these questions, multidisciplinary approaches are used which include neurophysiological recording of neuronal activity *in vivo*, in *in vitro* brain slice preparations, and from co-cultured explants; conventional light-, confocal, and electron microscopy of immunocytochemically identified proteins (e.g. receptors, cell adhesion molecules); in situ hybridization histochemistry to study gene expression and regulation in an anatomical context; and neuroanatomical tracing and neuron reconstruction. Recent studies have addressed: 1) the role of tactile experience in shaping motor cortex function during development and in adulthood; 2) the development and plasticity of thalamocortical and other major pathways of the cortex; 4) the role of intrinsic, horizontal axons in mediating shifts of cortical map borders between different body parts; 5) the role of adhesion molecules in the development of patterned synaptic connections in cerebral cortex; 6) the role of particular glutamate and GABA receptors in plasticity of adult and developing cerebral cortex; 7) cortical plasticity following spinal cord and peripheral nerve injury.

Yiannis A. Ioannou, PhD

Gene Therapy: Novel Vectors, Neural-Delivery and Neuronal Targeting

This lab is involved in a number of projects that center on the biology, function and diseases of the endosomal/lysosomal (E/L) system. In addition, they are developing methods to treat lysosomal diseases via enzyme and gene therapy approaches, as described below:

Project 1. Subcellular Cholesterol/lipid Transport: Although the intercellular transport of cholesterol from the liver to peripheral tissues has been intensively studied, little is known about its egress from the E/L system, its intracellular transport and the proteins involved in this process. The existence of such proteins is highlighted by the autosomal recessive disorders, Niemann-Pick C (NPC) disease, in which cholesterol accumulates in lysosomes and leads to progressive neurodegeneration, and Tangier disease in which cholesterol efflux at the plasma membrane is defective. The overall objective of this project is to identify and characterize the various components of the intracellular cholesterol and lipid transport machinery and determine their function and interactions.

Project 2. Proteomics of the E/L system; Cell Proliferation and Apoptosis: It is becoming clear that the lysosome has a greater role in cellular processes than was originally proposed. Our studies focus on the isolation and purification of intact endosomes and lysosomes. These organelles are then characterized for their membrane composition to identify new membrane

proteins. A novel method for the isolation and characterization of endosomes and lysosomes has been established in this lab. A long-term goal is to identify all the components of the endosomal/lysosomal apparatus using 2D electrophoresis and tandem mass spectroscopy.

Project 3. Gene Therapy for Diseases that Affect the CNS: Effective gene therapy strategies for the treatment of human disease still remain highly experimental due to difficulties encountered in the actual application of many gene therapy schemes. A non-viral approach to this problem by designing small peptides that can compact and deliver DNA to specific cell types is being developed. Recently, we were able to use such packages for the successful delivery of marker genes to the rat brain. In addition, they have been able to develop packages that can be endocytosed by target cells in an endosome-independent manner. This is the first step in developing non-viral DNA delivery packages for successful transduction of target cells both *in vitro* and *in vivo*.

Gordon Keller, PhD

Molecular and Cellular Control of Vascular and Neural Development During Embryogenesis

The focus of Dr. Keller's research program is to define and characterize the early events involved in the establishment, growth and maturation of the embryonic hematopoietic and vascular systems. In particular, he is interested in studying the common precursor of blood cells, a cell known as the hemangioblast. To address questions related to the early stages of lineage commitment, the mouse embryo is studied prior to blood island development. As access to the embryo at this stage of development is extremely difficult and the number of cells available limited, these early events are investigated using a model system based on the *in vitro* differentiation potential of embryonic stem (ES) cells. Previous studies have demonstrated that the establishment of the hematopoietic and endothelial lineages within the EBs parallels that of the embryo with respect to the kinetics of development and differential gene expression patterns. Using the ES differentiation model, he has recently identified a novel precursor that develops early within the EBs and displays the unique capacity to generate cells of both the hematopoietic and endothelial lineages. Given this potential, this cell has the characteristics of the hypothetical hemangioblast, and represents the earliest hematopoietic and endothelial precursor described to date. Using these early precursors, his group has recently carried out a subtractive hybridization between closely staged populations with the aim of identifying genes that are involved in the development of the hemangioblast and the subsequent generation of hematopoietic and endothelial progeny from it. With this approach, a number of novel genes have been isolated that are expressed at the stage of the hemangioblast as well as in the embryonic hematopoietic and endothelial lineages isolated from EBs. The current emphasis of his lab is to define the function of these genes and their role in developmental hematopoiesis and vascular biology.

R. Michael Linden, PhD

Site-Specificity of DNA Integration into the Human Genome

The primary focus of this laboratory is the site-specificity of DNA integration into the human genome based on the use of adeno-associated virus (AAV) as a vector for gene therapy. As evident from observations of wild type virus infections in man and primates as well as of experiments using recombinant virus in animal models, AAV holds an exciting promise as a vector for the treatment of inherited human diseases. Important characteristics for this aspect are: i) AAV has not been implicated as causing any human disease. ii) AAV has a wide host range. iii) Both growth-arrested and non-dividing primary cells can be efficiently infected by AAV. iv) Stable and efficient integration of viral DNA occurs into a specific site (AAVS1) within the host genome. This site is now characterized and therefore available for monitoring genetic alterations

which may follow integration. In addition, analysis of AAVS1 has led to the description of sequence requirements for site-specific integration and to the proposal of the underlying mechanism. However, several basic issues need to be addressed in order for AAV to provide a satisfactory system for therapeutic transgene delivery. The objective of our research is to study biochemical requirements for efficient targeting of site-specific integration of AAV. In extension, attempts will be made to achieve integration into the host genome at selected specific loci in addition to the human target sequence for wild type AAV integration (AAVS1). The general principle underlying this attempt is to replace a defective host gene by means of altered AAV site-specific integration.

Robert Margolskee, MD, PhD
Molecular Mechanisms Underlying Sensory Transduction

Dr. Margolskee's research is focused on sensory transduction using vertebrate taste as a model. Bitter and sweet are thought to be transduced by specific seven transmembrane-helix receptors linked to G proteins: their effects mediated via second messenger systems (e.g., adenylyl cyclase, phospholipase C and phosphodiesterase). Elucidating the molecular mechanisms underlying taste transduction is a major goal of our research. Until recently, most of the understanding of taste transduction came from physiological studies of taste buds and taste fibers and electrophysiological analysis of isolated taste cells. Biochemical and molecular biological characterization of the mechanisms of taste transduction had been hindered because taste receptor cells comprise only a small and inaccessible fraction of the lingual epithelium. During the past seven years, this group cloned taste specific cDNAs corresponding to proteins involved in sensory transduction, analyzed their expression patterns, expressed altered taste proteins in vitro and determined their functions in transgenic and knock out mice. Gustducin is a taste-specific G protein. We cloned a novel G protein ((alpha)) subunit, α-gustducin, from taste cells and showed by in situ hybridization and immunohistochemistry that it is only present within taste receptor cells. Remarkably, gustducin most closely resembles the transducins (the rod and cone photoreceptor G proteins), suggesting that gustducin's may play a role in taste transduction analogous to that of transducin in phototransduction. Subsequently, we found that rod transducin is also expressed in taste cells - this is the first demonstration of transducin expression in non-photoreceptor cells. The lab then went on to partially purified two novel phosphodiesterases from bovine taste tissue that can be regulated by gustducin or transducin. We have also purified a bitter responsive taste receptor that can activate exogenously added gustducin or transducin in vitro. Based on the biochemical data it is believed that gustducin and transducin couple taste receptors to taste phosphodiesterases. Using single cell PCR and differential hybridization gustducin's γ-subunit has been cloned and it has been demonstrated that gustducin's β-γ-subunits activate taste tissue phospholipase. Recently, homozygous knock out mice null for gustducin expression have been generated. These animals are viable and grossly normal in appearance. The knock out mice show profound deficits in their behavioral and neurophysiological responses to both bitter and sweet compounds. Transgenic experiments with specifically altered gustducin to analyze the gustducin-receptor and gustducin-phosphodiesterase interactions underlying sweet and bitter transduction are now being conducted. These studies will allow correlation of genetic defects (genotype) with behavioral and electophysiological changes (phenotype).

John Martignetti, MD, PhD
Gene Discovery for Inherited Diseases

Research in this laboratory is directed towards identifying the genes and molecular mechanisms underlying a number of diverse human diseases and sporadic and hereditary forms cancer. To achieve these aims, basic and advanced molecular biology/genomic methodologies are used and

developed, including linkage analysis, positional gene cloning, high-throughput and large-scale DNA sequencing and analysis, mutation detection, genotyping, automated fluorescent loss-of-heterozygosity (LOH), analysis of knockout and transgenic mouse models, and gene therapy. The most current translational-research projects have ranged from clinical description, gene identification, structure-function and genotype-phenotype correlations in a novel skeletal dysplasia/arthritis syndrome, to macrothrombocytopenia, deafness, renal syndromes. Several other projects based on inherited familial disorders are also underway. In addition, this group has developed the clinical and research infrastructure to actively pursue gene discovery projects in a number of important human cancers. These include sporadic and familial forms of prostate cancer, colon cancer, head and neck squamous cell carcinoma, and a familial thyroid and hereditary bone dysplasia/cancer syndrome.

John H. Morrison, PhD
Studies of Mammalian Brain Development

This laboratory's research program is focused on the neuroanatomic, cellular, and molecular analyses of non-human primate and human neocortex using various chemically-specific anatomic techniques (e.g. immunohistochemistry, and in situ hybridization), as well as transport and intracellular filling techniques to develop a detailed molecular/anatomic profile of identified neocortical neurons and circuits. The complex circuitry that is the hallmark of primate neocortex requires a high degree of neuronal specialization that is reflected in both the molecular and anatomic phenotype of a given cell or cell type. Such specialization may be seen in the expression of high levels of certain proteins, the use of a particular neurotransmitter or the presence of certain receptors, a unique dendritic arborization pattern, or axonal specializations that are related to the neuron's synaptic target. The goal of this laboratory is to develop a quantitatively accurate cellular model of neocortex that links both the molecular and anatomic characteristics of the various cellular constituents of neocortex. *In situ* hybridization and immunohistochemical studies are also being used to analyze the cellular distribution of neurotransmitter receptor molecules. Many of the histochemical and neuroanatomic analyses are quantitative and rely heavily on computer-assisted microscopy and image analysis. These data on basic cortical organization are then used to analyze the molecular/anatomic profile of the neurons that are vulnerable in Alzheimer's disease, and to determine which molecular and anatomic attributes of these neurons are responsible for their heightened vulnerability in Alzheimer's disease or schizophrenia. Attempts will be made to develop a means of protecting these neurons from the degenerative process that leads to the dementia of Alzheimer's disease, and to develop pharmacological interventions that are based on the cellular distribution of specific receptor subtypes. These methods can be used to investigate the neurodegenerative processes that occur in various genetic disorders with neurologic manifestations, including psychomotor mental retardation.

Angel Ortiz, PhD
Bioinformatics and Cheminformatics

Dr. Ortiz's research efforts are centered in the development of new computational approaches to use genome information in drug discovery. The goal is to better understand complex biological systems from a chemical and physical point of view and being able to use this understanding to design novel drugs. Her studies can be divided in three different areas: 1. Genome analysis and comparison: The avalanche of genome information available from genome sequencing projects is providing a plethora of data for which no biological meaning can be inferred yet. This group is trying to develop techniques for genome comparison that could make possible better annotation protocols by "function transference" from well annotated genomes. In addition, they are

developing techniques to detect, by means of genome comparison, genes of particular interest from a biological or pharmacological point of view. 2. Sequence-structure-function relationships in proteins: Deriving structural and functional information from sequence data is crucial in order to fully exploit genomic data. New techniques are being developed for structure prediction based on multivariate analysis of multiple sequence alignments coupled to Monte Carlo based protein folding algorithms. Rigorous methods for structure comparison also are being developed in order to better evaluate prediction success. The functional and structural implications of sequence variability patterns in protein families is being explored as well. 3. Ligand design and molecular recognition: Eventually, structure prediction algorithms and genome comparison methods will provide candidates of interesting pharmacological targets together with some approximate coordinates. Ligand design using these approximate models will be a challenge. This group is exploring new docking and scoring approaches in order to be able to design new leads from them.

Daniel Perl, MD
Environmental Factors in Neurodegenerative Disease

This laboratory is involved in the study of the role of potential environmental factors in the pathogenesis/etiology of neurodegenerative diseases, using Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis (Lou Gehrig's disease) as models. This group was the first to identify the accumulation of aluminum in the neurofibrillary tangles of Alzheimer's disease and in amyotrophic lateral sclerosis/parkinsonism-dementia complex seen in high frequency among the natives of the island of Guam. In this work highly precise and sensitive tissue microprobe techniques including laser microprobe mass analysis of LAMMA are used. Animal models of chronic neurotoxicity are also employed to understand mechanisms by which environmental factors may enter the central nervous system and produce chronic progressive neuronal degeneration. This lab also is involved in detailed descriptive studies to document the extent and distribution of various neuropathologic lesions in human brains which have been obtained at autopsy from patients affected by a wide range of neurodegenerative diseases. They are particularly interested in the extent of overlap between the morphologic changes encountered in what have been classically considered to be separate and distinct clinical disorders.

Stephen Salton, MD, PhD
Mechanisms of Action of Neurotrophic Growth Factors

Dr. Salton's group is interested in understanding how neural genes are regulated by neurotrophic growth factors in the developing and adult mammalian central and peripheral nervous systems. They have identified several gene products that are relatively selectively regulated by neurotrophic growth factors in PC12 cells, including the adhesion molecule NILE (rat L1) and the polypeptide VGF. VGF expression is rapidly and robustly induced in neurons and PC12 cells in vitro and is up-regulated in the brain following seizure and cortical injury. Unlike many immediate early genes with which vgf shares certain characteristics, this gene encodes a secreted polypeptide that is released from dense core vesicles and may be cleaved into bioactive fragments. In the normal mouse, VGF mRNA levels increase in the hypothalamus in response to fasting. Based on studies of VGF knockout mice, this molecule appears to play a critical role in regulating energy balance and hypothalamic function. Studies are currently underway to investigate the mechanisms by which VGF expression is targeted to neurons and regulated by neurotrophins, to better characterize the functions of VGF in the developing and adult nervous system, and to identify the mechanism(s) of action of VGF and putative cell surface receptors. Gene amplification techniques have been used to clone developmentally-regulated protein tyrosine phosphatases that are selectively expressed in the nervous system, one of which, RPTPzeta/beta, is abundantly expressed by astroglia during embryogenesis and is induced in

response to CNS and PNS injury. RPTPzeta/beta is a cell adhesion molecule with intracellular phosphatase domains that may signal binding to extracellular ligands (such as contactin and NILE/L1) by altering tyrosine phosphorylation. Studies are currently underway to identify RPTPzeta/beta substrates and to further characterize the regulation and signaling pathways of this receptor tyrosine phosphatase. In neurons, cell adhesion molecules such as NILE/L1 and telencephalin are selectively targeted to axons and dendrites, respectively. Current efforts are directed at trying to identify polypeptide signals that may be responsible for sorting of cell surface and secreted proteins into different intracellular compartments of the polarized neuron. It is hoped that the study of these gene products and their regulation will allow dissection of the mechanisms of action of neurotrophic growth factors during neural differentiation and regeneration.

Matthew Shapiro, PhD
Behavior Genetics and Memory Processing

This lab is interested in how the brain remembers; i.e., how ongoing information processing by neural circuits alters those circuits so that information is encoded, stored, and then later retrieved. In particular, we are interested in the neural mechanismsl of memory in the everyday senseof the world – the ability to learn new facts and remember recent events. Experiments are guided by cognitive, computational, physiological, and pharmacological hypotheses. The basic idea is that the properties of the NMDA receptor allows cells in the hippocampus to conjoin temporally overlapping cortical inputs into representations of events, and that recurrent connections within the hippocampus allow these events to be linked into the sequences that compromise episodic memories. Thus, trainees will study learning that requires the hippocampus, neural encoding by the hippocampus, and synaptic plasticity within the structure that depends upton NMDA receptor activation.

Hans-Willem Snoeck, MD, PhD
Hematopoietic Stem and Progenitor Cells

The focus of this laboratory is the biology of hematopoietic stem and progenitor cells. Since these cells are able to reconstitute the hematopoietic system of lethally irradiated recipients, they are key players in bone marrow transplantation, and ideal targets for gene therapy for the treatment of genetic, infectious and malignant diseases of the hematopoietic system. A fundamental question in stem cell biology is which mechanisms determine whether a hematopoietic stem cells will self renew or undergo differentiation. In other words, what determines the fate of hematopoietic stem cells. Many investigators have tried to induce in vitro self renewal using different cytokine and growth factor cocktails. Renewal in vitro has never been demonstrated, however. This lab is attempting to identify factors that govern renewal of stem cells by studying and comparing in vivo conditions where renewal is known to be differentially regulated. It is well known that the kinetic behavior of the primitive hematopoietic compartment changes upon aging, and is also genetically determined, at least in the mouse model. Investigating the mechanisms responsible for age-related changes and genetically determined differences in the stem cells kinetics, could lead to the elucidation of mechanism which are fundamental to the regulation of differentiation, renewal and kinetics of stem cells in vivo. Therefore the focus of this lab's studies are on aging and genetically determined differences in the stem cells pool size.

Martin Walsh, PhD
Molecular Genetics of Cystic Fibrosis

This laboratory studies the mechanism of cell cycle regulation of transcription, with specific emphasis on cystic fibrosis. In particular, recent studies have focused on transcription factors and cofactors involved in the regulation of the cystic fibrosis transmembrane conductance regulator (CFTR), cyclin-like uracil DNA glycosylase and myc genes. Our studies have provided evidence for the function of transcription factors to signal the gene-specific modification of chromatin structure via histone acetylation and shown that acetylating of histones within chromatin is a tightly regulated bio0chemical process directing the transcription of specific genes. Trainees have the opportunity to learn a wide range of approaches for studying gene regulation in mammalian cells, including RNA and promotor analysis, and analysis of chromatin structure *in vitro* and *in situ*. We are also using transgenic cells and animal models to identify the role of chromatin modification as a key function in the control of gene expression.

Rong Wang, PhD
Functional Proteomics in Neurodegenerative Diseases

The focus of the research in Dr. Wong's laboratory is functional proteomics and protein posttranslational modification in cancer and neurodegenerative diseases, using mass spectrometry in combination with biochemical cellular and molecular biological methods. These studies are focused on the identification of specific protein markers for early detection and diagnosis of Alzheimer's Disease (AD) and to discover key proteins involved in the pathologic al progression of AD. The long-term objective of this research project is to understand the molecular mechanism at the protein level of cellular changes that drive the evolution of AD. Currently, plasma proteins from AD patients are being studied and compared to those from normal controls to identify specific protein(s) that associate with AD. Biotransformation of amyloid β-peptide and proteomics of genetic mutations, presenilin 1 (PS1), amyloid precursor protein (APP), and apolipoprotein E (ApoE) are being investigated including: (1) amyloid β-peptide profiles; (2) both total and subcellular protein profiles and (3) protein complexes that associated with PS1, APP and ApoE proteins using cultured cells and transgenic mice.

Peter Warburton, PhD
Human Chromosome Structure

Dr. Warburton's research is focused on delineating the requirements for the formation of centromeres in mammalian cells. Centromeres appear to be epigenetically determined, not strictly dependent on a particular underlying functional DNA sequence, but instead dependent on formation and propagation of a specific centromeric chromatin structure. This lab employs cell biological and biochemical approaches to investigate the epigenetic requirements for human centromere formation, using both experimental- and patient-derived cell lines that contain variant centromeres. This research is not only important for the basic biology of chromosome function and cell division, but has broad ranging application to human health and disease, including aneuploidy and birth defects, cell cycle regulation and cancer, and gene therapy. The long term goals of the research program include development of mammalian artificial chromosome (MAC) vectors that are capable of efficient de novo centromere formation for use as autonomous gene expression vectors. The research program is ultimately directed towards the efficient construction and delivery of MACs, which by definition would be autonomous vectors that would not require integration into the host genome and could contain sufficient genomic DNA to provide correct temporal and tissue-specific gene expression.

Harel Weinstein, PhD
Molecular Biophysics of Neural-Specific DNA Binding Proteins

Dr. Weinstein's laboratory aims to discover the structure, dynamic and electronic determinants of biological processes underlying physiological functions through the development and application of methods in theoretical and computational biophysics. This group seeks a mechanistic understanding at the molecular level of detail, anchored in experimental information about structures and properties of cellular components and physiological mechanisms. The approaches used include theoretical determinations of molecular structure and properties, and computational simulations of molecular mechanisms and processes that can be studied with great accuracy. The theoretical studies are designed to complement experimentation in providing mechanistic insights about systems of ever increasing size and complexity, and to guide pointed experimental exploration of cellular processes and functions in numerous collaborative studies. A unifying theme is the understanding of mechanisms triggered by molecular recognition and leading to signal transduction. They study structural specificity and dynamics in three main areas in which such processes determine physiological mechanisms.

James G. Wetmur, PhD
Environmental Factors in MRDD

One of the interests of this laboratory is environmental genetics. For example, the second enzyme in the heme biosynthesis pathway, ALAD, is polymorphic. Dr. Wetmur's group has determined the complete human ALAD genomic sequence and has identified and demonstrated tissue-specific promoters for alternatively-spliced housekeeping and housekeeping mRNAs. A PCR-based method for genotyping human ALAD, which encodes ALAD isozymes based on two alleles, $ALAD^1$ and $ALAD^2$ has been developed. ALAD is inhibited when lead replaces zinc. The $ALAD^2$ allele has been shown to be associated with increased blood lead levels in exposed individuals, suggesting a population at greater risk from lead in the workplace or the environment. Having completed this work, this group has now focused on the study of other susceptibility genes affecting the response of children to environmental agents, including gene products of cytochrome P450 genes that detoxify organophosphate pesticides. The regulation of endonucleases associated with molecular motors involved in DNA replication, recombination and repair also is being studied. For example, a thermostable *in vitro* recombination system capable of enzyme-catalyzed branch migration is being assembled. The lab also has cloned and expressed thermostable RecA, the Holliday-junction binding protein RuvA, the helicase RuvB, and the resolvase RuvC from the hyperthermophile *Thermotoga maritima*. RuvA, RuvB and ATP are required for heteroduplex formation. RuvC cleaves at a consensus sequence in the Holliday junction. We are in the process of biochemical and structural characterization of the complete system, with ongoing collaborations in crystallography and atomic force microscopy. Similarly, the DNA replication system of *Methanococcus jannaschii*, a hyperthermophilic archaeon, which encodes a PCNA and a flap endonuclease more similar to eukaryotes than bacteria is being investigated. Some of these proteins have potential biotechnology applications.

Savio L. C. Woo, PhD
Gene Therapy For Metabolic Diseases

This laboratory is interested in the application of molecular biological and genetic tools to establish the basis of hereditary disorders in man as well as their ultimate cure by somatic gene therapy. Previous accomplishments include the initial cloning of the genes for phenylalanine hydroxylase and α1-antitrypsin, and the identification of the respective disease-causing mutations. More recent efforts have focused on the development of gene therapy strategies for genetic diseases and cancer. Replication defective recombinant retroviral and adenoviral vectors were successfully used to directly transfer genes into the liver of laboratory animals that resulted in their constitutive expression *in vivo*. These investigations have led to the long-term partial

correction of the bleeding phenotype in hemophilic dogs, as well as complete, but transient, correction of hypercholesterolemia in LDL-receptor deficient rabbits. More recently, replication-defective adeno-associated viral vectors have been used to transfer genes into the liver that resulted in their persistent expression. In order to further elevate gene expression levels *in vivo*, promoter/enhancers, mRNA stability and translational efficiency elements have been inserted into the transgene expression cassettes. These exciting new developments have provided the foundation for gene therapy of metabolic disorders secondary to hepatic deficiencies by *in vivo* gene delivery.

Ming-Ming Zhou, PhD
Structural Biology of Cellular Signaling and Chromatin Remodeling

This research is directed at understanding molecular mechanisms underlying cellular processes in two areas: 1) conserved modular protein binding domains that mediate protein-protein interactions in transmission of signals from the cell surface to the nucleus, and 2) enzymes that regulate function of proteins by phosphorylation and acetylation on specific amino acids. Nuclear magnetic resonance (NMR) spectroscopy in combination with other biophysical, biochemical and molecular biological techniques are used to determine three-dimensional structures of these proteins; to investigate their chemical properties for molecular recognition and modification; to elucidate their cellular functions; and to develop chemical molecules capable of modifying these functions.

Clinical Research Training in MRDD:

Alan M. Aron, MD
Neurofibromatosis and Hydrocephalus

Dedicated research activities in all aspects of neurofibromatosis have been consolidated into a Neurofibromatosis Center. This center incorporates primary basic research in neurofibromatosis with clinical research studies of a large patient population. The center provides services to about 300 pediatric patients and a similar number of affected adults. Laboratory studies have been directed to characterization of nerve growth factors and their interaction with neurofibromatosis tissue obtained at surgery. In addition, a bank of tissues has been developed over the years and new studies directed to elucidating the genetic defects in the NF-1 gene are being initiated. Interdisciplinary collaborations with dermatology, orthopedics, neurosurgery, psychiatry and genetics have permitted a multifaceted approach to all aspects of the management problems encountered in neurofibromatosis and provides trainees with in-depth experiences in the management of this genetic-developmental disability. Research in hydrocephalus has focused on developing physiological parameters which can detect early evidence of increased intracranial pressure in hydrocephalic patients with long-term indwelling shunts. Due to the loss of elasticity of the ventricular walls in many of these patients, CT and MRI scans may not have sufficient sensitivity to detect early ventricular dilatation. Brainstem auditory evoked responses are being used to correlate pre- and post-shun revision in an effort to detect mid-latency response prolongations which appear to correlate with increasing intracranial pressure. To further evaluate this approach, a murine model has been developed to test and correlate exact pressure increments with neurophysiologic alterations.

Monte Buchsbaum, MD
Use of Positron Emission Tomography to Study MRDD

This center operates one of the larges positron emission tomography (PET) laboratories in the world studying MRDD. We are dedicated to the study of Alzheimer's, Schizophrenia, Autism, and general questions regarding how the brain changes with age and behavior. The research is accomplished through co-registration of PET and MRI modalities. We have also developed software to aid in this research. Trainees receive comprehensive training in neuroimaging techniques and analysis.

Robert J. Desnick, PhD, MD, Margaret M. McGovern, PhD, MD and Ainu Prakash-Cheng, MD, PhD

Natural History and Treatment of Lysosomal Diseases

Project 1: Natural History and Treatment of Fabry and Shindler Diseases

Fabry and Schindler diseases are lysosomal disorders that result from the deficient activity of α-galactosidase (α-Gal) A and B, respectively. Fabry disease, inherited as an X-linked recessive disorder, and Schindler disease, inherited as an autosomal recessive trait, both have severe and milder forms. In Fabry disease, three phentypes have been identified, the classical form, and the cardiac and renal variants. In Schindler disease, two major phenotypes have been delineated, a neurodegenerative disease of infancy and a milder adult form which presents primarily with angiokeratoma and mild intellectual impairment. Studies are underway to determine the natural history and phenotype/genotype correlations for each of these disorders. These studies involved careful clinical evaluation, mutation analysis and characterization of the residual enzymatic activity, if any for each phenotype. In addition, efforts are focused on the development of effective therapies. In classical Fabry disease, enzyme therapy has proven effective in reversing the lysosomal pathology and its long-term clinical effectiveness in under study. For the cardiac variant of Fabry disease, galactose infusions have proven effective in improving the cardiac manifestations in one patient, and efforts will be directed to assess other non-toxic competitive substrates to determine if they can stabilize the mutant enzyme as well as galactose *in vivo*, and improve cardiac disease. For Schindler disease, preclinical studies are underway in the knockout mice to determine if the primary neurologic disease can be modified by bone marrow transplantation or stem cell gene therapy in the future.

Project 2: Natural History and Treatment of Niemann Pick Disease

The natural history of Niemann Pick B disease is being delineated in a clinical protocol designed to assess the clinical complications of this lysosomal storage disorder. This clinical protocol has enrolled over 40 patients who have been serially evaluated over a five-year period. Comprehensive evaluations at annual visits have been carried out to document: growth parameters, extent of organomegaly, pulmonary and cardiac status, and neurologic function. These detailed examinations include exercise tolerance testing, ultrafast CT scan of the chest to document sphingomyelin deposition in lung parenchyma, echocardiogram, and abdominal CT scan for liver and spleen volumes. In addition, studies are ongoing to define the hyperlipidemia that occurs in Niemann Pick disease, and to determine if it presents a risk factor for early atherosclerosis. Genotype phenotype studies also are being conducted.

Project 3: Natural History and Treatment of Gaucher Disease

The Department of Human Genetics has an active clinical and research program in the lysosomal storage disorders for over two decades, with specific programs for Gaucher, Fabry, Niemann Pick and Tay Sachs diseases. The Comprehensive Gaucher Disease Treatment Center follows over 400 patients and treats over 100 patients with type 1 and 3 Gaucher disease. We have several clinical protocols to investigate the progression of Gaucher disease in patients on enzyme replacement therapy. Project 1. Natural History, Pulmonary and Skeletal Complications and Treatment of Gaucher Disease with Imiglucerase The study of patients with Gaucher disease, both on enzyme

replacement therapy and untreated, has been ongoing for many years. We have been studying the relationship between the genotype, splenectomy status, symptoms, and various blood markers (such as chitotriosidase) of Gaucher disease. Further, a multi-center study is ongoing to clearly assess the skeletal complications of Gaucher disease. We have also been studying the incidence and prevalence of pulmonary hypertension in treated and untreated patients. Project 2. Phenotype/Genotype Studies in Gaucher Disease. This project investigates the effect of modifier genes on the varying phenotypes observed in patients with identical genotypes. Predoctoral or postdoctoral fellows would have the opportunity to further study these genes and identify particular mutations in the modifier genes.

Margaret. McGovern, M.D, PhD and Judith Willner, MD; Associate Program Directors
Clinical Genetics

Our Clinical Genetics Center offers a comprehensive program for the diagnosis, management, treatment, and counseling of patients with metal retardation, developmental disabilities and other birth defects. Unique clinical resources at the Mount Sinai School of Medicine include the Program for Inherited Metabolic Diseases, Comprehensive Gaucher Disease Clinic, Prenatal Diagnostic Center, Neurofibromatosis Center, Hemophilia Center, Hemoglobinopathy Clinic, Alzheimer's Disease Research Center, Center for Autism, and the Center for Jewish Genetic Diseases. These clinics and centers provide state-of the art medical management and counseling to some of the largest populations of patients in the world affected with these disorders. Therefore, these represent unique opportunities for clinical investigations into the natural history and progression of disease. Research opportunities in Clinical Genetics are available and include: 1) delineation of new syndromes, 2) natural history studies for the correlation of genotype with phenotype, 3) implementation of new methods of prenatal diagnosis for inherited disorders, 4) psychological and psychosocial evaluation of the effects of prenatal diagnosis and genetic counseling, 5) application of high resolution ultrasound for the prenatal detection of morphologic syndromes, and 6) evaluation of the clinical and biochemical effects of various therapeutic strategies, including enzyme replacement and gene therapy.

Eric Hollander, MD
The Management and Treatment of Autism

Dr. Hollander is the Director of the Seaver Center for Autism which is dedicated to unraveling the biological causes of autism and related disorders, and developing effective treatments for autism. The Seaver Center's interdisciplinary approach to the study of autism includes family/genetic studies, brain imaging studies, and biological, autoimmune, neuropsychiatric and psychosocial and medication treatment studies. The goal of the Family Studies Program of the Center is to understand better the genetic factors in autism, utilizing the latest advances in molecular biology and quantitative genetics in conjunction with the direct study of families of autistic individuals. A genetic linkage study of multiplex families (i.e., families with two or more autistic-related disorders, or siblings with genetically related disorders) is being conducted. Relatives of those who have autism-related disorders also are being studied to increase greatly the power to identify a genetic marker, so that fewer families are needed. These studies involve diagnostic assessment of autistic individuals, briefer assessment of unaffected relatives, and collection of blood samples from every relative studied to retrieve the DNA. Brain imaging studies using positron emission tomography (PET) and magnetic resonance imaging (MRI) make it possible to view a detailed structure of the brain, including its metabolic activity. This group has identified a specific area of the brain that has repeatedly shown marked differences between autistic and normal individuals. This brain region, called the cingulate gyrus, is especially rich in

the chemical neurotransmitter serotonin and may affect organization of behavior, verbal fluency and emotional activity. Preliminary studies have demonstrated that increased cingulate gyrus activity is associated with greater repetitive behaviors and a more favorable response to SSRIs such as fluoxetine. Attempts are now being made to link cingulate gyrus abnormalities with specific compulsive and social deficit symptoms, to evaluate predictors of fluoxetine treatment outcome, and to assess the effect of SRI treatment on anterior cingulate metabolic rate. In conjunction with Columbia-Presbyterian Medical Center, another study is being conducted using positron emission tomography (PET) and magnetic resonance imaging (MRI). This study measures a type of the chemical receptor, serotonin, in the brain of adults diagnosed with autistic disorders. An abnormality of serotonin function is implicated in the neurochemical abnormalities associated with autism. Alteration in serotonin function might be secondary to alteration in serotonin receptors, since serotonin receptors are crucial to mediate serotonin function. This study also involves an investigational drug that will allow researchers to identify a serotonin receptor in the brain. The goal of this project is to compare the density of a serotonin receptor subtype in the brain of autistic adults and normal adults.

Philip Landrigan, MD, PhD and Mary Wolff, PhD
Center for Environmental and Preventative Medicine

The goals of this center are to identify, elucidate, and prevent imprairments of neurological and behavioral functions in urban children that result from exposure to pesticides, polychlorinated biphenyls (PCBs), lead, and other developmental toxins in the inner city environment. The center sustains a highly integrated multidepartmental program that links epidemiological and basic biological research to community-based prevention efforts in East Harlem, New York City. We also maintain one of the country's leading centers for measuring the long term effects of lead exposure on bone development. Clinical trainees receive broad training in environmental and preventive medicine, and rotate through our multiple centers and clinics.

Deborah Marin, MD
Center for Memory Disorders

This is a federally funded center whose mission is to provide state of the art evaluations for memory problems, provide counseling for individuals with memory disorders and their families, and conducting research on the cause and treatments for Alzheimer's disease and other neurological disorders causing memory loss. Information gained from this research will help investigators understand the cause and ultimately to find a cure for these illnesses. Several FDA sponsored clinical trials are also being conducted by the center. Trainees will learn how to manage patients with memory disorders and how to conduct and interpret controlled drug trials for these individuals.

C. Warren Olanow, MD
Transplantation of Fetal Cells for Parkinson Disease: A Model System to Evaluate Transplantation of Fetal Cells to Improve Neurologic Metabolic Diseases

The primary objective of this project is to establish whether transplantation of fetal mesencephalic allografts into the putamen can safely improve and alter progression of disability in patients with metabolic brain diseases. The prototype for these studies is Parkinson's disease (PD). In addition, we will specifically determine whether benefits associated with fetal nigral grafts are influenced by the number of donor embryos implanted. The effect of nigral grafts will be evaluated in non-demented patients with advanced PD complicated by motor fluctuations. The study is designed as a prospective, randomized, double-blind trial comparing fetal nigral

transplantation to a cosmetic placebo operation. As clinical outcomes, we will assess disease status by measures of motor function during "on" and "off" stages, number and duration of "on-off" periods, activity of daily living scores, a quality of life instrument, neurophysiologic measures of motor function and neuropsychologic measures of cognitive function. Positron Emission Tomography (PET) will be employed as a measure of physiologic function and putative graft viability. Patients randomized to placebo surgery will be offered the most effective surgery. We hypothesize that bilateral transplantation of fetal nigral grafts into the post-commissural putamen will provide a safe and clinically efficacious treatment for PD, and that the magnitude of functional recovery following fetal nigral transplantation is dependent upon the number of fetal donors employed. Specifically, we will perform a prospective, randomized, double-blind, study to determine whether benefits obtained from bilateral post-commissural putamenal transplantation are enhanced when fetal grafts are derived from four versus one donor per side. These studies will provide essential information regarding the safety and efficacy of fetal nigral grafting in PD and the relative importance of using multiple donors in obtaining enhanced clinical benefits. Such studies will determine the feasibility of using fetal brain cells grafting for the treatment of genetic disorders characterized by severe neurologic involvement.

Melissa Wasserstein, MD, George Diaz, MD, and Claude Sansaricq, MD, PhD
Metabolic Disorders and Mental Retardation

The Program for Inherited Metabolic Diseases at Mount Sinai includes comprehensive diagnosis and treatment for the inborn errors of metabolism, and an active research program. The research activities of the program include: 1) the evaluation of the efficacy of long-term nutritional therapy for phenylketonuria (PKU) and the development of new therapeutic approaches, 2) the determination of genotype-phenotype relationships in PKU, 3) the delineation of the natural history of renal dysfunction in the organic acidemias, 4) long term outcome of patients with PKU who enter treatment after the third year of life, 5) studies of cognitive function and psychiatric symptoms in adolescents and young adults with PKU who refuse dietary therapy, and 6) the molecular basis of MSUD.

Judith Willner, MD, Richard Berkowitz, MD, Keith Eddleman, MD, and Randi Zinberg, MS
Reproductive Genetics

The reproductive genetics program provides state-of-the art prenatal diagnosis, management, treatment and counseling of patients with pregnancies at risk for birth defects and inherited disorders. This is a joint program with the Division of Perinatology in the Department of Obstetrics and Gynecology where sophisticated prenatal detection techniques and interventions are available including CVS, high resolution ultrasound, percutaneous blood sampling, etc. This group also is involved in a number of research efforts. For example, this group is one of eleven centers in the United States involved in a research study entitled FASTER (First And Second Trimester Evaluation of Risk), which is funded by the National Institutes of Health (NIH) and the National Institute of Child Health and Human Development (NICHHD).